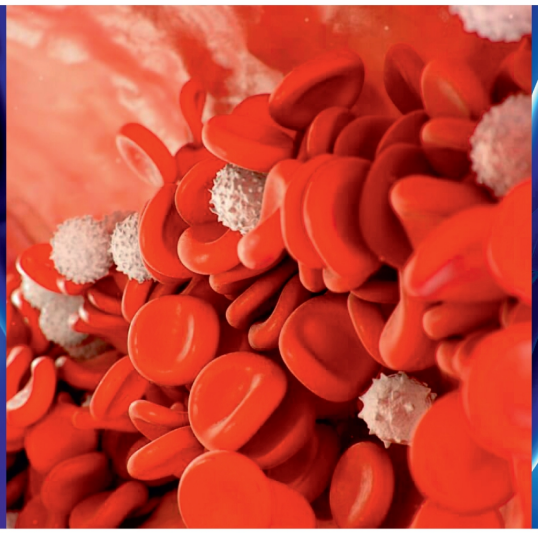
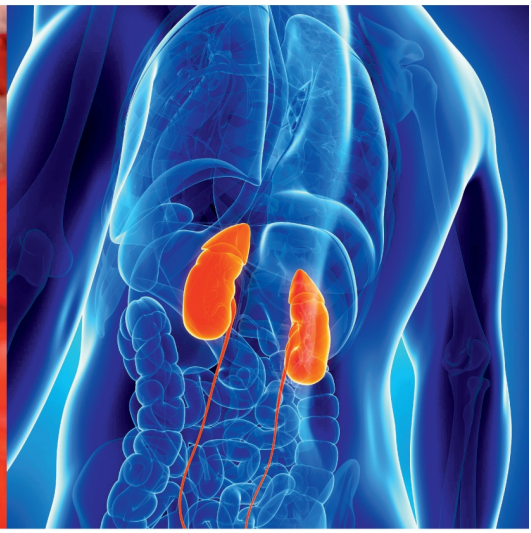
UNIVERSITATEA
DE MEDICINĂ ȘI FARMACIE
„VICTOR BABEȘ“ DIN TIMIȘOARA

PATHOPHYSIOLOGY

2 LECTURE NOTES FOR MEDICAL STUDENTS

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FOREWORD

Medicine, a continuously evolving science, is characterised by the symbiosis between clinical practice and scientific research, which allows the permanent optimisation of the diagnostic methods and therapeutic regimens for patient benefit.

Pathophysiology is a basic subject at the interface between preclinical and clinical disciplines which studies disease as the alteration of body's physiological functions and has continuously benefited from the information explosion in medicine.

The study of pathophysiology provides the knowledge necessary to understand the: causes (*etiology*), mechanisms (*pathogenesis*) that initiate and contribute to the evolution of a disease, and consequences (*clinical manifestations*) resulting from the impairment of the physiological functions. The lecture notes are purported to provide the 3rd year medical students with the information required for understanding the etiopathogenesis of diseases whose clinical manifestations and therapeutics are concomitantly studied at Medical Semiotics and Pharmacology, respectively. The present updated content matches the corresponding structure of the Pathophysiology manual for the Romanian section, in line with the syllabus for students in the IIIrd year, 2nd semester at the Faculty of Medicine.

To facilitate students' learning and preparation for the MCQ exam, information was concisely formulated and, when apropriately, included in summative tables. A brief overview of the main aspects taught in the first 2 years during the subjects that are curriculum prerequisites (anatomy, physiology, biochemistry, cell and molecular biology, biophysics, immunology) was introduced in the beginning of the chapters whenever considered useful. Also, when appropriate, both classic and novel pharmacological agents were mentioned in order to highlight the importance of correlating the pathophysiological mechanisms of diseases with the rationale of their therapy.

We trust that this second volume of the lecture notes, printed as e-book as the first one, will complement the traditional classroom courses and will be a useful study tool in order to gain a contemporary understanding of the pathophysiology of common diseases.

We will be grateful for any comment and remain receptive to all constructive opinions that may be used to improve the next edition.

The Authors

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1. PATHOPHYSIOLOGY OF RED BLOOD CELLS

Red blood cells (RBC) disorders include:

- **Anemias** – disorders with decreased red cell mass that are best described in terms of low hemoglobin concentration, since their pathophysiological impact is related to the low oxygen-carrying capacity of the blood.
- **Polycytemias (erythrocytoses)** – disorders with increased red cell mass that are best described best described in terms of increased hematocrit, since their pathophysiological impact is related to the high blood viscosity.

PATHOPHYSIOLOGY OF ANEMIAS

DEFINITION:

Anemia is defined as a **low hemoglobin (Hb) concentration** < 13.0 g/dL in adult men and < 12.0 g/dL in women, according to the World Health Organization (WHO).

MANIFESTATIONS:

- **Cause**: mainly, low **oxygen-carrying capacity of the blood** leading to **tissue hypoxia**
- **Consequences:**

A. In the <u>short-term</u>, COMPENSATORY changes occur:

a) Cardiovascular and respiratory:

- *increased heart rate and contractility* due to sympathetic stimulation $\Rightarrow$ increased cardiac output (CO) and redistribution of the blood to brain and heart (critical organs)
- *increased velocity of blood flow throughout the circulatory system* and *decreased blood viscosity* $\Rightarrow$ hyperdynamic circulation
- *increased respiratory rate* $\Rightarrow$ reflex tachypnea (to increase the O_2 intake)

b) Biochemical:

- *increased RBC production of 2,3-diphosphoglycerate (2,3-DPG)* $\Rightarrow$ right shift of the oxygen dissociation curve with lower Hb affinity for O_2 and increased O_2 delivery to the tissues

c) Hematological:

- *increased erythropoietin (EPO) production by the kidneys* $\Rightarrow$ stimulation of erythropoiesis with bone marrow erythroid hyperplasia; is <u>the most efficient compensatory mechanism</u> but it requires 3-5 days after the onset of anemia for the elevation of reticulocyte count in the peripheral blood.

B. In the <u>long-term,</u> if not corrected**,** anemia results in **CLINICAL MANIFESTATIONS**:

a) Manifestations (symptoms & signs):

i) Manifestations due to tissue hypoxia:

- *exertional dyspnea, cold extremities*
- *fatigue, weakness, headache, dizziness, cognitive impairment* (in severe forms)

ii) Manifestations due to skin, mucous membranes, hair and nails changes:

- *pale skin,* in iron-deficiency anemia or *yellow skin (jaundice)*, in vitamin B_{12} deficiency and severe hemolytic anemias

- *angular cheilitis (perlèche), glossitis,* and *dysphagia with esophageal web formation* (Plummer-Vinson syndrome or Paterson-Brown-Kelly syndrome, rare)
- *brittle nails, koilonychia, hair loss, alopecia,* in severe iron-deficiency anemia
- *premature greying of hair*, in severe vitamin B_{12} deficiency

iii) Digestive manifestations:

- *abdominal pain, bloating, constipation/diarrhea, nausea, anorexia, weight loss*

iv) **Manifestations due to the hyperdynamic circulation –** in chronic or severe, uncorrected anemias a rare complication may occur:

- **high-output cardiac failure** - leading to *palpitations, tachycardia, dyspnea, fatigue, elevated jugular venous pressure, ankle edemas*

CLASSIFICATION:

- **MORPHOLOGICAL classification** – according to the **erythrocyte size***,* i.e., *mean corpuscular volume (MCV)* on the peripheral blood smear, in:
  - **A. Microcytic** anemias: MCV < 80 fL
  - **B. Macrocytic/megaloblastic** anemias: MCV > 100 fL
  - **C. Normocytic** anemias: MCV 80-100 fL
- **FUNCTIONAL classification** – according to the peripheral **reticulocyte count**, in:
  - **A. Regenerative** anemias: *increased* reticulocyte count or *reticulocytosis*
  - **B. Non-regenerative** anemias: *decreased* reticulocyte count or *reticulocytopenia* (indicating the inability of the bone marrow to mount an appropriate regenerative response in the context of anemia)
- **ETIOPATHOGENIC classification** – according to the *causes and mechanisms* of anemias:
  - **A.** Anemia due to **decreased RBC production**, i.e. **DECREASED ERYTHROPOIESIS**
  - **B.** Anemia due to **increased RBC destruction**, i.e., **HEMOLYTIC ANEMIAS**

The etiopathogenic classification is further detailed.

A. ANEMIAS DUE TO DECREASED ERYTHROPOIESIS

According to the **mechanisms** responsible for the **decreased RBC production** by the bone marrow, the main anemias can be further classified as follows:

1. Anemia due to IMPAIRED IRON METABOLISM – microcytic, hypochromic anemias:

a) Iron-deficiency anemia
b) Sideroblastic anemia

2. Anemia due to ABNORMAL DNA SYNTHESIS – megaloblastic/macrocytic, normochromic anemias:

a) Vitamin B_{12} deficiency anemias
b) Folic acid deficiency anemias

3. Anemia due to DEFICIENT ERYTHROPOIESIS – normocytic, normochromic anemias:

a) Anemia due to bone marrow failure
b) Anemia of chronic inflammation
c) Anemia of chronic kidney disease

I. Anemias due to IMPAIRED IRON METABOLISM

Iron metabolism – Brief Overview

Iron is vital micronutrient in humans being present in two states, the divalent (Fe^{2+}) or ferrous iron and the trivalent (Fe^{3+}) or ferric iron. Its total body amount in adults is about **2-4 g** distributed as follows:

- the **functional iron** in: hemoglobin in RBC (65%), myoglobin and iron-containing enzymes and cytochromes in other tissues (10%)
- the **storage iron** (25%) in liver (hepatocytes), spleen and bone marrow (macrophages):
  - **ferritin** is the **soluble, rapidly available iron**, as hepatocytes and macrophages act as reservoirs, releasing iron for erythropoiesis and metabolic processes
  - **hemosiderin** is the **stable, insoluble, difficult to mobilize, iron-storage complex,** acting as the key long-term store

Iron intake. Dietary iron (15-20 mg/day) is present as the:

- *heme iron* (Fe^{2+}) in animal products – the easily absorbable form
- *non-heme iron* (Fe^{3+}) in vegetables – the major, yet more difficult to absorb form

Iron loss:

- in men: 0.5 - 1 mg/day through feces, urine, sweat
- in women: 1.5 - 2 mg/day due to associated menstrual losses

IRON METABOLISM – PATHOLOGICAL CHANGES

- **IRON ABSORPTION** - **10%** of the dietary iron, occurs in the **proximal duodenum**:
  - **heme iron (Fe^{2+})** is transported from the intestinal lumen into the enterocytes (via a heme transporter at the enterocytes brush-border membrane)
  - **non-heme iron (Fe^{3+})** is reduced into Fe^{2+} (ferrous) iron (by a ferric reductase at the enterocytes brush-border membrane) and then transported from the intestinal lumen into the enterocytes (via a divalent metal transporter). The low gastric pH is responsible for the reduction of the non-heme Fe^{3+} iron (highly insoluble) to the readily absorbable Fe^{2+} iron.

Observations!

Dietary reducing agents, e.g., ascorbic (vitamin C) and citric acid in citrus fruits significantly increase the absorption of non-heme iron. Dietary polyphenols (in tea and coffee), phytates (in grains and seeds), tannins (in tea, red wine, dark chocolate), and calcium supplements reduce non-heme iron absorption.

Pathological changes:

a. Iron absorption **decreases** under the following conditions:

i) *high iron stores*

ii) *reduced gastric acidity* (necessary for converting non-heme ferric iron to its absorbable ferrous form) due to: atrophic gastritis, Helicobacter pylori infection, chronic treatment with proton pump inhibitors, histamine-2 receptor blockers, and antacids

iii) *dysfunction of intestinal mucosa* in: celiac disease, inflammatory bowel disease, post-bowel resection

iv) diseases associated with chronic low-grade inflammation (increased hepcidin)

b. Iron absorption **increases** under the following conditions:

i*) low iron stores*, in iron deficiency

ii) after blood loss

- **IRON STORAGE**:

After absorption, iron is primarily stored within the hepatocytes and macrophages in the spleen and bone marrow. The **majority of iron** is stored as **ferritin** and, in cases of **iron overload**, as **hemosiderin**.

Pathological changes:

a. Iron DEFICIENCY (decreased iron stores) is frequent and **the hallmak laboratory finding** is the **low serum ferritin.**

Serum ferritin is the **screening test for diagnosing iron deficiency** and a level **< 30 µg/L** in healthy individuals indicate the **absence of bone marrow iron stores**.

Ferritin is an acute-phase reactant and its levels are elevated in the presence of inflammation from chronic diseases, conditions that can obscure true iron deficiency. In **chronic kidney disease**, **heart failure**, **inflammatory bowel disease, cancers** and **liver diseases**, **iron deficiency** is diagnosed as **serum ferritin < 100 µg/L.**

b. Iron OVERLOAD (increased iron stores, as hemosiderin) is less frequent and can be:

i) **localized** in the liver (often, after multiple transfusions), usually *without major damage* – a condition called **hemosiderosis**

ii) **systemic** in various tissues, e.g., liver, pancreas, heart, skin, which is associated *with organ/tissue damage* – a condition called **hemochromatosis.**

- **<u>IRON TRANSFER</u>** from **enterocytes, hepatocytes** and **macrophages** into the blood occurs at the level of a transmembrane channel protein called **ferroportin** and is controlled by a small peptide synthetised by the liver, named **hepcidin**. Hepcidin binds ferroportin causing its internalization and lysosomal degradation with subsequent inhibition of iron export into plasma and thus, **serum iron decreases.**

  Pathological changes:

  a. <u>Low</u> serum hepcidin (decreased liver synthesis):

  Causes:

  – *decreased iron stores*: e.g., iron deficiency anemia

  – *erythropoiesis stimulation*: e.g., severe hemolytic and posthemorrhagic anemias

  Consequences:

  i) *increased intestinal absorption of iron*

  ii) *high serum iron* (increased export mediated via ferroportin hyperexpression)

  b. <u>High</u> serum hepcidin (increased liver synthesis):

  Causes:

  – *increased iron stores*: e.g., sideroblastic anemia

  – *chronic inflammation* from: *autoimune diseases, chronic kidney disease, inflammatory bowel disease, obesity, cancers, etc.* where pro-inflammatory cytokines, mainly **IL-6**, increases hepcidin synthesis

  Consequences:

  i) *iron sequestration* with high ferritin in macrophages (and hepatocytes) that will leak into the circulation, thus *serum ferritin is high*

  ii) *low serum iron* (decreased export due to ferroportin internalization and degradation)

- **<u>IRON TRANSPORT</u> into the blood:**

  - **Transferrin & Total Iron Binding Capacity (TIBC)**

  Iron is transported bound to **transferrin** whose level is correlated with the **Total Iron Binding Capacity (TIBC)** defined as the maximum amount of iron that can be bound by serum transferrin. Transferrin releases iron to be either used for hemoglobin synthesis in the bone marrow (75%) or stored in hepatocytes and macrophages (25%).

Pathological changes:
a. <u>Low</u> transferrin (decreased liver synthesis):
i) acute inflammation - is a negative acute phase reactant
ii) chronic inflammation - in above-mentioned diseases)
iii) liver disease - a low transferrin level is a marker of poor prognosis in cirrhosis
iv) iron overload - as a compensatory response to excess iron
b. <u>High</u> transferrin (increased liver synthesis):
i) iron deficiency - a compensatory response to increase iron transport capacity

- **Serum iron (SI)** is the concentration of circulating iron bound to transferrin.
  It is not a reliable indicator of iron deficiency or overload, as its levels can fluctuate independently of total body iron stores.

- **Transferrin saturation (TSAT)** represents the degree to which the available transferrin sites are occupied by iron and is calculated as the ratio: **SI** / **TIBC x 100** (percent)
  ✓ Normal values: 20 – 45% (i.e., approx. 1/3 of transferrin is saturated with iron)
  Pathological changes:
  A decrease in **TSAT < 20%,** especially associated with **low serum ferritin is a reliable indicator of iron deficiency.**

  Remember !
  In patients with **chronic heart failure,** TSAT is currently used as **the most sensitive indicator of iron deficiency**, often preceding the decrease in serum ferritin **in early NYHA stages,** and the low values predict **poor outcomes.**

a) IRON-DEFICIENCY Anemia

DEFINITION: microcytic, hypochromic anemia characterized by **<u>depleted</u> iron stores, low ferritin** and/or **low transferrin saturation**, with thresholds adjusted for the presence of inflammation.
It is the **most common type of anemia worldwide.**

ETIOLOGY:
Iron deficiency can be either ABSOLUTE of FUNCTIONAL:
1. ABSOLUTE iron deficiency is due to either increased losses or decreased absorption:
i) Increased iron losses - the ***<u>main cause of iron deficiency</u>***, due to:
- **chronic occult *digestive* bleeding in:**
  - ***gastro-intestinal malignancies***, e.g. **colon cancer - the most frequent etiology**
  - **gastritis, peptic ulcers** mainly due to **chronic treatment with non-steroidal anti-inflammatory drugs (NSAIDs)**
  - intestinal parasites
- **chronic *genital* bleeding in pre-/postmenopausal women**

ii) Decreased intestinal absorption:
- **malabsorption post-gastrectomy, gastric bypass surgery or due to autoimmune atrophic gastritis** (HCl deficit)
- **infection with Helicobacter pylori**

- malabsorption due to bacterial proliferation (Small Intestinal Bacterial Overgrowth Syndrome, SIBO) or intestinal reseaction
- chronic treatment with proton pump inhibitors (low HCl secretion will be responsible for the decreased iron absorption)

2. FUNCTIONAL iron deficiency is due to increased sequestration, with decreased iron availability for erythropoiesis (See Anemia of Chronic Inflammation)

i) Increased sequestration occurs in **chronic inflammatory diseases** - currently, the ***second cause of iron deficiency***, in:

- **congestive heart failure**
- **chronic kidney disease**
- inflammatory bowel disease
- autoimmune diseases
- cancers
- elderly

PATHOGENESIS:

Iron deficiency occurs in **3 stages:**

1. **Iron stores depletion** characterized by:
   - **decreased serum ferritin < 30 μg/L if no inflammation** and **< 100 μg/L in the presence of inflammation**
   - **decreased number of sideroblasts** (iron containing RBC precursors) and of **iron stores in bone marrow macrophages** (assessed by the Perls' Prussian blue stain for ferric iron)
   - normal serum iron, Hb, RBC number and morphology
2. **Iron-deficient erythropoiesis** characterized by:
   - **decreased transferrin saturation** < 20%
   - **decreased serum iron**
   - Hb, RBC number and morphology remain normal ***or***
   - **normocytic normochromic anemia** may occur
3. **Iron-deficiency anemia** characterized by:
   - **progressive decrease of Hb and RBC number**
   - **microcytic hypocromic anemia** occur with variable symptomatology

b) SIDEROBLASTIC Anemia

DEFINITION: microcytic, hypochromic anemia with **overloaded iron stores**, **high ferritin** and **high transferrin saturation.**

ETIOLOGY:

1. **HEREDITARY sideroblastic anemia**
   - rare, caused by *defects of genes encoding enzymes involved in the*: i) *heme synthesis*, most commonly, the enzyme *amino-levulinic acid synthase* (ALA) whose co-factor is pyridoxine (vitamin B_6) or in ii) *mitochondrial metabolism,* e.g., Pearson marrow-pancreas syndrome - characterized by mitochondrial DNA deletion associated with exocrine pancreatic failure and malabsorption (in which anemia may also be macrocytic).

2. ACQUIRED sideroblastic anemia

- more frequent, caused by the *inhibition of enzymes involved in heme synthesis* due to:
  - ✓ **primary causes:**
    - myelodysplastic syndromes and myeloproliferative neoplasms
  - ✓ **secondary causes** - anemia can be reversed by removing the underlying cause:
    - **chronic alcoholism**
    - **drug-induced:** isoniazide (tuberculostatic agent), antibiotics, e.g., chloramphenicol, linezolid
    - vitamin B_6 (pyridoxine) or copper deficiency
    - lead poisoning (saturnism) or zinc toxicity

PATHOGENESIS: defective heme synthesis during erythropoiesis is responsible for:
i) the appearance of "**ring sideroblasts**" and ii) the **erythroid progenitor cell failure in the bone marrow.**

Consequences:

- ✓ ***In the bone marrow aspirate:***
  - **increased deposits of non-heme iron in mitochondria in the erythroid precursors** forming a **ring-like distribution** around the nucleus, hence the term **ring sideroblasts** (Perls stain) - **pathognomonic for diagnostic**
  - **erythroid hyperplasia** - defined as increased number of erythroblasts
  - ***ineffective* erythropoiesis** - defined as ***the premature destruction (apoptosis) of precursors in the bone marrow*** and subsequent inability to produce a sufficient number of mature RBCs (despite an increased production of erythroblasts since they fail to mature properly)
- ✓ ***In the peripheral blood:***
  - increased ferritin
  - increased serum iron
  - increased transferrin saturation
  - decreased reticulocytes (reticulocytopenia)
- ✓ ***At tissue level:***
  - **iron overload** with parenchymal iron load - **hemosiderosis**, mainly in the liver with fibrosis and in evolution, cirrhosis

II. Anemias from ABNORMAL DNA SYNTHESIS

DEFINITION: a group of megaloblastic / macrocytic, normochromic anemias.

PATHOGENESIS:

The main pathophysiological mechanism is **ineffective erythropoiesis** secondary to **intramedullary apoptosis of hematopoietic cell precursors**, which results from abnormal DNA synthesis.

Defective DNA synthesis is responsible for:

- **abnormal or delayed nuclear division** (and chromatin condensation), while the **cytoplasm maturation proceeds at a normal rate** in the developing erythroid precursors

- the dissociation between nuclear and cytoplasmic maturation, called **nucleo-cytoplasmic maturation asynchrony** is: i) **the hallmark of megaloblastic anemias** due to vitamin B12 or folate deficiency (essential cofactors for the synthesis of thymidine in DNA), and ii) the reason for the appearance of **megaloblasts** (large cells with looser chromatin and a rim of normal cytoplasm) in the **bone marrow** and **macrocytes/macro-ovalocytes** in the **peripheral blood**
- macrocytes have **normal mean corpuscular hemoglobin concentration per cell (MCHC) explaining normochromia**, although the mean corpuscular hemoglobin (MCH) is usually increased (due to cell size)
- **ineffective erythropoiesis** due to **increased intramedullary apoptosis** of the large hematopoietic cell precursors
- the impaired DNA synthesis affects **all the hematopoietic precursors in the bone marrow - megaloblastic hematopoiesis** and, also the rapidly renewing tissues, such as the **gastrointestinal tract**, which explains the association of **hematological manifestations** (macrocytes, hypersegmented neutrophils, abnormal platelets) with **digestive manifestations** in **megaloblastic anemias**

a) Anemias due to vitamin B_{12} DEFICIENCY

DEFINITION – megaloblastic/macrocytic, normochromic anemias caused by vitamin B_{12} (cobalamin) deficiency

Vitamin B_{12} or cobalamin - Brief overview

- **Source**: food of *animal origin, e.g., meat, fish, eggs, dairy products*
- **Required amount**: 2-3 µg/day
- **Absorption**: in the **terminal ileum** where it arrives after binding proteins with protective role:
  - in mouth and stomach, dietary vitamin B_{12} binds to the *R-binder (transcobalamin I or haptocorrin)* that protects the acid-sensitive vitamin from degradation by the stomach acid
  - in the dudenum, vitamin B_{12} is released from the R-factor by the pancreatic proteases and binds to the *intrinsic factor (IF)*, a glycoprotein secreted by the *gastric parietal cells*, forming a stable vit. B_{12} - IF complex, which is transported to the **terminal ileum**; at this level, the cobalamin-IF complexes are processed by a receptor (cubulin), cobalamin is released, binds to its carrier protein called **transcobalamin II (TCII)** and is absorbed in the portal circulation.
- **Plasma transport**: bound to transcobalamin II (TC-II). The TCII-B_{12} complex is called *holotranscobalamin*, and is responsible for delivering vitamin B_{12} to the body cells. After cellular uptake, cobalamin is converted to adenosyl-cobalamin and methyl-cobalamin, the cofactors of B_{12}-dependent enzymatic reactions.
- **Liver deposits**: sufficient for 2-4 years (if intake is lacking)

VITAMIN B_{12} METABOLISM – PATHOLOGICAL CHANGES

Metabolic roles: vitamin B_{12} has **two active forms** that serve as **enzymatic co-factors**: **methyl-cobalamin** and **adenosyl-cobalamin**.

a) Methyl-cobalamin*:*

- **Characteristics**: is the co-enzyme of *methionine synthase* that catalyzes the synthesis of **methionine from homocysteine**, a reaction in which N^5-methyl-tetrahydrofolate (N^5-methyl THF) serves as the methyl group donor (and becomes tetrahydrofolate - THF). THF will further accept a methylene group and participates as $N^{5,10}$ - methylene THF in the

synthesis reaction of deoxythymidine monophosphate (dTMP), the precursor of **thymidine in the DNA structure**. THF is also converted into the tissue storage form (polyglutamate). Lack of methyl-cobalamin causes folate to become 'trapped' in the 5-methyl-THF form, i.e. "folate trap" with no generation of THF (Fig. 1).

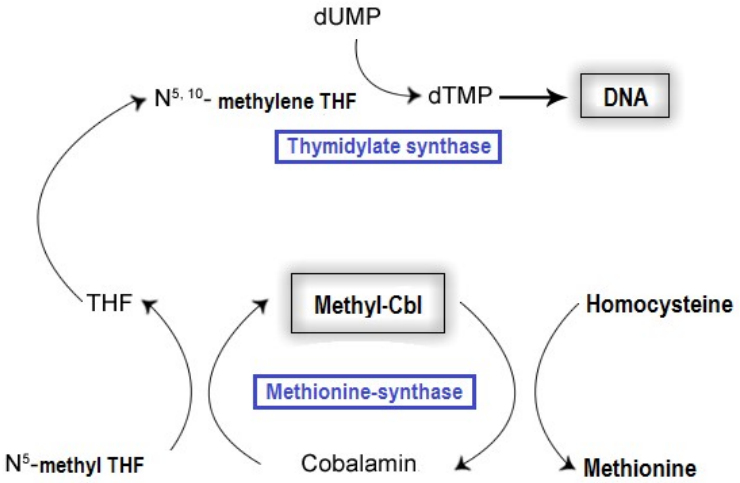

Figure 1. The metabolic role of **METHYL-COBALAMIN (methyl-Cbl)** and **TETRAHYDROFOLATE (THF)** - explanations in the text.
(dUMP - deoxyuridine monophosphate, dTMP - deoxythymidine monophosphate)

- **Consequences of METHYL-COBALAMIN deficiency:**
  - **impairment of DNA synthesis** - by reduced THF availability
  - **decreased tissue folate stores** - by lack of polyglutamate generation
  - **chronic hyperhomocysteinemia** - risk factor for ATS & vascular thrombosis and also, for neurodegeneration (due to neurotoxicity)
  - methyl-cobalamin deficiency is responsible for the **hematological and digestive manifestations** of megalobastic anemias, which are completely *reversible* with B_{12} replacement therapy.

b) Adenosyl-cobalamin*:*

- **Characteristics**: is the the co-enzyme of *methyl-malonyl CoA mutase* that catalyzes the isomerization of **methyl-malonyl coenzyme A to succinyl coenzyme A** (Fig. 2)

Figure 2. Metabolic role of adenosyl-cobalamin (adenosyl-Cbl).

- **Consequences of adenosyl-cobalamin deficiency:**
  - **defective myelin synthesis and repair**, which affects the **dorsal and lateral columns of the spinal cord**, **peripheral nerves**, and **cerebral white matter**, and leads to spinal cord degeneration, sensory neuropathy, and cognitive dysfunction

- **accumulation of methyl malonic acid** (metabolite of methyl-malonyl CoA), which is neurotoxic and contributes to the **axonal degeneration** and **neuronal injury**; its increased serum and urinary levels have a **diagnostic role**
- adenosyl-cobalamine deficiency is responsible for the **neurological manifestations** from B_{12} deficiency, which are *partially* or *fully reversible* with timely B_{12} therapy.

ETIOLOGY of vitamin B_{12} deficiency:

- **Decreased intake:** veganism
- **Impaired absorption**:
  - Intrinsic factor (IF) deficiency in:

  - Addison-Biermer pernicious anemia caused by autoimmune gastric atrophy
  - gastric surgery
  - Intestinal disorders:
    - ✓ localized at terminal ileum, e.g., regional ileitis (Crohn's disease), ileal resection
    - ✓ diffuse, e.g., lymphomas
  - Chronic pancreatitis – insufficient pancreatic proteases
  - Zollinger-Ellison syndrome (gastrinoma with gastrin hypersecretion) – the massive acid production requires long-term treatment with proton pump inhibitors, which severely reduce stomach acid. Hypo-/achlorhydria prevents vitamin B_{12} from being released from food proteins, leading to deficiency (despite normal intrinsic factor).
- **Competition for vitamin B_{12}** in:
  - ✓ blind loop syndrome (with stasis and bacterial overgrowth)
  - ✓ fish tapeworm infestation

PERNICIOUS ANEMIA or ADDISON-BIERMER ANEMIA

DEFINITION: the prototype of **megaloblastic, macrocytic anemia due to vitamin B_{12} deficiency** diagnosed in the **elderly** (up to 20% of individuals over 60 years old)

PATHOGENESIS: decreased vitamin B_{12} absorption due to intrinsic factor (IF) deficiency, due to an **autoimmune atrophic chronic gastritis** (type A) characterized by:

- **chronic infiltrate of gastric the corpus and fundus** (it spares the antrum) **with cytotoxic T lymphocytes and plasma cells that secrete autoantibodies**, i.e., pathogenesis involves **both type II and IV hypersensitivity reactions**
- presence of **autoantibodies:**
  - ✓ **anti-gastric parietal cells – non-specific screening test** (positive in other autoimmune diseases and in some healthy elderly), should be assesed alongside other specific tests. The anti-parietal cell antibodies destroys the parietal cells, which produce IF and hydrochloric acid. In turn, achlorhydria causes a decrease in the release of cobalamin bound to dietary protein, aggravating the deficiency.
  - ✓ **anti-IF – specific test,** should be tested in **all** patients with unexplained vitamin B12 deficiency, megaloblastic anemia
- **association of anemia with other autoimmune diseases**: Hashimoto thyroiditis, Addison disease, type 1 diabetes mellitus etc.

MANIFESTATIONS: result from the deficiency of vitamin B_{12} active forms:

- **methyl-cobalamin deficiency** is responsible for the **hematological** and **digestive manifestations (a, b)**
- **adenosyl-cobalamin deficiency** is responsible for **neurological manifestations (c)**

a) <u>HEMATOLOGICAL manifestations:</u>

① ERYTHROCYTES:

- *in the bone marrow:*
  - ✓ **megaloblastic erythropoiesis**
  - ✓ **ineffective erythropoiesis** with apoptosis of the erythroid precursors
- *in the peripheral blood:*
  - ✓ **macrocytic, normochromic anemia**
  - ✓ **aniso-poikilocytosis**
  - ✓ erythrocytes with **nuclear fragments**: Jolly bodies and Cabot rings
  - ✓ hemolysis responsible for: **increased unconjugated bilirubin (UB), elevated lactate dehydrogenase (LDH)** and **decreased haptoglobin** in severe forms
  - ✓ **low reticulocyte count** that responds to B_{12} therapy - reticulocytosis that occurs after the treatment confirms the diagnostic

② LEUKOCYTES:

- *in the bone marrow* – **giant metamyelocytes** and morphologically abnormal nuclei
- *in the peripheral blood* – **leukopenia** and **<u>pathognomonic</u> <u>hypersegmented granulocytes</u>** (may precede macrocytic anemia)

③ PLATELETS:

- *in the bone marrow* – **giant megakaryocytes** and atypical mitoses
- *in the peripheral blood* – **abnormal platelets** and **thrombocytopenia**

b) <u>DIGESTIVE manifestations</u>:

- **Hunter glossitis**: red, tender, smooth tongue
- **gastric achylia**: decreased gastric secretion volume
- **increased incidence** of **gastric cancer**: due to metaplasia of the gastric mucosa

c) <u>NEUROLOGICAL manifestations</u>:

- **peripheral neuropathy** due to ***axonal degeneration*** and ***segmental demyelination***, occurs **early,** and is responsible for:
  - ✓ *sensory neuropathy*, i.e. decreased ability to feel sensations: touch, vibration, pain
  - ✓ *symmetrical paresthesias*, mainly of the lower limbs
  - ✓ loss of proprioception with *gait disturbance* and *ataxia* (uncoordinated movement)
- **myelopathy**, due to ***degeneration of the spinal cord*** – the ***hallmark of vitamin B_{12} deficiency***, occurs in **later stages**
- **cognitive impairment**: *memory and mood disturbances* in severe forms

POSITIVE DIAGNOSIS comprises: **low serum B_{12} level** (< 200 ng/L) **plus the presence of anti-IF antibodies, increased homocysteine and methyl malonic acid**. However, the gold standard for diagnosis is endoscopy with **gastric biopsy** that provides the **histopathological confirmation of autoimmune atrophic gastritis**.

Remember !

Parenteral lifelong vitamin B_{12} replacement, primarily by intramuscular injections and more recently, also by oral high doses, results in the remission of hematological manifestations and alleviation/remission of the neurological ones, but it will NOT treat the *cause* of malabsorption, which is the autoimmune gastritis responsible for the gastric atrophy and the risk of gastric neoplasia (requires ongoing surveillance and upper endoscopy within 6 months of diagnosis).

a) Anemias due to FOLIC ACID DEFICIENCY

DEFINITION: **megaloblastic / macrocytic, normochromic anemia**

Folic acid (folate) or vitamin B9 - Brief overview

- **Source:** foods of *plant origin, e.g. legumes, leafy greens, fruits, seeds, fortified grains* (as *polyglutamate*) Polyglutamates are 60-90% destroyed during food preparation.
- **Required amount:** 100 µg/day minimally
- **Absorption:** in the **proximal jejunum**, as *monoglutamate*
- **Plasma transport**: as *N^5-methyl tetrahydrofolate (THF)*
- **Liver deposits:** as *polyglutamate*, sufficient for 4-6 months (if intake is lacking)
- **Metabolic role**: *transfer of 1 carbon atom groups (methyl, methylene, formyl) to organic compounds*, acting as a co-enzyme for the production of:
  - pyrimidines required for DNA synthesis
  - methionine, in the presence of vitamin B_{12}.

Methionine is then converted into S-adenosyl-methionine, which act as universal methyl donor for almost all cellular methylation reactions, including of DNA and neurotransmitters.

ETIOLOGY:

- **<u>chronic alcoholism</u>** disrupts folate absorption and hepatic metabolism/storage – **<u>the major cause</u>**
- **impaired use**: drugs interfering with folate metabolism ('folate antagonists'), e.g., methotrexate - cytostatic that inhibits dihydrofolate reductase, enzyme required for THF regeneration, anticonvulsants (phenytoin), antibiotics (trimethoprim)
- **increased requirements**: pregnancy - where supplementation is mandatory to prevent neural tube defects in the fetus, and also, in rapid growth periods (infants, adolescents)
- **malabsorption:** gastrointestinal diseases, e.g., celiac disease or bowel surgery
- **decreased intake**: especially in the elderly and people with poor socio-economic conditions and malnutrition
- **vitamin B_{12} deficiency** ("folate trap")

MANIFESTATIONS:

1. HEMATOLOGICAL and **DIGESTIVE MANIFESTATIONS** are the similar to those from vitamin B_{12} deficiency.

2. NEUROLOGICAL MANIFESTATIONS (less frequent, less severe) are represented by:

- depression and cognitive decline due to: ***hypomethylation of neuronal genes and impaired neuronal maturation***, ***altered neurotransmitter synthesis*** *(mainly, of serotonin and dopamine),* ***neuroinflammation,*** *and* ***homocysteine toxicity*** (at variance from B_{12} deficiency, demyelination, myelopathy and peripheral neuropathy are rare).

POSITIVE DIAGNOSIS: **low serum folate level** (< 2 ng/mL), **increased plasma homocysteine, normal methyl malonic acid, and normal vit B_{12}**.

Observations !
The primary diagnostic laboratory criteria of folate deficiency are *low serum folate* and *low red cell folate* concentration. A **low red cell folate** is **more specific for deficiency** because reflects the tissue stores over the preceding months, whereas the serum folate reflects the recent intake.

III. Anemia due to DEFICIENT ERYTHROPOIESIS

a) Anemia due to BONE MARROW FAILURE

APLASTIC anemia

DEFINITION: failure of the pluripotential stem cell that results in a loss of blood cell precursors, **hypoplasia or aplasia of bone marrow, and peripheral blood cytopenias** in two or all three hematopoietic lineages – the latter condition being known as **pancytopenia,** i.e., decreased number of RBC, white blood cells, and platelets.

ETIOLOGY:
1. **Inherited aplastic anemias** – are **rare**, typical is **Fanconi anemia**, a genetic mutation of an enzyme involved in DNA repair that leads to bone marrow hypocellularity and peripheral cytopenia associated with: short stature, microcephaly, skeletal abnormalities, heart and kidney anomalies, etc.
2. **Acquired aplastic anemias** - the **majority** of cases, of 2 types:
   a. **Idiopathic** – in up to 50% of the cases a triggering factor cannot be identified
   b. **Secondary** – caused by:
   – drugs:
      ✓ antibiotics, e.g., chloramphenicol
      ✓ antineoplastic drugs: alkylating agents, e.g. cyclophosphamide, antimetabolites, e.g., methotrexate – dose-dependent effect
      ✓ others: penicillamine, carbamazepine, NSAIDs, acetazolamide
   – chemicals/toxins: benzene, organophosphates, chlorinated hydrocarbons
   – radiation: therapeutic or accidental intensive radiation of the marrow-bearing bones
   – viral infections: hepatitis virus, Epstein-Barr virus, HIV
   – connective tissue disorders: systemic lupus erythematosus, Hashimoto thyroiditis, rheumatoid arthritis

PATHOGENESIS:
Two major **defects in pluripotential stem cells** contribute to the disease:
– **an <u>extrinsic</u> defect of stem cells**, which become **autoantigenic** when injured by exposure to drugs, toxins, viruses, and trigger a **cellular immune response** characterized by proliferation of **autoreactive T lymphocytes** and **immune suppression of hematopoietic stem cells** – in acquired forms.
– an **inherited <u>intrinsic</u> defect of stem cells** that have **reduced proliferation and differentiation capacity** – in Fanconi anemia**.**

CONSEQUENCES:
- **bone marrow hypocellularity and progressive replacement with adipose tissue**
- **pancytopenia** in the peripheral blood with:
  - ✓ **leukopenia** ⇒ increased risk of *recurrent infections*
  - ✓ **thrombocytopenia** ⇒ increased risk of *hemorrhages*, e.g., purpura, petechiae, gingival bleeding, epistaxis, gastrointestinal bleeding
  - ✓ **anemia with the absence of reticulocytes (aregenerative anemia)**

Since leukocytes and platelets have a shorter lifespan as compared to erythrocytes, the symptomatology related to their deficiency, i.e., bacterial infections and bleeding tendency) usually occur before anemia becomes evident.

Remember !
Differential diagnosis should exclude other causes of peripheral pancytopenia, such as leukemias and myelodysplastic neoplasms, in which the bone marrow is ***hypercellular*** (not hypocellular) as result of infiltration with malignant precursors.

b) Anemia of CHRONIC INFLAMMATION (ACI)

DEFINITION: also known as ***anemia of chronic diseases (ACD)***, is a **normocytic, normochromic** anemia that becomes, in evolution, **microcytic, hypochromic**. Is is mainly a **functional anemia** due to iron-restricted erythropoiesis.

ETIOLOGY:
ACD is **the <u>most frequent anemia</u>** in **<u>hospitalized / chronically ill patients</u>** with:
- **congestive heart failure**, where *absolute* iron deficiency caused by reduced intestinal absorption secondary to the intestinal edema can be associated to the functional deficiency
- **chronic pulmonary diseases:** COPD, chronic respiratory failure
- **inflammatory bowel disease**: Crohn disease, ulcerative colitis
- **autoimmune diseases**: SLE, rheumatoid arthritis
- **infections**: osteomyelitis, bacterial endocarditis, pulmonary abscess
- **cancers and hematological malignancies**
- **obesity**
- **end-organ failure:** advanced CKD

PATHOGENESIS:
The common feature of chronic diseases is the **<u>systemic inflammation</u>**, which **activates the immune cells and their pro-inflammatory cytokines production**, mainly **IL-6**, but also IL-1, and TNFα that are directly responsible for:

1. Iron restriction for erythropoiesis:
- **increased hepatic synthesis of hepcidin** (with subsequent ferroportin internalization and degradation), which causes a **FUNCTIONAL iron deficiency** by:
  - ✓ **blocking dietary iron absorption** from duodenal enterocytes into the blood leading to:
    - **reduced serum iron**
  - ✓ **blocking iron export from macrophages in spleen and liver** with 2 consequences:
    - **reduced serum iron**

- **increased iron stores** and **serum ferritin**, respectively

The typical changes in ACI are **hypoferremia** and **hyperferritinemia**.

2. Decreased hepatic synthesis of transferrin (negative acute-phase protein).

Remember !

Iron sequestration in macrophages is **the major pathophysiological mechanism of functional iron deficiency in anemia of chronic inflammatory diseases** because recycling of iron released from macrophages accounts for > 90% of the daily requirements for Hb synthesis.

2. Inflammatory suppression of erythropoiesis:

- **reduced renal synthesis** and/or **reduced biological activity of erythropoietin (EPO)**
- **inflammation-driven low responsiveness of the EPO receptors** on the erythroid cells

3. Decreased erythrocyte lifespan:

- shortened erythrocyte survival occurs due to enhanced phagocytosis of erythrocytes sensitized by deposition of antibody and complement by splenic and hepatic macrophages, and is a secondary mechanism in chronic diseases

Observation!

A particular type of chronic anemia is **anemia in elderly persons,** which is frequent and has multifactorial origin, being the consequence of: chronic inflammation/disease (including CKD), iron deficiency (with or without blood loss), vitamin B12/folate deficiency, hypothyroidism, hypersplenism, myelodysplastic syndromes, myeloproliferative diseases, leukemias, lymphomas or has unexplained etiology (up to 30% of cases).

c) Anemia of CHRONIC KIDNEY DISEASE (CKD)

DEFINITION: also known **as *anemia of chronic renal disease***, is a **normochromic, normocytic anemia** constantly associated with **end-stage CKD** that may become, in evolution, **hypochromic microcytic**. While sharing similarities with anemia from chronic inflammation, it has as **key feature** the **severe erythropoietin (EPO) deficiency.**

PATHOGENESIS:

Anemia is **multifactorial**, the pathomechanisms related to CKD being associated with *abnormal iron metabolism*, the hallmark of *chronic inflammation*.

The **chronic renal disease** is responsible for the:

- **decreased EPO production** due to the **reduction of renal parenchyma** is resposible for the impaired erythroid maturation in the bone marrow **– the major pathomechanism**
- **increased Fibroblast Growth Factor 23 (FGF23) level** - a hormone produced by osteocytes and osteoblasts in CKD with mineral and bone disorder, which further suppresses EPO production and erythropoiesis
- **increased hepcidin levels** due to decreased renal clearance with the progressive fall of the glomerular filtration rate
- **accumulation of uremic toxins** in the end-stage renal disease, the final CKD stage, with:
  - ***inhibition of erythropoiesis*** by direct bone marrow toxicity

- ***RBC deformity*** (membrane rigidity and decreased deformability) and ***early hemolysis*** due to splenic clearance (RBC lifespan is shortened to 60-90 days)
- ***platelet dysfunction*** responsible for bleeding tendency

Besides these pathomechanisms, the **chronic inflammation** impairs iron homeostasis via the same pathways priorly described, namely:

– **increased proinflammatory cytokines** that stimulate the hepatic synthesis of **hepcidin**, which causes:
  - ✓ inhibition of intestinal iron absorption
  - ✓ iron sequestration and **functional iron deficiency** (decreased iron availability for erythropoiesis)
  - ✓ increased serum ferritin
  - ✓ further impairment of the EPO renal secretion

In evolution, absolute iron deficiency occurs, being determined by **impaired hemostasis** and **blood loss** due to hemodialysis.

Nutritional deficiencies, such as vitamin B12 and folate, due to anorexia and/or dialysate losses, can also contribute to anemia.

B. HEMOLYTIC ANEMIAS

DEFINITION:

Hemolytic anemias are characterized by the following general features:

– **premature peripheral destruction of RBC** with shortened lifespan (< 120 days)

– **increased erythropoiesis with compensatory bone marrow hyperplasia/hypercellularity** (the capability of the bone marrow to increase RBC production is very high and when it compensates for their peripheral destruction, **hemolysis will be compensated**, without anemia)

– **peripheral reticulocytosis** is **constant** (with polychromatophilia/polychromasia and possible macrocytosis on the blood smear due to the bluish/grayish appearance of reticulocytes and their large size).

PATHOGENESIS:

Two major types of hemolysis occur:

✓ **EXTRAVASCULAR hemolysis**: is the most *frequent*, occurs in the ***spleen and liver macrophages*** (similarly to the physiological one but in an accelerated manner) and is characterized by: **jaundice** due to increased unconjugated (indirect) bilirubin released from Hb breakdown, **normal/mild decreased haptoglobin**, **lack of hemoglobinuria** (dark urine). In **chronic** hemolysis, **splenomegaly and bilirubin gallstones** occur.

✓ **INTRAVASCULAR hemolysis**: is *rare*, occurs in the ***blood vessels*** and leads to the release of large amounts of free hemoglobin into plasma (that may exceed the haptoglobin-binding capacity) and is characterized by: **jaundice, severely decreased/absent haptoglobin, elevated lactate-dehydrogenase (LDH), hemoglobinemia** and **hemoglobinuria.** In **acute** hemolysis, **acute kidney injury may occur** (Hb precipitation in

the renal tubules), while in the **chronic** forms, iron and hemosiderin deposition in the kidney may occur. **Splenomegaly is absent in pure intravascular hemolysis.**

CLASSIFICATION:

I. Hemolytic anemias due to INTRACORPUSCULAR (INTRINSIC) defects:

1. Hemolytic anemia due to MEMBRANE defects (membranopathies):

a) Hereditary spherocytosis

2. Hemolytic anemia due to ENZYMATIC defects (enzymopathies):

a) Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency

b) Pyruvate kinase (PK) deficiency

3. Hemolytic anemias due to HEMOGLOBIN defects (hemoglobinopathies):

a) Qualitative hemoglobinopathies

b) Quantitative hemoglobinopathies

II. Hemolytic anemias due to EXTRACORPUSCULAR (EXTRINSIC) defects:

1. IMMUNE hemolytic anemias:

a) Autoimmune hemolytic anemias

b) Drug-induced immune hemolytic anemias

c) Alloimmune hemolytic anemias

2. NON-IMMUNE hemolytic anemias:

a) Mechanical hemolytic anemias

I. Hemolytic anemias due to INTRACORPUSCULAR (INTRINSIC) defects

1. Hemolytic anemias due to MEMBRANE defects (membranopathies)

a. HEREDITARY SPHEROCYTOSIS

- **Definition**: autosomal dominant or recessive disease, but some cases occur spontaneously (*de novo* mutation) characterized by defects in RBC membrane proteins that compromise cell deformability and survival.
- **Pathogenesis:**

– **mutations of the genes encoding proteins in the RBC membrane and cytoskeleton**, mainly ***spectrin*** and ***ankyrin*** disrupt the vertical interaction between the membrane skeleton and the lipid bilayer (responsible for the normal biconcave RBC shape and membrane stability) and lead to loss of surface area with the formation of **spherocytes**, which are less deformable and also, osmotically fragile

– inability of spherocytes to traverse the splenic microvasculature results in their premature sequestration and destruction by splenic macrophages with **EXTRAVASCULAR hemolysis and decreased RBC lifespan** (to 10-20 days)

– the **chronic hemolysis** is responsible for: anemia, reticulocytosis, jaundice (from increased unconjugated bilirubin), splenomegaly, and predisposition to bile gallstones.

– **the direct antiglobulin (Coombs) test is negative** – necessary for differential diagnosis with autoimmune hemolytic anemias where spherocytes are frequently present

- **Complications**: **aplastic crisis** triggered by viral infections – typical by the parvovirus B19 that temporarily suppress hematopoiesis and results in severe anemia (sudden drop in Hb) and absence of peripheral reticulocytosis

2. Hemolytic anemias due to ENZYMATIC defects (enzymopathies)

a. GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G-6-PD) DEFICIENCY

- **Definition:** inherited X-linked mutation in the G-6-PD gene that results in reduced activity or stability of the enzyme
- **Pathogenesis**:
  - G-6-PD catalyzes the first step of the pentose phosphate pathway, generating **NADPH**, which is essential for maintaining a **normal level of reduced glutathione** that protects the RBC membrane and hemoglobin from oxidative damage (the pentose phosphate pathway is their sole source of NADPH since erythrocytes lack mitochondria)
  - in G-6-PD deficiency, **acute, intermittent hemolysis** is triggered by exposure to oxidative stressors, such as:
  - ✓ *acute infections*, e.g., viral hepatitis, pneumonia
  - ✓ *pro-oxidant drugs*, e.g., sulfonamides, nitrofurantoin, antimalarials
  - ✓ *foods* that trigger oxidative stress through metabolization, e.g., "fava" beans that cause *favism* (that is endemic to the Mediterranean basin and some areas of Africa)

  when red cells undergo premature hemolysis due to their inability to neutralize the excess of reactive oxygen species.
  - oxidized hemoglobin forms sulfhydryl cross-links and precipitates as **Heinz bodies**, small, round, dark inclusions - a **pathognomonic finding** during the acute hemolytic episodes
  - during these episodes, **hemolysis** is predominantly **INTRAVASCULAR** but Heinz bodies damage the erythrocyte membrane and decrease its deformability, thus leading also to a degree of EXTRAVASCULAR hemolysis (removal by the splenic macrophages)

b. PYRUVATE KINASE DEFICIENCY

- **Definition:** autosomal recessive mutation in the gene that encodes the red cell isoform of pyruvate kinase (PK)
- **Pathogenesis:**
  - PK catalyzes the final step of anaerobic glycolysis (converting phosphoenolpyruvate to pyruvate) and generating ATP (anaerobic glycolysis is their sole source of ATP, since erythrocytes lack mitochondria)
  - PK deficiency leads to reduced ATP production, impairs red cell membrane stability and ion transport, resulting in RBC dehydration, echinocyte formation, and premature destruction of erythrocytes by the spleen, i.e., **EXTRAVASCULAR hemolysis**
- **Complications:** are those of **chronic hemolysis**, i.e., splenomegaly, gallstones, jaundice, and iron overload, with exacerbations during infections

3. Hemolytic anemias due to HEMOGLOBIN defects (hemoglobinopathies)

a) QUALITATIVE hemoglobinopathies

- **Definition:** inherited disorders characterized by **mutations in globin genes that alter the amino acid sequence of the globin chains**, resulting in production of **structurally abnormal hemoglobin** molecules.
  The classic example is **sickle cell disease** where the abnormal hemoglobin polymerizes and precipitates within the RBC, leading to hemolytic anemia.

SICKLE CELL DISEASE (SCD)

- **Definition**: also known as *hemoglobin S disease (HbS), falciform anemia or drepanocytic anemia,* is caused by a mutation in a β-globin gene that results in substitution of valine for glutamic acid at position 6 that produces hemoglobin S (HbS, $\alpha_2\beta^S_2$).

Observation!
This frequent hereditary hemoglobinopathy mainly affects the black population of African origin, mainly in the regions where malaria is endemic. The heterozygous form of the disease is asymptomatic and provides protection against malaria.

- **Pathogenesis**:
  - under deoxygenated conditions, HbS undergoes polymerization within the RBCs
  - the HbS polymers are rigid and distorts RBCs into the classic sickle shape, reducing their deformability and promoting cellular dehydration
  - **characteristics** of RBC sickling:
  - ✓ **is aggravated by:** i) **hypoxia** e.g., from high altitude, physical exertion; ii) **dehydration** that increases HbS concentration and its polymerization; iii) **acidosis** that decreases Hb affinity for O_2; iv) **chronic systemic inflammation**, which decreases blood flow in the microcirculation, thus promoting persistent hypoxia
  - ✓ **is alleviated by increased fetal Hb** as HbF inhibits HbS polymerization (this explains why children become symptomatic only after 6 months of life, when level of HbF drops).

  The sickling process is initially reversible by increasing the PaO_2, but in time the cycle of sickling and unsickling irreversibly damages the RBC membrane, causing the loss of deformability and resulting in their removal by macrophages, primarily in the spleen and liver, i.e. **EXTRAVASCULAR hemolysis is the primary mechanism of anemia.**

- **Complications** are both chronic and acute:
  i) Chronic complications are represented by:
    - ✓ **chronic hemolytic anemia,** responsible for: *increased unconjugated bilirubin with jaundice*, *gallstones*, hepatomegaly, painful *splenomegaly* (due to microcirculation obstruction) with onset in childhood, which is replaced in the adult life with *progressive retractile fibrosis*, responsible for *functional asplenia* and increased lifelong infection risk
    - ✓ **progressive organ damage** due to **repeated vaso-occlusive episodes** of an inflammed vascular bed that result in **microvascular obstruction** in almost every organ/tissue, leading to: **chronic sicke nephropathy**, **pulmonary hypertension**, **retinopathy**, **hepatobiliary dysfunction**, chronic bone pain and avascular necrosis (mainly of the femoral head) and neurocognitive impairment

  ii) Acute complications are represented by: **pain crises** due to acute vaso-occlusion (with intense pain in the limbs, chest, abdomen, lumbar region, etc.), **acute chest**

syndrome, **stroke, aplastic crisis** (triggered by parvovirus B19), and **severe infections with sepsis,** e.g., meningitis from encapsulated bacteria due to functional asplenia.

b) QUANTITATIVE hemoglobinopathies - THALASSEMIAS

- **Definition:** inherited disorders characterized by mutations that reduce or block the synthesis of specific globin chains, α or β (that otherwise are structurally normal)
- **Pathogenesis**:

– **the decreased synthesis of the involved chain** results in **decreased Hb production** and **microcytic, hypochromic anemia**

– **the normal synthesis and accumulation of the unaffected chains** result in their **precipitation** and the appearance of **Heinz bodies**, responsible for:

- ✓ oxidative damage, **apoptosis** and **ineffective erythropoiesis** of the **erythroid precursors in the bone marrow**
- ✓ alteration of the RBC membranes in the peripheral blood and **EXTRAVASCULAR hemolysis** in the spleen.

ALPHA - THALASSEMIA

- **Definition:** reduced or absent synthesis of the **α-globin chains** due to deletion/mutations in the genes located on **chromosome 16** (on each chromosome 16 there are 2 genes encoding for the α-globin chains, i.e., α α / α α). This will lead to excess synthesis of β- or γ-globin chains, forming unstable tetramers known as Hb H and Hb Bart's, respectively.
- **Pathogenesis**:

The imbalance between α- and non-α-globin chains results in the synthesis of aberrant hemoglobin tetramers; excess of γ chains yield hemoglobin Bart (γ_4) in fetuses, while excess of β chains produce hemoglobin H (β_4) in children and adults. Both exhibit elevated oxygen affinity and decreased oxygen delivery, leading to tissue hypoxia.

The disease severity is correlated with the number of affected alleles:

– 1 allele: (- α / α α), resulting in:
- ✓ silent α-thalassemia
- ✓ asymptomatic carrier status

– 2 alleles: (- α /- α) *or* (- - / αα), resulting in:
- ✓ specific α-thalassemia ("trait")
- ✓ **chronic mild microcytic anemia**

– 3 alleles: (- - / - α), resulting in:

✓ HbH (β4) tetramers generation, which precipitate within the RBC leading to the formation of Heinz bodies, responsible for:
- **inefficient erythropoiesis, apoptosis of erythroid precursors** and compensatory bone marrow expansion
- damage of the RBC membrane, **EXTRAVASCULAR hemolysis**
- **chronic moderate to severe hypochromic, microcytic anemia**
- **complications:** gallstones, splenomegaly, iron everload with liver and cardiac and endocrine complications in patients receveing transfusions

– 4 alleles: (- - / - -), resulting in:

- ✓ Bart's Hb (γ4) generation, with very high affinity for O_2, with severe tissue anoxia, responsible for:
  - **hydrops fetalis,** defined as pleural, pericardial effusions, generalized edema (anasarca), massive hepatosplenomegaly, and cardiac failure

  In the past, death in utero or shortly after birth was the rule, today the survival of neonates is possible with intrauterine transfusions, but the may present several anomalies, neurocognitive impairment, and require transfusion support for the lifetime.

BETA - THALASSEMIA

- **Definition**: reduced or absent synthesis of the **β-globin chains** due to point mutations in the genes located on **chromosome 11** (on each chromosome 11, there is 1 gene encoding for the β-globin chain synthesis, i.e., β / β)
- **Pathogenesis:**

  ① **Beta - thalassemia minor** (*heterozygous* defect, thalassemia "trait"), characterized by:
  - **mild, hypochromic, microcytic anemia** (with normal serum ferritin and iron stores, unlike iron-deficiency anemia)
  - asymptomatic
  - do not require transfusions

  ② **Beta - thalassemia major or Cooley anemia** (*homozygous* defect), characterized by:
  - **severe hypochromic, microcytic anemia** caused by precipitation of excess unpaired α-globin chains and Heinz bodies formation in:
    - erythroid precursors with their apoptosis and ineffective erythropoiesis
    - erythrocytes with their injury, **EXTRAVASCULAR hemolysis**, splenomegaly, gallstones
  - requires **regular transfusions for survival**
  - **complications:**
    - ✓ due to **chronic anemia**:
      - *compensatory bone marrow expansion* and bone deformities, growth retardation and risk of fractures
      - *extramedullary hematopoiesis with hepato-splenomegaly*
    - ✓ due to **iron overload/hemocromatosis**, from increased intestinal iron absorption and repeated transfusions, leading to organ dysfunction:
      - *liver fibrosis/cirrhosis*
      - *cardiomyopathy*
      - *endocrine disorders* (diabetes, hypothyroidism)

II. Hemolytic anemias due to EXTRACORPUSCULAR (EXTRINSIC) defects

1. IMMUNE HEMOLYTIC anemias

- **Definition**: acquired anemias caused by premature RBC destruction via immune-mediated mechanisms.
- **Classification:**

  a) AUTOimmune hemolytic anemias

b) DRUG-induced immune hemolytic anemias
c) ALLOimmune hemolytic anemias

a) AUTOimmune hemolytic anemias (AIHA)

- **Definition:** hemolytic anemias caused by **autoantibodies** (autoAb) **directed towards antigens on the RBC** surface and characterized by a **positive direct antiglobulin (Coombs) test**
- **Types:**
  i) WARM AIHA
  ii) COLD AIHA (Cold Agglutinin Disease)

i) WARM AIHA – is most common type

- **Etiology**:
  i) primary (idiopathic) - anemia occurs without any apparent cause
  ii) secondary to an underlying condition:
  – autoimmune diseases: systemic lupus erythematosus (SLE), rheumatoid arthritis
  – lymphoproliferative disorders: chronic lymphocytic leukemias, lymphomas, monoclonal gammopathies
  – infections: Epstein-Barr virus, hepatitis C and B viruses, parvovirus B19, HIV, SARS-CoV2
  – drugs (see below)
- **Pathogenesis**:
  – autoAb are typically polyclonal **IgG** that bind to RBC surface at **37ºC** (the maximal binding affinity is at body temperature)
  – the IgG-coated erythrocytes are recognized by the Fc receptors on *splenic* macrophages with phagocytosis and predominant **EXTRAVASCULAR hemolysis**
  – the **direct Coombs test** is **positive** for the bound **IgG** antibodies
  – **markers of hemolysis** are present: *reticulocytosis, indirect hyperbilirubinemia, jaundice, elevated LDH, splenomegaly, gallstones*
  – some of the IgG autoAb activate the classical complement pathway resulting in C3b deposition and the formation of small, round cells called **spherocytes**
  – in **acute** severe cases, complement activation progresses to the surface assembly of the membrane attack complex (C5b-9), causing **INTRAVASCULAR hemolysis** and *hemoglobinuria* (dark urine) and *low haptoglobin*
  – **autoimmune thrombocytopenia** and **neutropenia** can also occur (Evans syndrome)

ii) COLD AIHA or Cold Agglutinin Disease

- **Etiology:**
  i) primary (idiopathic)
  ii) secondary to an underlying condition:
  – autoimmune diseases: SLE
  – lymphoproliferative disorders: lymphoid B-cell malignancy, monoclonal gammopathies
  – infections: Mycoplasma pneumoniae, Epstein-Barr virus, influenza, SARS-CoV2

- **Pathogenesis:**
  - autoAb are typically monoclonal **IgM** that bind RBCs at **lower temperatures** (the optimum temperature being **4°C** *in vitro*), i.e., in the cooler part of the circulation, such as the extremities, *in vivo*
  - IgM binds to multiple RBCs, causing **agglutination (clumping) at cold - the hallmark of COLD AIHA,** responsible for the clinical manifestations
  - the IgM autoAb activate the classical complement pathway, resulting in C3b formation on the RBC surface, with opsonization that renders them more susceptible to phagocytosis by macrophages, particularly in the *liver* (Kupffer cells), resulting in primary **EXTRAVASCULAR hemolysis**
  - the **direct antiglobulin (Coombs) test** typically detects **C3** on the RBC surface (because IgM detaches from RBC in warmer areas of the circulation)
  - **markers of hemolysis** (as mentioned for warm AIHA) are present in association with **acrocyanosis** and **Raynaud-like phenomena** under cold exposure
  - acute conditions may also lead to **INTRAVASCULAR hemolysis** if terminal complement activation occurs with direct RBC lysis and Hb release, leading to *hemoglobinuria* and *low haptoglobin*

b) DRUG-induced immune hemolytic anemias

- **Definition**: hemolytic anemias caused by anti-RBC antibodies induced by drugs (currently there are over 130 incriminated drugs) whose diagnosis is based on:
  - drug-induced alteration of normal membrane components, leading to immune hemolysis weeks to months after drug initiation
  - resolution of anemia upon discontinuation of therapy with the suspected drug
  - **positive direct antiglobulin (Coombs) test**
- **Pathogenesis**: anemia occurs via 3 mechanisms:

  i) Drug-*independent* antibody-mediated hemolysis:
  - **Drugs: alpha-methyldopa** (historical hypotensive drug), **antineoplastics** (fludarabine), **diuretics** (hydrochlorothiazide), **tuberculostatics** (izoniazid)
  - **Mechanism**:
    - the drug induces an autoimmune reaction towards the self-RBCs, with the production of anti-RBC **IgG** antibodies, **independent of drug presence** (hence the name *drug-independent immune hemolytic anaemia*)
    - the IgG auto-Ab cause Fc-mediated **EXTRAVASCULAR hemolysis** by splenic macrophages, which is indistinguishable from WARM AIHA
    - the **direct Coombs** test is **positive for IgG**

  ii) Drug-*dependent* antibody-mediated hemolysis - type II HS reaction:
  - **Drugs: antibiotics** (cephalosporins, penicillins), **NSAID** (diclofenac, ibuprofen)
  - **Mechanism**:
    - the drug firmly binds to the RBC membrane, generating a neoantigen that triggers the immune response (the drug acts as an incomplete Ag - hapten, which becomes a complete Ag by RBC covalent binding)

- auto-Ab antibodies, **IgG** or **IgM**, recognize this RBC-drug complex and trigger **EXTRAVASCULAR** (macrophage-mediated) or **INTRAVASCULAR** (complement-mediated) hemolysis depending on the antibody class and complement activation
- the **direct Coombs** test is **positive for IgG** or **C3**

iii) Drug-*dependent* immune complex-mediated hemolysis - type III HS reaction:

– **Drugs: quinidine, quinine, phenacetin**

– **Mechanism**:

- the drug forms soluble immune complexes with anti-drug Ab from **IgM** class
- the immune complexes bind to the RBC surface, activate complement with acute **INTRAVASCULAR hemolysis**
- these complexes may also adsorb onto leukocytes and platelets causing concomitant *leukopenia and thrombocytopenia*
- the **direct Coombs** test is positive for **C3**

c) ALLOIMMUNE hemolytic anemias

- **Definition:** hemolytic anemia in which anti-RBC Ab of one individual react with eRBC of another individual
- **Etiology:**
  – **hemolytic disease of the newborn:** exposure of a Rh (-) mother to fetal erythrocytes expressing paternally inherited Rh (D) antigen (via prior pregnancy, transfusion) leads to a secondary immune response with IgG antibodies that are actively transported across the placenta, bind to fetal erythrocytes, and trigger splenic and hepatic extravascular hemolysis (HS type II)
  – **hemolysis during blood transfusions**
  – **hemolysis after allogeneic transplantation** of bone marrow, kidney, liver, heart, intestine when donor lymphocytes transferred by allograft ("passenger lymphocytes") can lead to the formation of anti-RBC Ab against recipient erythrocytes

2. NON-IMMUNE HEMOLYTIC anemias

MECHANICAL hemolytic anemia

- **Definition**: lysis of erythrocytes due to **trauma** with: i) *intravascular hemolysis* in the case of immediate cell lysis in the circulation and/or ii) *extravascular hemolysis* in the case of distortion or fragmentation of erythrocytes that can thus circulate for a period of time prior to premature destruction by splenic macrophages
- **Etiology**:
  – **dysfunctional artificial heart valves**
  – **microangiopathic hemolytic anemia** – erythrocyte fragmentation occurs at the level of an abnormal microcirculation in association with multiple pathologies: malignant hypertension, eclampsia, thrombotic thrombocytopenic purpura, disseminated intravascular coagulation

PATHOPHYSIOLOGY OF POLYCYTHEMIA (ERYTHROCYTOSIS)

DEFINITION: increase in RBC mass above the normal range for age and sex, diagnosed by **elevated hemoglobin** (Hb) >16.5 g/dL in men and >16.0 g/dL in women **or increased hematocrit (Ht)** > 49% in men and > 48% in women, according to WHO.
The general consequences include:

- **increased blood viscosity** and risk of **arterial and venous thromboembolism with:** deep vein thrombosis, pulmonary embolism, myocardial infarction, transient ischemic attack (TIA) and stroke
- **hyperuricemia** and risk of **gout**

CLASSIFICATION:

I. Relative (spurious) polycythemia
II. Absolute (true) polycythemia

I. RELATIVE polycythemia

- **Definition**: increase in Ht due to **volume contraction** with a **normal red cell mass**
- **Etiology: conditions associated with severe dehydration**: diarrhea, severe vomiting or increased diuresis

II. ABSOLUTE polycythemia

- **Definition**: increase in Ht due to **elevated absolute red cell mass**
- **Etiology**:
  1. Primary polycythemia, **polycythemia vera** (Vaquez disease)
  2. Secondary polycythemia

1. PRIMARY Polycythemia or Polycythemia Vera

- **Definition:** increase in RBC mass caused by a **clonal disorder of the hematopoietic stem cell** that leads to **uncontrolled proliferation of erythroid precursors and elevated peripheral mature RBCs**, despite a **low erythropoietin** (EPO) level.

- **Etiopathogenesis**:
  The clonal proliferation is driven by **mutations in the JAK2** (Janus Kinase 2) **gene** that result in constitutive activation of a signaling pathway in hematopoietic stem cells, responsible for increased EPO sensitivity and uncontrolled proliferation of erythroid precursors but also of the other two lineages (panmyelosis). Since the process is independent of EPO stimulation, patients typically have **low** or normal **serum EPO** levels. The same mutation can also lead to proliferation of myeloid precursors with **leukocytosis** and of megakaryocytes with **thrombocytosis** in the peripheral blood.

  Observations!
  Polycythemia vera, **essential thrombocytosis**, **primary myelofibrosis** and **chronic myeloid leukemia** are the **4 entities** belonging to the group **myeloproliferative neoplasms (MPN)** characterized by somatic gain-of-function mutations responsible for the *constitutive activation of the JAK/STAT pathway*, which drive *excessive production of mature erythrocytes*, myeloid cells, platelets, alone or in combination and are These syndromes share overlapping features: marrow hypercellularity, increased peripheral counts,

splenomegaly, risk of thrombosis and hemorrhage and, in advanced disease, progression to myelofibrosis or risk for acute myeloid leukemia. The first 3 entities are referred to as **Philadelphia (Ph) chromosome negative (Ph-)** at variance from the **chronic myeloid leukemia,** where the **presence of Philadelphia chromosome (Ph+)** and **BCR-ABL1 fusion gene** (that encodes for a constitutively active tyrosine-kinase) are the **diagnostic hallmarks** (see further).

The **identification of the Ph chromosome is essential for diagnosis**, as it directly impacts therapeutic strategy—JAK inhibitors are used for the treatment of Ph-negative MPNs, while tyrosine kinase inhibitors are indicated for Ph-positive CML.

2. SECONDARY Polycythemia

- **Definition:** increase in RBC mass caused by stimulation of erythroid progenitor cells proliferation in the bone marrow due to **elevated circulating EPO** levels
- **Etiopathogenesis**:

a. Physiological, appropriate/compensatory EPO increase as response to tissue hypoxia in:
- High-altitude residence (decreased partial pressure of O_2)
- Chronic lung disease, e.g., COPD with cor pulmonale
- Cyanotic congenital heart disease with right-left shunt, e.g., Fallot tetralogy
- Sleep apnea
- Abnormal hemoglobins, e.g., methemoglobin, carboxyhemoglobin

b. Non-physiological, innapropriate/autonomous EPO increase of tumor origin, as a paraneoplastic syndrome in:
- Renal cell carcinoma (90% of cases)
- Hepatic carcinoma

2. PATHOPHYSIOLOGY OF WHITE BLOOD CELLS

HEMATOLOGIC MALIGNANCIES

DEFINITION

Hematologic malignancies or malignant hemopathies represent a heterogeneous group of **clonal disorders** originating from the hematopoietic lineages in the **bone marrow** or from immune cells in the **lymphoid tissues,** which are characterized by **uncontrolled proliferation and/or impaired differentiation of myeloid or lymphoid cells** and **specific genetic and molecular alterations** with diagnostic, therapeutic and prognostic roles.

CLASSIFICATION

Current WHO classification based on lineage (myeloid *vs* lymphoid), cell maturity (precursor *vs* mature), and genetic & molecular features (not presented) includes two major categories:

I. MYELOID neoplasms:

1. **Myelodysplastic neoplasms** (formerly, myelodysplastic syndromes) — clonal myeloid neoplasms characterized by a the presence of dysplastic cells (≥10%) in one or more bone marrow cell lineages, ineffective hematopoiesis, peripheral blood cytopenias, and a risk of progression to acute myeloid leukemia.
2. **Acute myeloid leukemias (AML).**
3. **Myeloproliferative neoplasms (MPN)**

II. LYMPHOID neoplasms:

1. **Acute lymphoblastic leukemias (ALL)**
2. **Mature B-cell neoplasms**
3. **Mature T-cell and NK-cell neoplasms**
4. **Hodgkin lymphomas**
5. **B-cell neoplasms with plasmacytic differentiation:**
   - Multiple myeloma

Observations !

1. The lymphoid neoplasms entities from points 2 and 3 of the WHO classification (mature B-cell, T-cell, and NK-cell lymphoid neoplasms) were previously classified as **non-Hodgkin lymphoma**.
2. **Hodgkin lymphoma** include the: *i) classic type*, with Sternberg-Reed cells as the pathognomonic morphological feature (95%) and *ii) nodular lymphocyte predominant type* (5%).

ETIOPATHOGENESIS

Malignant hemopathies arise from the combination between **genetic** & **environmental** factors.

a) GENETIC predisposition – rare

- refers to diseases in which inherited gene variants and somatic mutations are found
- are characterized by onset in childhood and concordance in monozygotic twins
- they may occur in the evolution of congenital syndromes, e.g. Down syndrome (trisomy 21), Fanconi anemia (primary bone marrow failure)

b) ACQUIRED mutations – frequent

- are represented by chromosomal abnormalities (deletions, translocations, insertions, inversions) or point gene mutations that drive leukemogenesis via **impaired apoptosis** and/or **dysregulated signaling**, e.g.:
  - *overexpression of anti-apoptotic proteins*, e.g., BCL2 in chronic lymphocytic leukemia (CLL), which prevent the death of malignant cells
  - *constitutive activation of growth pathways*, e.g., BCR-ABL1 in chronic myeloid leukemia (CML), which promote uncontrolled proliferation of the malignant cells
- are characterized by increased incidence with the age
- may result from ***prior cytotoxic therapy or radiotherapy of solid tumors*** (breast, lung cancer), e.g., therapy-related acute and cronic myeloid leukemias
- **diagnosis and treatment decisions** are currently **guided by the cytogenetic and molecular features**

c) ENVIRONMENTAL factors

- **ionizing radiation** – therapeutic or accidental exposure increased the risk of *acute and chronic myeloid leukemia* (the latter in survivors of Hiroshima atomic bombing)
- **chronic exposure to certain chemicals:** benzene, toluene, pesticides, dyes in the chemical industry – risk of *acute myeloid leukemia*
- **heavy smoking** – risk of *acute myeloid leukemia*

d) VIRUSES – certain viruses with lymphotropism increase the risk for lymphoid neoplasms:

- Human T Cell Leukemia Virus type 1 (HTLV-1)– risk for adult T cell leukemia/lymphoma
- Epstein-Barr virus – risk for Hodgkin lymphoma

e) ABNORMAL IMMUNE RESPONSE

- **chronic stimulation of the immune response** in:
  - ✓ **chronic infections** – e.g. infection with Helicobacter pylori is associated with a type of *B cell gastric lymphoma* called *gastric MALT (Mucosa-Associated Lymphoid Tissue) lymphoma*
  - ✓ **autoimmune diseases** – e.g. celiac disease is associated with *intestinal T cell lymphomas*
- **chronic deficiency of the immune response** in:
  - ✓ **AIDS** – is associated with the risk of *B-cell lymphomas* (T-lymphocyte dysfunction usually leads to B-lymphocyte hyperplasia within the germinal centers)

LEUKEMIAS

DEFINITION: clonal malignancies of hematopoietic stem or progenitor cells, characterized by **uncontrolled proliferation and impaired differentiation of leukocytes in the bone marrow**, **WITH the discharge of the malignant leukocytes into the peripheral blood** (hence the name given back to 1874 by Virchow, "leuk-ema" = "white blood", Gr.).

CLASSIFICATION:

I. According to the ONSET - into:

A. ACUTE leukemias - characterized by:

- ✓ *rapid* onset

- ✓ proliferation and predominance of **immature blasts**

B. CHRONIC leukemias - characterized by:

- ✓ *insidious* onset
- ✓ proliferation and predominance of **mature cells**

II. According to the CELL LINEAGE - into:

A. LYMPHOID (lymphocytic) leukemias (either acute or chronic)

B. MYELOID (granulocytic) leukemias (either acute or chronic)

ACUTE LEUKEMIAS

DEFINITION: clonal malignancies characterized by **rapid proliferation of immature blasts** with impaired differentiation in the bone marrow and their appearance in the peripheral blood.

GENERAL MANIFESTATIONS – consist of a classic triad: **anemia, hemorrhages and infections** that arises from the **leukemic cell overgrowth and infiltration of the bone marrow** that profoundly disrupts the normal hematopoiesis.

1. **Anemia** with fatigue, pallor, dyspnea, decrease exercise tolerance, results from the:
   - suppression of erythroid precursors proliferation
   - ineffective erythropoiesis due to increased apoptosis of erythroblasts mediated by leukemic cell-derived cytokines
   - premature destruction of RBC due to splenomegaly and hypersplenism
2. **Hemorrhages**, e.g., petechiae, epistaxis, or gingival bleeding, are due to:
   - thrombocytopenia caused by:
     i) impaired megakaryocyte differentiation and maturation, and
     ii) splenic sequestration due to splenomegaly with hypersplenism
   - activation of coagulation and fibrinolysis leading to disseminated intravascular coagulation (DIC) in certain subtypes, such as *acute promyelocytic leukemia*
3. **Recurrent infections** (bacterial, fungal, and viral) occur due to:
   - neutropenia and dysfunctional granulocytes impair innate immunity
   - lymphocyte abnormalities, especially in acute lymphoblastic leukemia (ALL) result in hypo-gammaglobulinemia and impaired cell-mediated immunity
   - chemotherapy-aggravated immunosuppression and mucosal barriers disruption

Other MANIFESTATIONS may include**:**

- **Weight loss, asthenia, fatigue** – due to anemia and increased basal metabolism
- **Leukostasis in acute leukemias** – refers to the **accumulation of large numbers (up to 100,000/mm^3) of leukemic blasts in the peripheral blood leading to microvascular obstruction** (and tissue hypoxia) of the **small vessels in the retinal, pulmonary and cerebral circulation** responsible for: *retinal hemorrhages, progressive dyspnea, headache, confusion,* and finally, coma; it is a life-threatening complication with indication for plasmapheresis.
- **Tumor lysis syndrome** – refers to the **rapid destruction of malignant cells, typically after initiation of cytotoxic therapy** resulting in the release of the cellular content (potassium, phosphate, and nucleic acids) into the circulation with:

- **metabolic disturbances**: *hyperkalemia, hyperphosphatemia* and secondary *hypocalcemia* (due to precipitation of calcium phosphate), *hyperuricemia* (due to catabolism of nucleic acids)
- **acute complications** – risk of *acute kidney injury* (due to uric acid/calcium phosphate precipitation manifested as elevated creatinine and oliguria) due to uric acid/calcium phosphate precipitation and of *cardiac tachyarrhythmias* (due to hyperkalemia or hypocalcemia)

– **Tumor infiltration** – refers to the **invasion of leukemic blasts into extramedullary tissues** mediated by the cytokine-driven increased vascular permeability in the bone marrow microenvironment, matrix degradation and transendothelial migration of blasts leading to: bone pain, hepato-splenomegaly, adenopathies, skin and central nervous system infiltration.

CLASSIFICATION:
1. ACUTE LYMPHOID (LYMPHOBLASTIC) leukemia
2. ACUTE MYELOID (MYELOBLASTIC) leukemia

1. ACUTE LYMPHOID leukemia (ALL)

- **Definition:** clonal proliferation and **accumulation of immature lymphoid progenitor cells (lymphoblasts)** in the bone marrow and the peripheral blood
- **General features:**
  - most common in **children** and **young adults**
  - pathogenesis involves acquired genetic translocations (t(12;21), t(9;22))/mutations that block normal lymphoid maturation and lead to uncontrolled proliferation of lymphoblasts
  - in **80%** of cases the malignant proliferation involves **precursor B lymphocytes** and diagnosis is confirmed by identifying **≥ 20% lymphoblasts in the bone marrow**
  - the leukemic blasts crowd out normal hematopoietic cells and lead to bone marrow failure (anemia, hemorrhage, infections), high circulating blasts, and extramedullary infiltration
  - prognostic is good in childhood ALL (5-year survival rate > 80%)
  - in **young adults**, **the presence** of the **t(9;22) translocation** or **the Philadelphia chromosome** (which is the hallmark of chronic myeloid leukemia is associated with a **poor prognosis**

2. ACUTE MYELOID leukemia (AML)

- **Definition:** clonal proliferation and **accumulation of immature myeloid progenitor cells (myeloblasts)** in the bone marrow and peripheral blood
- **General features**:
  - most common in **adults** but the incidence increases with the age
  - pathogenesis involves acquired pathogenic genomic aberrations (translocations, mutations), which block normal differentiation and confer a proliferative advantage
  - prognosis is primarily determined by the cytogenetic and molecular profile at diagnosis, with specific abnormalities stratifying patients into adverse or favorable risk groups
  - adverse profiles are associated with poor outcomes and resistance to standard therapy, whereas favorable cytogenetic/molecular profiles predict higher remission and survival rates

- a distinct subtype of AML is the **acute promyelocytic leukemia** in which abnormal promyelocytes accumulates in the bone marrow and peripheral blood, and has as particular features:
  i) a **specific translocation** (t(15;17)) that is responsible for the **block in myeloid differentiation at the promyelocyte stage**
  ii) **disseminated intravascular coagulation is the most frequent complication** (due to tissue factor release by the leukemic promyelocytes) and lead to **severe bleeding diathesis**, with the risk of life-threatening intracranial or pulmonary hemorrhages.

CHRONIC LEUKEMIAS

DEFINITION: clonal hematologic malignancies characterized by the **slow, progressive accumulation of mature but dysfunctional leukocytes** (lymphoid or myeloid) in the bone marrow, peripheral blood, and lymphoid tissues; these disorders are distinguished from acute leukemias by a **more indolent clinical course.**

CLASSIFICATION:

1. CHRONIC LYMPHOID (LYMPHOCYTIC) leukemia
2. CHRONIC MYELOID (GRANULOCYTIC) leukemia

1. CHRONIC LYMPHOID leukemia (CLL)

- **Definition:** clonal malignancy of **mature B lymphocytes**, characterized by the accumulation of **small, immunologically dysfunctional B cells**.
- **General features:**
  - **most common** in **elderly** people
  - pathogenesis involves genetic and epigenetic alterations that disrupt apoptosis (overexpression of anti-apoptotic proteins BCL2), and promote clonal expansion of B lymphocytes with impaired immune function, which accumulate leading to:
    - **bone marrow infiltration** - lymphocytes represent at least 30% of the cellularity
    - **chronic lymphocytosis** with persistent (≥ 3 months) absolute B-lymphocyte count ≥ 5,000 cells/μL in the peripheral blood
    - **absence of blasts** on the peripheral blood smear
  - most patients with CLL are asymptomatic at diagnosis, being incidentally identified by the discovery of an **elevated white blood cell count and lymphocytosis**
  - symptomatic patients may present: **lymphadenopathy, hepatosplenomegaly, recurrent infections due to hypogammaglobulinemia**, and **autoimmune hemolytic anemia (positive Coombs test)** or immune thrombocytopenia
  - the disease progression is more slowly than in other types of leukemia and has **the highest survival rate**
  - the prognosis is highly dependent on the cytogenetic profile, with specific mutations/ chromosomal abnormalities stratifying patients into favorable and adverse categories

Observation!

A distinct subtype of CLL is **hairy-cell leukemia**, characterized by the presence of mature B lymphocytes with abundant pale cytoplasm and irregular cytoplasmic projections, which infiltrate the bone marrow and

spleen, leading to cytopenias and splenomegaly; the disease is driven by a specific mutation, commonly in adult males and has a remitting–relapsing course, but patients achieving near-normal life expectancy with current therapies.

2. CHRONIC MYELOID leukemia (CML)

- **Definition:**
  - clonal myeloproliferative neoplasm of hematopoietic stem cells, characterized by the **presence of the Philadelphia chromosome**, which results in **uncontrolled proliferation of the myeloid lineage cells**, leading to **marked myeloid hyperplasia in the bone marrow and peripheral blood.**
- **General features:**
  - most common in **adults** (median age between 45-55 years)
  - the specific marker of the disease is the **Philadelphia chromosome (Ph1)**, which is a **short chromosome 22** resulting from the reciprocal translocation of genetic material between the long arms of chromosomes 9 and 22 in the pluripotent stem cell (and all precursors of the granulocyte, erythrocyte and megakaryocyte lineages) and leads to the formation of a **fusion gene** called the **BCR-ABL1 oncogene**.
  - the t(9;22) translocation leads to the formation of a **fusion gene** called the **BCR-ABL1 oncogene** that encodes a **constitutively active tyrosine kinase** responsible for aberrant proliferation, survival and reduced apoptosis of the malignant myeloid cells.

Observation!

The **tyrosine kinase inhibitors** (TKI, e.g., imatinib, dasatinib, nilotinib, bosutinib, ponatinib, asciminib), the **mainstay therapy of CML**, are highly effective at suppressing the BCR-ABL1 activity of the leukemic cells and induce sustained remissions but **are not curative** (they do not eradicate the leukemic stem cells); **allogeneic hematopoietic stem cell transplantation is the only curative treatment.**

- **Pathogenesis**:

According to the International Consensus Classification (ICC, 2022) diagnostic criteria, the CML course is **triphasic:**

a) The CHRONIC phase - is the **initial, common presentation**, being characterized by one ore more of the following:

- **proliferation of the myeloid lineage** with **leukocytosis and left-shifted granulopoiesis**
- **blasts < 10%** in the bone marrow or peripheral blood
- **basophilia < 20%** in the peripheral blood
- **platelets normal or increased (thrombocytosis)**
- **Ph chromosome is present** but no additional chromosomal abnormalities
- patients are often asymptomatic or have mild symptoms such as: fatigue, exertional dyspnea, weight loss, splenomegaly
- treatment with TKI typically keeps the disease under control for long periods

Observations !

Other laboratory markers may be abnormal, e.g. **high serum vitamin B_{12}** due to **increased transcobalamin I** production by proliferating granulocytes and **low leukocyte alkaline phosphatase** (reflecting the functional abnormalities of malignant neutrophils) but are not clinically relevant for diagnosis or disease monitoring in the era of genetic and molecular testing (they may be useful for a differential diagnosis with a leukemoid reaction).

b) The ACCELERATED phase – signals **rapid disease progression** and is characterized by one or more of the following:

- **excessive proliferation of the myeloid lineage** with **marked leukocytosis** and/or splenomegaly unresponsive to therapy
- **blasts 10-19%** in the bone marrow or peripheral blood
- **basophilia ≥ 20%** in the peripheral blood, ± eosinophilia
- **persistent thrombocytopenia** (< 100,000/μL) unrelated to therapy
- **new additional chromosomal abnormalities in Ph-positive cells** (clonal evolution)
- progressive clinical symptoms: *symptomatic splenomegaly*, bone pain, infiltration/dysfunction of other organs (intestine, kidneys, lungs)
- laboratory abnormalities: hyperuricemia (secondary to tumor lysis) with the risk of gout and renal lithiasis (urate stones)

c) The BLAST CRISIS phase – signals **disease "acutization"/ terminal stage** and is characterized by **one** of the following:

- **blasts ≥ 30%** in the bone marrow or peripheral blood and may be myeloid (*myeloblastic crisis*) *or lymphoid* (*lymphoblastic crisis*) – **pathognomonic feature**
- presence of **extramedullary blast proliferation**, e.g., in lymph nodes, skin, or other tissues
- **marked persistent leukocytosis with** basophilia ± eosinophilia
- **symptoms and signs similar to acute leukemia**: **severe anemia and thrombocytopenia, infections,** bone pain, weight loss, massive splenomegaly, fever
- **additional cytogenetic abnormalities**
- the disease is frequently **refractory to standard therapies** and has **poor prognosis** (median survival less than one year despite intensive therapy)

Observation!

The management of chronic myeloid leukemia utilizing tyrosine kinase inhibitors (TKI) is as a model for the successful development of cancer therapies. Nowadays the majority of patients with CML experience a normal quality and lifespan with TKI therapy, and only a subset progresses to the accelerated and blast phases, both with poor prognosis. The progression rates have markedly decreased from over 20% in the pre-TKI era to less than 5% currently, primarily due to advancement in CML treatment (new generations of TKI).

LYMPHOMAS

DEFINITION: **solid tumors of lympho-reticular tissue, WITHOUT the discharge of malignant cells into the peripheral blood at the onset of the disease**, but which may evolve towards their presence with the disease progression.

CLASSIFICATION:

1. **HODGKIN lymphomas**
2. **NON-HODGKIN lymphomas**

1. HODGKIN Lymphoma (HL)

- **Definition:** malignant lymphoid neoplasm characterized by **the presence of a pathognomonic morphological feature, the Reed-Sternberg cell**.
- **General features:**

- **bimodal age distribution**, with two peaks: in **young adults** (20–35 years) and **older adults** (50-60 years)
- HL originates from **germinal-center B cells** in the lymph nodes that have undergone clonal transformation
- the **histopathological hallmark** is the **Reed-Sternberg (RS) cell,** a large, binucleated "owl's eye" cells, with prominent basophilic nucleoli within an abundant microenvironment of inflammatory and immune cells that is actively shaped by the RS cells to support their growth. RS cells secrete cytokines/chemokines that contribute to the: i) lymphadenopathy, ii) systemic inflammatory (B type) symptoms, and iii) tissue remodeling and fibrosis in some subtypes.
- the onset is with **painless isolated lymphadenopathy**, most often in the **cervical, supraclavicular, axillary regions** or as **a mediastinal mass** causing chest discomfort, dyspnea or cough
- **in HL extension occurs by contiguity, via the lymphatic system**, typically spreading in a predictable way, **starting in lymph nodes and extending to nearby areas**, such as spleen, liver, lungs and bone marrow
- **extranodal involvement** (extralymphatic sites involvement) **is <u>rare</u>**
- **systemic features are frequent** – a classic triad consists of: **fever** (>38°C) + **night sweats** + **unintentional weight loss** (>10% in 6 months)
- in the diagnosis, the absence of systemic symptoms is marked with **A**, and their presence with **B** (association with poor prognosis)
- positive diagnosis requires *excisional lymph node biopsy* and *immunohistochemistry* to identify RS cells and their characteristic surface markers, respectively
- **CT/PET scans** (neck, chest, abdomen, pelvis) are the **gold standard for staging and assessing treatment response**
- HL is **one of the most curable malignancies in early stages**; long-term survival is achieved in approx. 80–90% of cases using modern chemotherapy with or without radiotherapy

- **Pathogenesis**:
  - the central element in the pathogenesis of HL consists in the **activation of the transcription factor NF-kB signaling** by viral infections (in particular, with the Epstein-Barr virus), which triggers the **clonal transformation** of the **germinal-center B cells** into the **RS cells** within the lymph nodes and their uncontrolled proliferation
  - **RS cells** secrete: i) **cytokines** (IL-4, IL-5, IL-10 and IL-13), and ii) **growth factors** – (TGFbeta, bFGF/basic Fibroblast Growth Factor) responsible for the:
    - **chemotactic effect** for granulocytes/neutrophils, monocytes/macrophages and eosinophils $\Rightarrow$ *chronic inflammatory tumor microenvironment*
    - **inhibition** of the **cellular immune response**, i.e., suppression of Th1 and cytotoxic T lymphocytes
    - **stimulation** of the **humoral immune response**, i.e., activation of Th2 lymphocytes, formation of plasma cells and auto-antibodies $\Rightarrow$ risk for *immunohemolytic anemias*
    - **proliferation of fibroblasts** $\Rightarrow$ *local fibrosis*

2. NON-HODGKIN Lymphoma (NHL)

- **Definition:** heterogeneous group of **malignant clonal proliferations of lymphoid B-cells, T cells, or NK cells** that arise in **lymph nodes, extranodal tissues, or bone marrow** and **lack Reed–Sternberg cells** as distinct feature from HL.
- **General features:**
  - **unimodal age distribution in elderly people** (highest rate is seen in people aged 65-75 or older)
  - they are **mainly derived from B cells (85-90%)**, T cells and NK cells (10-15%)
  - onset with **painless polyadenopathies** and **non-contiguous spread (***cervical/axillary* and *inguinal / mesenteric*) with duration depends on the histological subtype:
    - ✓ in *aggressive* NHL - onset is rapid (weeks, months)
    - ✓ in *indolent* NHL - onset is slow (years)
  - **extranodal involvement is <u>frequent</u>** and **a hallmark feature** - onset with tumors located in the gastrointestinal tract, Waldeyer ring, skin or central nervous system
  - **other manifestations:** hepatomegaly, splenomegaly, recurent infections
  - **systemic "B symptoms"** (fever, night sweats, and unintentional weight loss) occur **in aggressive NHL subtypes**
  - positive diagnosis requires adequate tissue biopsy for *histopathology, immunophenotyping and molecular studies*
  - **variable prognosis** depending disease subtype, extent of involvement, and molecular features
- **Pathogenesis**:

NHL arises from malignant transformation lymphocytes at different stages of maturation driven by: i) genetic (point) mutations, ii) chromosomal translocations, iii) inhibition of apoptosis allowing prolonged survival, and iv) constitutive activation of signaling pathways responsible for uncontrolled proliferation and immune escape.

PLASMA CELL NEOPLASMS

DEFINITION: a heterogenous group of malignant disorders characterized by **proliferation of a clone of plasma cells** and **excessive production** of **abnormal immunoglobulins leading to specific end-organ damage**. The abnormal immunoglobulins are produced by a single clone of plasma cells and migrate in the gamma region on serum protein electrophoresis, hence the term **monoclonal gammopathies** used for these disorders.

Multiple myeloma is the most clinical significant disease due to **widespread bone marrow infiltration** and **several systemic complications**.

MULTIPLE MYELOMA (Plasma cell myeloma, Kahler's disease)

- **Definition: clonal proliferative disorder** characterized by **uncontrolled growth of plasma cells in the bone marrow** (multiple tumors) and **abnormal increase of monoclonal paraprotein**, which occurs **in geriatric population** (median age at diagnosis of 70 years).

- **Pathogenesis**:
  - the disease is caused by *chromosomal translocations* (especially, on chromosome 14) and *activation of oncogenes*, which are responsible for the **malignant transformation of a clone of B cells in plasma cells** that **infiltrate the bone marrow** (≥10% clonal bone marrow plasma cells) and lead to **osteolytic focal bone lesions** (of the skull, vertebrae, ribs)
  - plasma cell proliferation in the bone marrow microenvironment leads to secretion of: ***osteoclast-activating factors,*** including pro-inflamatory cytokines, resulting in *excessive bone resorption* and ***osteoblast-inhibitory factors*** that suppress osteoblast function with *reduced bone formation,* ultimately leading to **focal bone destruction and the formation of lytic lesions** with **bone pain, fractures, and hypercalcemia** as hallmarks of skeletal (end-organ) damage
  - malignant plasma cells excessively produce whole **immunoglobulins IgG** and **IgA** (rarely IgM) or **immunoglobulins** components, the **light chains (κ or λ)**; their increased plasma and urinary levels result in:
    - **paraproteinemia** responsible for the: i) ***hyperviscosity syndrome*** (visual disturbances, headache), ii) ***organ deposition and dysfunction*** (neuropathy, cardiomyopathy) and iii) ***immunosuppression aggravation*** (as the abnormal immunoglobulins suppress normal B-cell function and production of polyclonal immunoglobulins)
    - **paraproteinuria** - the urinary excretion of the free light chains is responsible for **direct renal damage** and **progression towards renal failure via: i) *cast nephropathy*** (precipitation and obstruction of the distal tubules), ii) ***amyloidosis*** (deposition of light chains as amyloid fibrils) and **nephrotic syndrome**, iii) ***thrombotic microangiopathy*** (via endothelial injury and complement activation).
- **Manifestations**:
  - **'CRAB' features**, the classic mnemonic for multiple myeloma manifestations due to the end-organ damage, include:

C: Hypercalcemia (serum calcium ≥ 11 mg/dL)
R: Renal insufficiency (increased serum creatinine and/or decreased creatinine clearance)
A: Anemia (Hb < 10 g/dL)
B: Bone lesions (one or more osteolytic round, radiolucent focal lesions, each ≥5 mm in size)

  - **recurrent** and **severe infections** that are a **leading cause of morbidity and mortality** in multiple myeloma via several pathomechanisms:
    - **compromised humoral immunity** due to *disease-induced hypogammaglobulinemia* leads to increased susceptibility to **bacterial infections** (with encapsulated pathogens, e.g., Streptococcus pneumoniae and Haemophilus influenzae)
    - **impaired cellular immunity** due to *global dysfunction of T cells, NK cells, dendritic cells* increases vulnerability to **viral infections** (e.g., influenza, herpes virus)
    - **therapy-related immunosuppression** causes *neutropenia* and further impairs the defense capacity.

3. PATHOPHYSIOLOGY OF HEMOSTASIS

HEMOSTASIS – BRIEF OVERVIEW

Hemostasis or the cessation of bleeding, is a **compex biological process** that requires the intricated participation of 3 main components: the **VASCULATURE and PLATELETS, which are responsible for the PRIMARY hemostasis,** and **the PLASMA COAGULATION FACTORS, which are responsible for the SECONDARY hemostasis**.

PRIMARY hemostasis encompasses **vasoconstriction** and **platelet plug formation** via 4 coordinated processes: **adhesion, activation, secretion,** and **aggregation**. Platelet **adhesion** to the subendothelium occurs via the interaction of the **GPIb receptor** with **von Willebrand factor (vWF)**, while platelet **aggregation** is mediated by the **GPIIbIIIa receptor** binding to fibrinogen and vWF. Platelet **activation** occurs after the binding of various mediators on the platelet surface, such as: *epinephrine*, *adenosine diphosphate* (ADP, via P2Y1 and P2Y12 receptors), *thrombin* (via PAR1 and PAR4 receptors), and *collagen* (via GPVI receptor). Upon activation, platelets undergo important morphological changes and degranulate with the **secretion** of several mediators from the *α-granules (fibrinogen and vWF)*, and *δ-granules (serotonin, ADP, and calcium*).

SECONDARY hemostasis aims at reinforcing the platelet plug via a classic cascade of progressive proenzymes activation, encompassing the parallel *extrinsic and intrinsic pathways* that end up into the *common (final) pathway*. Tissue factor promotes FVII activation in the extrinsic pathway, subsequently activating FX. The intrinsic pathway entails the sequential activation of FXII, FXI, FIX, and FVIII, also leading to FX activation. Ultimately, in the final pathway, activated FX interacts with FV, platelet phospholipids, and calcium ions to catalyze the conversion of prothrombin into thrombin, which subsequently cleaves fibrinogen into fibrin. Factor XIII (FXIII) reinforces the fibrin clot. These pathways predominantly represent *ex vivo* mechanisms described in laboratory (where the coagulation tests have been standardized) but fail to encompass the complex *in vivo* processes. As such, the current paradigm supports the **cellular model of hemostasis**, wherein all interactions occur on the surface of the endothelial cells and platelets, according 3 overlapping stages: i) **initiation**, triggered by tissue factor interaction with FVII to generate a complex called *extrinsic tenase* responsible for FX activation and the *initiation of coagulation*, ii) **amplification**, in which thrombin generation occur and further amplifies FX activation (both directly and indirectly via the F.VIIIa - F.IXa complex called *intrinsic tenase*), and iii) **propagation**, driven by the activated platelets, which provide the substrate for the continuation of thrombin generation and the fibrin network formation.

Hemostasis also involves the dynamic balance between procoagulant and anticoagulant systems. The latter prevent excessive clot formation, thus averting thrombosis, and are represented by:

1. **FIBRINOLYSIS**, i.e. the **<u>breakdown of the existing fibrin clot</u>** mediated by plasmin, which cleaves:
   - **fibrinogen** and **soluble** fibrin (*monomer*) releasing the peptides **peptides X, Y, D, E,** collectively known as the **fibrin degradation products (FDP)** that inhibit fibrin polymerization and platelet aggregation
   - **insoluble** fibrin (*polymer*) with the generation of **D-dimers**

2. SOLUBLE inhibitors of the activated clotting factors that **<u>prevent novel fibrin clot formation</u>**; they include **protein C and its cofactor, protein S**, which inactivate FVa and FVIIIa, and **antithrombin III (AT III)**, which inactivates **thrombin**, as well as factors FXa, FXIIa, FXIa, FIXa. The inactivation reaction is accelerated (by approx. 1000-fold) in the presence of heparin by inducing a conformational change in ATIII that enhances its reactivity with thrombin and the other clotting factors). This acceleration is a central mechanism underlying the *anticoagulant effect of heparin*, the oldest anticoagulant drug in clinical use.

Abnormal hemostasis refers to disruptions in blood clotting, which lead to either:

- **deficient hemostasis** resulting in **HEMORRHAGIC SYNDROMES (excessive bleeding)**
- **exaggerated hemostasis** resulting in **HYPERCOAGULABILITY SYNDROMES (excessive clotting)**
-

HEMORRHAGIC SYNDROMES (BLEEDING DIATHESES)

According to the *mechanisms that disrupt normal hemostasis* and lead to bleeding, there are:
I. VASCULAR hemorrhagic syndromes or VASCULAR purpuras
II. PLATELET-related hemorrhagic syndromes or PLATELET purpuras
III. CLOTTING FACTORS-related hemorrhagic syndromes or COAGULOPATHIES

I. VASCULAR HEMORRHAGIC SYNDROMES / VASCULAR PURPURAS

DEFINITION: disorders caused **abnormalities of the vessel walls** due to vascular *malformation* or *damage* that result in increased vessel fragility and blood leaking from the **small vessels** leading to **superficial cutaneous or mucosal purpura**.
The term "**purpura**" is used to describe *subcutaneous bleeding* related to either *blood vessel wall abnormalities*, i.e. **vascular purpura** or *platelet abnormalities*, i.e. **platelet purpura**.

GENERAL manifestations of purpuras:
- red or purple lesions on the skin or mucous membranes, as a result of red blood cell extravasation from abnormal/damaged capillaries lead to the main clinical manifestations that occur **spontaneously** or **immediately after minimal trauma**:
  - **petechiae** are pinpoint red/purple flat spots less than 2 mm in diameter
  - **purpura** refers to larger, red/purple lesions measuring 4 to 10 mm in diameter, which may be symmetrically distributed (frequently, on the lower extremities) and become palpable (raised) if associated with inflammation (vasculitis)
  - **ecchymoses** are larger (more than 10 mm), diffuse areas of skin discoloration resulting from more extensive bleeding into the subcutaneous tissue
- the skin lesions are non-blanching (do not disappear under pressure), as the hallmark of the blood presence in the cutaneous tissues, while mucosal involvement lead to oral or conjunctival hemorrhage
- severe vascular purpuras may progress to cutaneous ulceration or digital necrosis, whereas extensive purpura may be a sign of associated internal bleeding
- **laboratory: platelet count and function** (assessed with aggregometry) and **coagulation tests**, activated partial thromboplastin time (aPTT) and prothrombin time (PT) **are normal**

Observation !
Bleeding time was historically used as screening test of primary hemostasis (to assess vascular integrity and platelet function) being typically prolonged in vascular and platelet purpuras. Currently is not anymore routinely measured due to lack of standardization and limited clinical utility.

CLASSIFICATION:
A. INHERITED vascular purpuras
B. ACQUIRED vascular purpuras

A. INHERITED vascular purpuras

1. HEREDITARY HEMORRHAGIC TELANGIECTASIA (Rendu-Osler-Weber disease)
- **Definition: autosomal dominant** inherited vasculopathy characterized by the development of **muco-cutaneous telangiectasias** and **visceral arteriovenous**

malformations due to impairment of signaling pathways that control vascular integrity and angiogenesis. It can affect both sexes but it has a higher diagnostic prevalence in women.

- **Pathogenesis:**
  - mutations in genes encoding for proteins that regulate TGF-β signaling in the vascular endothelial cells are responsible for the:
    - abnormal angiogenesis and formation of dilated vessels and larger arteriovenous malformations in mucosal, skin and internal organs
    - disruption of the endothelial cell function and loss of capillary beds results in shunting, increased wall stress in the small vessels, which are fragile and at risk for bleeding
  - *telangiectasias* occur in the nasal mucosa and skin, while *visceral arterio-venous malformations* may occur in the gastro-intestinal tract, lungs, liver and brain
- **Manifestations**:
  - **spontaneous, recurrent epistaxis** (onset in childhood)- the most common (90%)
  - chronic **gastrointestinal bleeding** leading to **iron deficiency anemia**
  - rarely, hemoptysis, meningeal hemorrhage
  - acute complications include: pulmonary hemorrhage, embolic or hemorrhagic stroke

2. PURPURA ASSOCIATED WITH CONNECTIVE TISSUE DISEASES: Ehlers-Danlos syndrome, Marfan syndrome

B. ACQUIRED vascular purpuras

1. HENOCH-SCHONLEIN purpura (anaphylactoid purpura, IgA vasculitis)

- **Definition**: **small-vessel, systemic vasculitis** characterized by **deposition of IgA-containing immune complexes in the vascular walls,** most common in children and young adults.
- **Pathogenesis**:
  - **vascular purpura** triggered by **an exogenous antigen** that leads to the formation of **IgA containing circulating immune complexes** that are deposited in the small vessels of the skin, joints, gastrointestinal tract and kidneys with subsequent **neutrophil recruitment**, **endothelial injury, inflammation (vasculitis) and complement activation** leading to marked increase in vascular permeability and extravasation of red blood cells, responsible for extensive ecchymotic, necrotic purpura
  - the **upper respiratory tract infections are the common environmental triggers**, but also, **genetic susceptibility** contributes
  - classic, the onset of the disease was described 2-3 weeks after the infection with group A β-hemolytic streptococcus but nowadays the onset post-viral infections or
- **Manifestations –** classic **triad:**
  ① **cutaneous purpura** - palpable, symmetric on the lower extremities and buttocks
  ② **arthralgia or arthritis** - transient swelling of large joints (knees, ankles)
  ③ **abdominal pain**, and **gastrointestinal bleeding** (hematemesis/melena)
  - **renal involvement** may also occur, ranging from isolated proteinuria/hematuria to acute glomerulonephritis and progression towards chronic kidney disease (rare)

- the disease course is usually self-limited in children, but renal complications can cause significant morbidity, especially in adults

2. AUTOIMMUNE DISEASE-related purpura

- **Definition: small-vessel, systemic vasculitis** associated with systemic connective tissue diseases: systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, dermatomyositis
- **Pathogenesis: multifactorial,** comprising:
  - *immune complex deposition*, *complement activation,* and *direct vascular injury* from inflammatory cells and autoantibodies result vessel wall damage, RBC extravasations and palpable purpura
  - the immune dysregulation and pro-inflammatory cytokines released in the bloodstream can directly damage endothelium, aggravating the vascular injury/hyperpermeabilization

3. INFECTIOUS purpuras

- **Definition:** infection-associated vascular injury with a wide range of manifestations from localized petechiae to extensive purpura fulminans, occurring typically in severe bacterial infections (mainly, with encapsulated bacteria) associated with sepsis, and less frequent in viral infections (varicella, parvovirus B19).
- **Pathogenesis: multifactorial**, comprising:
  - *direct endothelial injury by microbial toxins* (e.g., endotoxin in meningococcal meningitis) and *vascular inflammation (vasculitis)*
  - *indirect amplification of the endotheliitis via disseminated intravascular coagulation* (DIC) responsible for: widespread thrombi formation in the microcirculation, thrombosis, consumption of circulating anticoagulants (proteins C and S) resulting in hemorrhagic necrosis, and secondary extravasation of RBC into the skin

Observation !

Purpura fulminans is a rapid evolving, life-threatening condition severe purpura in the course of infections, which initially manifests massive symmetric purpura on the trunk and lower extremities, and then progresses to bullous and necrotic lesions that may lead to gangrene, frequent in patients with splenectomy or functional hyposplenism.

4. Purpura from **CUSHING syndrome** or from **CHRONIC STEROID THERAPY**

- **Definition:** non-palpable purpura, ecchymoses, and bruises, most commonly on the trunk and extremities, which occur spontaneously or after minimal trauma are the hallmarks of hypercortisolism-related vascular fragility (not to vasculitis)
- **Pathogenesis:**
  - the chronic excess of glucocorticoids increases protein catabolism, impairs proliferation of the dermal fibroblasts, inhibits collagen synthesis/turnover proliferation, all resulting in the structural weakness of subcutaneous vessels and thinning of the skin
  - the increased fragility of cutaneous blood vessels and connective tissue causes blood extravasation into the dermis with purpura, which is accompanied by other typical findings such as, wide purple striae and poor wound healing

5. SENILE purpura

- **Definition**: ecchymotic, non-palpable purpura in the elderly, which appears on the hands and forearms, spontaneously or after minimal trauma, in the absence of vasculitis or coagulopathy

- **Pathogenesis:**
  - age-related atrophy of the dermis/epidermis, with loss and disorganization and of both collagen and elastic fibers are responsible for the increased fragility of dermal blood vessels, rendering them susceptible to rupture from minimal trauma
  - extravasation of the blood in the subcutaneous tissues produces ecchymotic purpura or bruises that may be aggravated by sun exposure

6. SCURVY purpura

- **Definition: small-vessel, systemic vasculitis** associated with severe vitamin C deficiency responsible for non-blanching petechiae or purpura, especially on the legs.
- **Pathogenesis:**
  - vitamin C is required for hydroxylation of proline and lysine during collagen synthesis in the absence of vitamin C, defective collagen synthesis result in weak connective tissue especially in the capillary walls of the skin and gums
  - since capillaries lack a structural support and are fragile they will be prone to spontaneous rupturing leading to bleeding typically around the hair follicles and into the skin of the lower limbs
- **Manifestations**: skin petechiae/purpura, bleeding gums, weakness, fatigue, anemia

II. PLATELET HEMORRHAGIC SYNDROMES / PLATELET PURPURAS

CLASSIFICATION:

A. Thrombocytopenias
B. Thrombocytopathies

A. THROMBOCYTOPENIAS

DEFINITION: decreased platelet count < 100,000/μL

GENERAL manifestations:

- **immediate and excessive bleeding from the small vessels** occurring spontaneously and/or after minor trauma or surgical procedures (tooth extraction, tonsillectomy), as:
  - **skin bleeding:** petechiae, purpura, ecchymoses, bruises
  - **mucosal bleeding:** epistaxis, gums bleeding, hematuria, menorrhagia (increased menstrual bleeding), gastro-intestinal bleeds
  - **internal bleeding** in *severe cases*: retinal bleeding, intracranial hemorrhage (vital risk)
- **laboratory: low** platelet count, **normal** coagulation tests (aPTT, PT)

PATHOGENESIS – there are 3 major mechanisms:

1. Decreased BONE MARROW PRODUCTION due to bone marrow failure, toxicity and/or infiltration:

- Aplastic anemia
- Megaloblastic anemia (vitamin B_{12} or folic acid deficiency)
- Viral infections (hepatitis, HIV)
- Anticancer chemotherapy/radiotherapy

- Toxic drugs / Drug addiction (e.g., alcohol, cocaine)
- Viral infections (hepatitis, HIV)
- Inflitrative malignancies: leukemia, lymphoma, plasma cell neoplasm, metastases

***Key feature*:** *Low platelets in periphery with decreased and/or abnormal megakaryocytes*

2. Increased PERIPHERAL DESTRUCTION or CONSUMPTION:

a) IMMUNE-mediated destruction (antiplatelet antibodies)
- **Idiopathic (or immune) thrombocytopenic purpura (ITP)**
- Systemic lupus erythematosus
- Chronic lymphoid leukemia
- Drug-induced immune thrombocytopenia: e.g., **heparin-induced thrombocytopenia**

b) NON-IMMUNE CONSUMPTION
- **Thrombotic thrombocytopenic purpura**
- Disseminated intravascular coagulation (DIC)

***Key feature*:** *Low platelets in periphery, increased megakaryocytes in bone marrow*

3. ABNORMAL DISTRIBUTION / SEQUESTRATION
- Hypersplenism due to splenomegaly from: portal hypertension, liver cirrhosis

Key feature: *Low platelets in periphery, normal megacaryocytes, enlarged spleen*

Three types of thrombocytopenia are further detailed.

IDIOPATHIC (IMMUNE) THROMBOCYTOPENIC PURPURA (ITP)

- **Definition**: acquired autoimmune disorder characterized by immune-mediated platelet destruction and isolated thrombocytopenia.
- **Pathogenesis**:
  - **IgG antiplatelet auto-antibodies** formation against surface membrane antigens (GpIIb/IIIa or GpIb) - type II hypersensitivity reaction
  - the antibody-coated circulating platelets are prematurely destroyed via phagocytosis by:
    - the splenic macrophages that have receptors that recognize the Fc fragment of IgG
    - binding complement with C3b opsonization, which enhance phagocytosis by splenic and liver macrophages
  - marked decrease of platelet lifespan (from 7-10 days to 1-2 days or even hours)
- **Manifestations**:
  - ✓ **ACUTE ITP** (most common form in children):
    - occurs in **children** (2-6 years)
    - sudden onset after a viral infection (measles, chickenpox) or vaccination
    - manifestations: mild-moderate cutaneous petechiae/purpura, mucosal hemorrhages
    - course: self-limiting with remission up to 6 months
    - prognosis: favorable, most recover spontaneously or response to corticotherapy
  - ✓ **CHRONIC ITP** (most common form in adults):
    - occurs in **adults** (20-40 years), **women** predominance (W/M ratio: 3/1)
    - insidious onset
    - association with other autoimmune diseases, e.g., SLE, autoimmune thyroiditis

- novel evidence has showed that in many adult patients with ITP bone marrow platelet production is also decreased (besides the increased peripheral destruction) due to low thrombopoietin
- manifestations: recurrent purpura, epistaxis, hematuria, menorrhagia
- course: relapsing, persistent > 6 months to years
- prognosis: variable, chronic disease, risk of death from cerebral hemorrhage

THROMBOTIC THROMBOCYTOPENIC purpura (TTP)

- **Definition:** life-threatening microangiopathy characterized by **widespread occlusion of small vessels by platelet-rich microthrombi** causing organ ischemia and thrombocytopenia due to increased consumption (not increased destruction as in ITP).
- **Pathogenesis**:
  - **decreased plasma level of ADAMTS13** (**A D**isintegrin **A**nd **M**etalloproteinase with **T**hrombospondin Motifs), a metalloprotease responsible for the cleavage of the very large multimers of von Willebrand factor (vWf) into monomers, which can be:
    - **acquired** (most common): **IgG autoantibodies against the ADAMTS13 enzyme**
    - **inherited** (rare): congenital ADAMTS13 gene mutation
    - **persistence of the vWF multimers in the circulation** results in excessive and widespread platelet adhesion and aggregation with 2 cose:
    - formation of **platelet-rich thrombi in the small vessels** from kidney, heart, brain
    - subsequent **fibrin deposition** with occlusion of microcirculation with **hemolytic anemia** and **ischemic organ damage**
  - **normal** coagulation tests (aPTT, PT)
- **Manifestations** - classic **PENTAD**:
  ①**Thrombocytopenia** with: petechiae, purpura and muco-cutaneous bleeding
  ②**Microangiopathic hemolytic anemia** due to fragmentation of erythrocytes when passing through the fibrin network in small vessels with: schistocytes on blood smear, increased indirect bilirubin, jaundice
  ③**Neurological symptoms (may be severe)**: headache, confusion, stroke-like deficits
  ④**Renal dysfunction**: proteinuria, hematuria or even acute kidney injury
  ⑤**Fever**

HEPARIN-INDUCED THROMBOCYTOPENIA

- **Definition:** severe complication of anticoagulant therapy with unfractionated heparin caused by **antibodies formed against heparin–platelet factor 4 (PF4) complexes**, which lead to platelet activation, **thrombocytopenia**, and **thrombosis**.
  It occurs in less than 5% of patients treated with heparin as a 30-50% decrease in platelet number between 5-10 days after the therapy onset, and may evolve as *heparin-induced thrombocytopenia (HIT)* or *heparin-induced thrombocytopenia with thrombosis (HITT).*
- **Pathogenesis:**

– **platelet factor 4 (PF4)** released from the alpha granules **forms with heparin** an **antigenic complex** that trigger an humoral **immune response** with generation of **IgG** against the **heparin-platelet factor 4 (PF4) complex**, with two consequences:

- **IgG-coated complexes** bind to **Fc receptors on platelets** causing **their activation, aggregation** and **consumption** with **thrombocytopenia**
- in HIT – formation of **platelet thrombi** leads to the so-called "*white clot syndrome*"
- in HITT – PF4 interacts with heparin-like subendothelial structures and triggers the **marked activation of coagulation** and increased risk of:
  - ✓ **venous thrombosis**: deep vein thrombosis, pulmonary embolism
  - ✓ **arterial thrombosis:** limb ischemia, myocardial infarction, stroke

Heparin therapy should be stopped and replacing it with another anticoagulant (e.g., argatroban, bivalirudin - direct thrombin inhibitors).

THROMBOCYTOPATHIES

DEFINITION: impaired platelet function with **normal platelet count**

PATHOGENESIS - 3 mechanisms of thrombocytopathies:

1. Platelet **ADHESION** defects
2. Platelet **AGGREGATION** defects
3. Platelet **SECRETION (RELEASE)** defects

1. Platelet ADHESION defects

- **Definition: impaired initial platelet binding to the vessel wall** /adhesion to the subendothelial matrix, at the sites of vascular injury.
- **Causes:**

  a) Bernard-Soulier Syndrome - primary ADHESION defect, with autosomal recessive transmission, caused by the *glycoprotein Ib deficiency*, the *receptor for vWF.* Patients have large platelets and sometimes, mild thrombocytopenia.

  b) Von Willebrand disease - the most frequent inherited bleeding disorder (see below)

2. Platelet AGGREGATION defects

- **Definition: impaired platelet to platelet cohesion**, i.e. the ability of platelets to bind to each other and form aggregates after activation.
- **Causes:**

  a) GLANZMANN thrombasthenia - *primary* AGGREGATION defect, with autosomal recessive transmission, caused by *glycoprotein IIb-IIIa deficiency,* the *receptor for fibrinogen,* which is required for fibrinogen-mediated platelet-platelet cohesion.

  b) DRUG-induced:
  - **ADP receptor antagonists** (selective inhibitors of P2Y12 receptors) - e.g. ticlopidine, clopidogrel, ticagrelor – elicit *secondary* AGGREGATION defect to ADP
  - **platelet fibrinogen receptor inhibitors** (glycoproteins IIb/IIIa inhibitors) - e.g., abciximab, eptifibatide, tirofiban – prevent fibrinogen binding to activated platelets

  c) UREMIA complication – due to *functional inhibition of GPIIb-IIIa* by the uremic toxins

3. Platelet SECRETION (RELEASE) defects

- **Definition: impaired release of the granules content** after activation**,** leading to **reduced or absent secondary aggregation in response to agonists,** despite normal primary aggregation.

- **Causes:**

a) DRUG-induced:

  - **aspirin** - *irreversible* inhibition of cyclooxygenase-1 (COX-1) and thromboxane A2 synthesis – elicits *secondary* aggregation defect by impaired amplification of platelet aggregation; bleeding persists 3-7 days after therapy discontinuation (for the platelet lifespan)
  - **other NSAIDs** - *reversible* inhibition of COX1 during treatment; bleeding ceases upon drug discontinuation.

b) UREMIA complication: ± associated primary aggregation defect

III. CLOTTING FACTORS-RELATED HEMORRHAGIC SYNDROMES / COAGULOPATHIES

CLASSIFICATION:

A. INHERITED/CONGENITAL coagulopathies
1. **von WILLEBRAND DISEASE**
2. **Hemophilia A**
3. **Hemophilia B**

B. ACQUIRED coagulopathies
1. **Hemorrhagic syndromes due to VITAMIN K DEFICIENCY**
2. **Hemorrhagic syndromes due to CHRONIC LIVER DISEASE**
3. **DISSEMINATED INTRAVASCULAR COAGULATION (DIC)**

A. INHERITED/CONGENITAL COAGULOPATHIES

1. von WILLEBRAND DISEASE

- **Definition**: the **most common inherited bleeding disorder** caused by a ***quantitative* or *qualitative* defect in von Willebrand factor (vWF),** a plasma protein with dual role in hemostasis, platelet adhesion and stabilization of coagulation factor VIII (FVIII).
- **Pathogenesis**:
  - vWF is a multimeric glycoprotein produced by the vascular endothelial cells and megakaryocytes that is released into circulation with 2 major functions:
    - ✓ **platelet adhesion** to sites of vascular injury (role in **primary** hemostasis)
    - ✓ **FVIII transport** and **stabilization** (role in **secondary** hemostasis); the defect causes aPTT prolongation due to F VIII dysfunction/deficiency
  - the vWF defect **can be quantitative or qualitative** – the disease encompasses three major subtypes: **type 1** and **type 3** are **quantitative deficiencies**, and **type 2** - **qualitative defects** resulting in various degrees of vWF deficit and FVIII dysfunction
- **Manifestations**:
  - impaired **primary** hemostasis results in *cutaneous and mucosal hemorrhages* with *immediate onset*: easy bruising, epistaxis, menorrhagia, excessive bleeding after surgery and minor trauma
  - impaired **secondary** hemostasis results in *late onset* hemorrhages: bleeding from skin cuts that may stop and restart over hours or deep bleeding into tissues – muscle

hematomas and joint bleeds – *hemarthroses,* which are due to marked FVIII deficiency, similar to hemophilia (and occur in the 3rd and the most severe type of disease)

- **Laboratory: normal** platelets count, **prolonged** aPTT, and **normal** PT

2. Hemophilia A

- **Definitions**: an X-linked recessive congenital bleeding disorder characterized by **deficiency/dysfunction** of **coagulation factor VIII** due to mutations of the F8 gene.
- **Pathogenesis**:
  - the F8 gene is on the X chromosome, and mutations affect FVIII synthesis or function
  - males (XY) are typically affected and females (XX) are asymptomatic or symptomatic carriers
  - FVIII is synthesized by hepatocytes (mainly) and vascular endothelial cells and circulates bound to vWF, which stabilizes it
  - **FVIII insufficient activity** disrupts the **intrinsic pathway of coagulation**, leads to impaired thrombin generation and defective stable fibrin clot formation resulting in **bleeding** whose severity is correlated with the residual plasma activity of FVIII
  - thus, hemofilia can be: **mild** (5% to 40%), **moderate** (1–5%), and **severe** (<1% activity)
- **Manifestations**:
  - since the **primary** hemostasis is **normal, petechiae and purpura are absent**
  - impaired **secondary** hemostasis leads to **deep hemorrhages into soft tissues**, **muscles** and **joints** (ankles, knees, elbows), such as:
    - ✓ subcutaneous/intramuscular **hematomas**
    - ✓ **recurrent hemarthroses** with **synovitis**, leading to fibrosis, joint deformation/destruction and disability, known as **hemophilic arthropathy** are the **hallmark clinical manifestations**
    - ✓ hematuria, gastrointestinal bleeding and intracranial hemorrhages (life-threatening)
  - a typical feature is **delayed and persistent bleeding after trauma and surgery**
  - **severe hemophilia** typically causes **severe bleeding throughout life,** beginning in early childhood (but may be evident immediately after birth, e.g., scalp hematoma after delivery) with **frequent spontaneous** bleeding and **after minor trauma**, **surgery/dental procedures**
  - **moderate hemophilia** presents with less **frequent spontaneous bleeding and abnormal bleeding after minor trauma**
  - **mild hemophilia** manifests as **excessive bleeding only after significant trauma** or surgery and is frequently diagnosed late in life
- **Laboratory: normal** platelet count, **prolonged** aPTT and **normal** PT, **factor VIII assay** determine the type and severity of the disease

3. Hemophilia B

- **Definition:** a rare X-linked recessive congenital bleeding disorder characterized by **deficiency/dysfunction** of **coagulation factor IX** caused by mutations in the F9 gene (also on the X chromosome).
- **Pathogenesis:** insufficient **factor IX activity** in the intrinsic pathway of the coagulation impairs thrombin generation and formation of a stable fibrin clot resulting in bleeding
- **Manifestations:** similar to those of hemophilia A, but less frequent

- **Laboratory: normal** platelet count, **prolonged** aPTT and **normal** PT, **factor IX assay** determine the type and severity of the hemophilia B (same degrees as in Hemophilia A).

B. ACQUIRED COAGULOPATHIES

1. Hemorrhagic syndromes due to VITAMIN K DEFICIENCY

- **Definition**: a group of bleeding disorders collectively termed **vitamin K deficiency bleeding (VKDB)** characterized by **defective coagulation due to insufficient activity of vitamin K-dependent coagulation factors II (prothrombin), VII, IX, X**, which is correctable by vitamin K administration.
- **Pathogenesis:**
  - the vitamin K sources are *primarily the diet*, mainly the green leafy vegetables, which are rich in vitamin K_1 (phylloquinone) and *secondary, synthesis by intestinal bacteria*, which produce vitamin K_2 (menaquinone) as an inactive form, requiring activation by the enzyme epoxide-reductase in the liver
  - vitamin K is required for the γ-carboxylation of glutamic acid residues in the structure of **vitamin K-dependent coagulation factors: II, VII, IX** and **X** and **proteins C and S** in order to become *functional* (the residues of γ-carboxyglutamic acid bind Ca^{2+} ions)
  - in vitamin K deficiency, the **impaired γ-carboxylation** leads to the production of **non-functional coagulation factors,** defective hemostasis and bleeding
- **Etiology**:

  1. In **newborns/infants**, VDKB is **frequent** due to:
  - poor placental transfer of vitamin K (liposoluble)
  - during the first few days of life the neonatal gut is sterile
  - immaturity of the neonatal liver with respect of the vitamin K-dependent factors synthesis
  - low vitamin K stores in the liver
  - low vitamin K content in breast milk (occurs mainly in in exclusively breastfed infants in the absence of prophylaxis)

  According to the age of the onset, the clinical spectrum in neonates include: *early, classic, and late VKDB* and manifestations comprise: bleeding from the umbilical stump or the puncture sites, epistaxis, hematuria, gastrointestinal bleeding, and intracranial hemorrhage (life-threatening).

  2. In **adults**, VDKB is less frequent, with deficiency resulting from:
  - **Decreased intake or synthesis** in:
    - poor intake in restrictive diets, malnutrition (rare)
    - prolonged use of broad-spectrum antibiotics that disrupt intestinal flora and reduce vitamin K2 synthesis
  - **Decreased absorption** in conditions that **impair fat absorption** (vit. K - liposoluble):
    - cholestatic liver diseases: obstructive jaundice, primary biliary cholangitis
    - chronic pancreatitis and cystic fibrosis
    - celiac disease and Crohn disease
  - **Impaired utilization** – **functional deficiency** in:

  - vitamin K antagonists – coumarin derivatives and warfarin inhibit the vitamin K epoxide reductase complex resulting in *functional deficiency*
  - chronic liver disease – decreased hepatic storage and thus, utilization
- **Laboratory:**
  - **prolonged PT** or **INR** (factor VII drops early) – the **most sensitive test**
  - **normal** aPTT initially, may become abnormal in evolution/severe forms
  - **low activity levels of vitamin K–dependent factors (II, VII, IX, X),** with normal levels of other factors, e.g., factor V and fibrinogen – differential diagnosis with liver failure where all clotting factors are usually reduced
  - **normal** platelet count, **normal** fibrinogen – differential diagnosis with DIC where thrombocytopenia and hypofibrinogenemia are common

Key feature: rapid correction of PT (and aPTT, if prolonged) after vitamin K administration by the Koller test or vitamin K correction test – PT corrects or significantly shortens after parenteral vit. K (and does not correct in liver disease)

2. Hemorrhagic syndromes due to CHRONIC LIVER DISEASE

- **Pathogenesis**: **multifactorial,** comprising:
  - decreased synthesis of all *coagulation factors* (except factor VIII), *soluble anticoagulants (proteins C, S, ATIII*) and *fibrinolytic proteins* (plasminogen)
  - impaired primary hemostasis due to *acquired defects in platelet adhesion and aggregation*
  - portal hypertension induces:
    - ✓ *splenomegaly and hypersplenism* with platelet sequestration ⇒ *thrombocytopenia*
    - ✓ *esophageal varices* prone to rupture ⇒ risk for *major gastrointestinal hemorrhages*
  - *decreased clearance of activated clotting factors* with risk of *DIC* in severe liver failure

Key feature: unstable balance between coagulation and fibrinolysis, increased risk for hemorrhagic and thrombotic complications

3. DISSEMINATED INTRAVASCULAR COAGULATION (DIC)

- **Definition**: an acquired, life-threatening syndrome characterized by **primary activation of coagulation, secondary activation of fibrinolysis, and systemic endothelial injury**, causing various degrees of both **thrombosis and bleeding.**
- **Pathogenesis:**
  - the **hallmark** of CID is **excessive, uncontrolled and widespread generation of thrombin in the circulation**, primarily initiated by *tissue factor expression* on the endothelial cells and activated monocytes
  - the consequence is the **diffuse fibrin deposition in the microcirculation**, resulting in widespread **microthrombi formation** and organ dysfunction
  - **primary activation of coagulation** is responsible for the **consumption of platelets and coagulation factors** – hence the term **consumption coagulopathy**, and **secondary activation of fibrinolysis** contribute to the bleeding diathesis
  - **endothelial injury** contributes to both thrombosis and hemorrhage
- **Etiology**:

DIC always results from an underlying disorder that **firstly** triggers **systemic coagulation activation**:

- ✓ **Tissue factor expression/release** that causes activation of the **extrinsic pathway** in:
  - **obstetric syndromes**: amniotic fluid embolism, placental abruption, retained dead fetus
  - **malignancies**: adenocarcinomas of stomach, lung, breast, prostate, pancreas,
  - **acute promyelocytic leukemia**
  - **severe ischemia**: acute myocardial infarction, acute pancreatitis
  - **shock** of any etiology
  - **major trauma** with severe tissue damage: burns, head trauma
- ✓ **Endothelial lesions** that cause activation of the **intrinsic pathway** in:
  - **severe infections**, e.g. **bacterial sepsis** with Gram-negative germs (e.g., meningococci) that secret endotoxins responsible for *endothelial injury*.
    Sepsis-related DIC has a **multifactorial pathogenesis**: *acute inflammatory response* induces tissue factor expression on monocytes and endothelial cells with *activation of extrinsic pathway*, *downregulates anticoagulant systems* (protein C, ATIII) and the *neutrophil extracellular traps (NETs)* provide a scaffold for fibrin deposition and *microthrombi formation/propagation*
  - **severe intravascular hemolysis** due to increased release of RBC microparticles that provide the phospholipid surface required to amplify thrombin generation

- **Laboratory:**
  a) **PRIMARY activation of coagulation** results in:
     - **thrombocytopenia** due to platelet consumption in microthrombi
     - **prolonged aPTT, PT, and marked decrease in fibrinogen and all clotting factors** due to their consumption (decreased FVIII is useful for the differential diagnosis with severe liver disease, where FVIII is normal)
     - **schistocytes on the blood smear** due to microangiopathic hemolytic anemia (mechanical disruption of RBC when passing through the fibrin network)
  b) **SECONDARY activation of fibrinolysis** results in:
     - plasmin-mediated degradation of the fibrin polymer with **increased D-dimers level**

HYPERCOAGULABILITY SYNDROMES (EXAGGERATED HEMOSTASIS)

DEFINITION: an heterogenous group of diseases associated with an **increased tendency toward thrombosis** due to an imbalance between procoagulant and anticoagulant mechanisms.

The risk of thrombosis is usually the result of gene-environment interaction. Most **inherited thrombophilias are associated with venous thrombosis**, while **acquired hypercoagulability syndromes** may lead to **either venous or arterial thrombosis** depending on the underlying risk factors.

CLASSIFICATION:

A. INHERITED hypercoagulability syndromes or THROMBOPHILIAS

B. ACQUIRED hypercoagulability syndromes

INHERITED HYPERCOAGULABILITY SYNDROMES / THROMBOPHILIAS

- **Definition**: conditions associated with **genetic defects that impair the quality or quantity of proteins involved in coagulation**, and primarily increase the risk of **VENOUS thrombosis** and embolism.
- **Pathogenesis**:
  Inherited thrombophilias result from mutations that determine the:
  1. Increased activity of procoagulant factors
  2. Loss of function of soluble anticoagulants

1. Increased activity of procoagulant factors – is the pathomechanism underlying the:

a) Factor V Leiden or activated protein C resistance:

- factor V Leiden is **the most common inherited thrombophilia**
- mutation in the factor V gene results in a **variant of factor V protein that is resistant to inactivation by activated protein C (APC)**, known as **Factor V Leiden**
- normally, APC inactivates FVa by proteolytic cleavage at a specific AA, while in the presence of mutation, APC cannot inactivate anymore FVa
- the prolonged activity of FVa results in increased thrombin generation, and a persistent procoagulant state, and increased risk of **deep venous thrombosis** and **pulmonary embolism**
- most individuals with the mutation remain asymptomatic
- the thrombotic risk is higher in homozygotes than heterozygotes and increases in the presence of additional acquired risk factors, e.g., surgery, pregnancy, estrogen therapy
- in affected women there is an increased risk of pregnancy complications, such as recurrent miscarriage

2. Loss of function of soluble anticoagulants – is the pathomechanism underlying the:

a) Antithrombin III (ATIII) deficiency

- ATIII is the main physiological inhibitor of thrombin and factor X
- gene mutations lead to either a quantitative or qualitative deficiency
- reduced level or decreased function of ATIII impairs the inhibition of thrombin and factor Xa, resulting in excessive thrombin generation, and a markedly increased risk of venous thromboembolis
- **acquired ATIII deficiency** can occur in conditions such as **nephrotic syndrome** (increased urinary loss) and **liver disease** (decreased synthesis)
- because ATIII is necessary for the action of heparin, people with ATIII deficiency may be relatively resistant to heparin

b) Protein C or protein S deficiency

- hereditary or acquired thrombophilias caused by **reduced levels or impaired function of protein C, and/or its cofactor, protein S** (both vitamin K–dependent)
- proteins S and C are soluble, circulating anticoagulants, which act as the physiological inhibitors of factors Va and VIIIa, and IXa respectively
- the pathogenesis involves *gene mutation* (hereditary from) or *decreased synthesis* due to vitamin K deficiency, liver disease, pregnancy, inflammation (acquired form), resulting in impaired anticoagulant activity and increased thrombin generation

- manifestations range from asymptomatic to recurrent venous thromboembolism, (deep vein thrombosis and pulmonary embolism) in homozygotes

ACQUIRED HYPERCOAGULABILITY SYNDROMES

- **Definition**: conditions in which acquired (non-genetic) factors disrupt the balance between procoagulant and anticoagulant mechanisms, leading to a increased tendency for VENOUS or ARTERIAL thrombosis.

- **Classification:**
  A. ARTERIAL Thrombosis
  B. VENOUS Thrombosis

Thrombosis occurs as a result of the interaction between the **3 factors** that constitute the **Virchow triad**:

1. **Changes** in **blood flow: stasis or turbulence**
2. **Vascular wall dysfunction/damage**
3. **Hypercoagulability**

The interplay among these factors is critical: for example, stasis and endothelial dysfunction can induce a hypercoagulable state, while hypercoagulability can amplify the effects of stasis or vascular injury. The importance of the components of the Virchow triad differs in arterial and venous thrombosis:

- **Turbulent flow** and **damage to the vascular wall**, produced by atheromatous plaques, are factors favoring **ARTERIAL thrombosis**
- **Stasis** and **hypercoagulability** are the primary factors favoring **VENOUS thrombosis**

ARTERIAL THROMBOSIS

- **Definition:** formation of thrombi within the arterial system, typically resulting in partial or complete obstruction of blood flow and tissue ischemia.
- **Type of thrombi:** arterial thrombi are typically "**white thrombi"**, composed **predominantly of platelet aggregates** interspersed with few thin strands of fibrin due to their formation under *high-flow* conditions.
- **Etiology**: **atherosclerosis** whose progression is accelerated by the co-existence of:
  - hyperlipidemias
  - diabetes mellitus
  - smoking
  - advanced age
- **Pathogenesis**:
  - the central pathomechanism is represented by the **erosion of an atherosclerotic plaque**, which exposes subendothelial collagen and tissue factor to circulating blood
  - the platelet adhesion, activation, and aggregation occurs rapidly under high shear conditions / turbulent blood flow together with the activation of the coagulation via both the extrinsic and intrinsic pathways, leading to thrombin generation and fibrin deposition
  - non-occlusive thrombi will be incorporated into the vessel walls, thus accelerating the atherosclerotic plaque growth, while occlusive thrombi will trigger the complications

- **Complications:**
  - **thrombosis** on the atheromatous plaque with:
    - *partial* occlusion ⇒ unstable angina
    - *total* occlusion ⇒ acute myocardial infarction
  - remote **embolization,** especially in the **cerebral** vasculature with:
    - *temporary* ischemia ⇒ transient ischemic attack (TIA)
    - *permanent* ischemia ⇒ stroke

VENOUS THROMBOSIS

- **Definition:** formation of thrombi within the venous system, most commonly manifesting as deep vein thrombosis or pulmonary embolism when the thrombus embolizes to the lungs.
- **Type of thrombi**: venous thrombi are typically **"red thrombi"** composed predominantly of **fibrin** and trapped red blood cells with variable amounts of platelets due to their formation under *low-flow* conditions.
- **Etiopathogenesis**:
  Stasis and **hypercoagulability** are the main factors favoring **VENOUS thrombosis.**
  Venous blood stasis allows the accumulation of activated coagulation factors in the deep veins of the lower extremities or pelvis, and is favored by:
    i) prolonged immobilization:
    - after a major surgical intervention on the lower limbs or pelvis
    - absolute bed rest > 3 days (e.g., plaster cast)
    - recent long-distance travel (sitting in a chair, duration > 4 hours)

    ii) obesity (BMI over 30 kg/m^2)
    iii) pregnancy and the postpartum period
    iv) cardiac diseases - congestive heart failure, atrial fibrillation, prosthetic valves

  Hypercoagulability is favored by:
    i) increased release of tissue factor/production of procoagulant factors in:
    - **cancers**
    - **antiphospholipid syndrome**

    ii) increased resistance to circulating anticoagulant factors - in:
    - administration of estrogens (hormonal contraceptive combinations, oral hormone therapy)

    iii) low-grade chronic inflammation with the release of pro-inflammatory cytokines (IL-1, IL-6, TNF-alpha) in:
    - inflammatory diseases, e.g. inflammatory bowel disease, SLE
    - COVID-19 (where venous and arterial thrombosis are associated)
    - old age ("inflammaging")
- **Complications**: remote embolization especially in the **lungs** ⇒ pulmonary embolism

4. PATHOPHYSIOLOGY OF THE GASTROINTESTINAL SYSTEM

DISORDERS OF THE ESOPHAGUS AND STOMACH

DYSPEPSIA

DEFINITION: the feeling of discomfort associated with the act of eating, consisting of the appearance of one or more of the following *symptoms*:
- postprandial abdominal fullness/distension
- early satiety
- retrosternal pain/heartburn

CLASSIFICATION:

A. FUNCTIONAL dyspepsia – represents 70-80% of cases
- **Definition**: dyspepsia lasting **over 3 months** (not necessarily consecutive) **in the past year, in the absence of an organic** (on endoscopic examination) **or metabolic disease** underlying the symptomatology.
- **Clinical forms** – there are 2 types:
  i) Dysmotility/dyskinesia functional dyspepsia
  Manifestation: *postprandial discomfort* – patients mainly present: *postprandial fullness (or epigastric heaviness), early satiety, eructations*
  ii) Ulcerative functional dyspepsia
  Manifestation: *epigastric pain* – patients mainly present: *pain, painful hunger, heartburn*
- **Etiopathogenesis** – incompletely elucidated
  - **Dysmotility functional dyspepsia** – involves:
    - delayed gastric evacuation
    - psychological factors
  - **Ulcerative functional dyspepsia** – involves:
    - Helicobacter pylori infection
    - presence of a hypersecretory status

B. ORGANIC dyspepsia – represents 20 - 30% of cases
- **Definition**: **dyspepsia determined by the presence of an *organic* condition** evident on endoscopic examination that associates discomfort at the level of *upper* abdomen, "alarm" manifestations such as: dysphagia, vomiting, upper digestive bleeding (UDB, hematemesis or melena), anorexia, weight loss.
- **Etiopathogenesis**:
  - ***Main causes:***
    iv) Eso-gastric diseases:
    - Gastroesophageal reflux disease (GERD) with or without esophagitis
    - Peptic ulcer
    - Gastric/esophageal cancer

    v) Other digestive diseases:
    - Gallstones
    - Chronic pancreatitis/pancreatic cancer

– ***Secondary causes:***
  vi) Gastroparesis: in diabetes mellitus
  vii) Intestinal parasites: giardiasis, oxyuriasis
  viii) Medications: non-steroidal and steroidal anti-inflammatory drugs, iron preparations, antiosteoporotic drugs

Observation!
In a broader sense, the term dyspepsia (indigestion) also includes:
– food intolerance/allergy associated with nausea, vomiting
– gaseous dyspepsia: belching, abdominal bloating, bloating, flatulence

DYSPHAGIA

- **Definition**: the feeling of difficulty swallowing food
- **Classification** - according to etiology:

A. ESOPHAGEAL dysphagia
i) functional - by alteration of esophageal motility
ii) organic - by stenosis of the esophageal lumen by: strictures, tumors, Schatzki esophageal ring

B. EXTRA-ESOPHAGEAL Dysphagia
1. Oropharyngeal dysphagia:
– inflammatory/tumor lesions of the oral cavity: tonsillitis, pharyngitis, glossitis, lingual carcinoma
– Zenker diverticulum (inferior pharyngeal)
2. Dysphagia associated with mediastinal pathology (extrinsic compression):
– tumors and mediastinal adenopathies
– plunging thyroid goiter
– mitral valve diseases with left atrial dilation
3. Dysphagia associated with other conditions:
– collagenoses: scleroderma, rheumatoid arthritis
– diabetes mellitus (DM) with neuropathy
– neurological disorders: Parkinson's disease, myasthenia gravis
– iron deficiency anemia (rare, Plummer-Vinson syndrome)

- **Pathogenesis**

According to the **mechanism** of dysphagia, we distinguish:
a) MECHANICAL (obstructive) dysphagia - manifests itself for solids
– **Cause**: *reduction of the pharyngo-esophageal lumen* of an organic nature by:
  o foreign bodies
  o intrinsic narrowing: inflammations, strictures, diverticuli
  o extrinsic compression: tumoral mediastinal mass, dilated left atrium

b) MOTOR (neuromuscular) dysphagia - manifests itself predominantly for liquids
– **Causes:**
  o *reduction or lack of coordination of peristaltic contractions*
  o *impairment of the relaxation of the esophageal sphincter* in:
    ✓ difficulty initiating the swallowing reflex from:

- damage to the swallowing center in the brainstem: stroke, intoxications, coma
- hyposalivation

✓ pharyngo-esophageal neuromuscular diseases in:
- *striated* muscle damage: myopathies, myasthenia gravis, poliomyelitis
- *smooth* muscle damage: achalasia, scleroderma, diabetes

ESOPHAGEAL DISORDERS

ACHALASIA

- **Definition**: **MOTOR** disease of the esophageal *smooth* muscle characterized by **progressive DILATION of the esophageal body** (radiological image of "bird's beak")
- **Etiology**: viral, autoimmune and neurodegenerative causes
- **Pathogenesis**: incompletely elucidated, incriminated factors being:
  - **pathological increase in tone (hypertonia) of the lower esophageal sphincter (LES)** at rest and/or **incomplete relaxation of the LES** during swallowing
  - **absence of normal peristalsis of the esophageal body** due to *defective innervation of the esophageal smooth muscle* (in the lower 2/3) determined by:
    - ✓ inflammation of the myenteric plexus (Auerbach) and decrease in the no. of ganglia
    - ✓ selective loss of nitrergic neurons containing nitric oxide synthase (NOS) with an inhibitory role on intestinal peristalsis

Observation!
The symptomatology is similar to that of Chagas disease - infection with Trypanosoma cruzi, a neurotropic pathogen that causes the destruction of the myenteric plexus.

- **Clinical manifestations:**
  - dysphagia for liquids and solids (from the onset of the disease)
  - postprandial regurgitation in the supine position
  - odynophagia
- Complications:
  - episodes of nocturnal aspiration of regurgitated gastric contents ⇒ risk of aspiration pneumonia
  - spontaneous retrosternal pain (occasional esophageal spasm) ⇒ differential diagnosis with angina pain
  - erosions and ulcerations of the esophageal mucosa ⇒ risk of bleeding
  - progressive dilatation of the esophagus with worsening symptoms ⇒ malnutrition and weight loss

GASTRO-ESOPHAGIAN REFLUX DISEASE (GERD)

- **Definition**: condition determined by recurrent reflux of gastric contents into the lower esophagus, common in Western countries (with a prevalence of 10-20%)
- **Pathogenesis**:

1. PRIMARY impairment of LES function by:

- **permanent decrease in basal LES tone** (hypotonia) – the *opposite of achalasia*
- repeated episodes of **transient relaxation**

2. SECONDARY impairment of LES function – hypotonia, determined by:

- increased intra-abdominal pressure: obesity, pregnancy, massive ascites
- symptomatic hiatal hernia
- scleroderma
- medications: anticholinergics, calcium channel blockers, nitrates
- dietary factors that:
  - decrease LES tone: chocolate, coffee, menthol, alcohol, fats
  - cause hyperacidity: carbonated drinks, tomato juice, citrus fruits
- smoking
- stress

- **Clinical manifestations:**

A. DIGESTIVE manifestations:

- **Heartburn and acid regurgitation**: the main symptoms, which must be present at least 2 times a week for 3-4 weeks for a positive diagnosis of GERD.
  Heartburn characteristics:
  - ✓ onset 30-60 minutes postprandial
  - ✓ worsening in the supine position/at night and with the ingestion of spicy, hot foods, alcohol
  - ✓ transient relief with antacids/fluid ingestion
  - ✓ not correlated with the severity of mucosal lesions
- **Dysphagia** (difficult swallowing)
- **Odynophagia** (painful swallowing)

B. EXTRADIGESTIVE manifestations:

- **Chronic cough**
- **Dysphonia (hoarseness)**
- Sensation of a pharyngeal foreign body ("lump in the throat")
- Pharyngodynia
- Tracheobronchitis
- Asthma
- Retrosternal pain (differential dg. with angina pain)

- **Complications:**
  - **Reflux esophagitis**: moderate inflammation/hyperemia of the mucosa, hemorrhagic ulcerations and benign fibrous strictures
  - **Reflux laryngitis**: chronic hoarseness + irritating dry cough
  - **Barrett's esophagus**: complication of ***chronic*** gastroesophageal reflux
    - ✓ replacement of normal squamous epithelium of the lower esophagus with its transformation into columnar epithelium secondary to chronic inflammation
    - ✓ requires repeated endoscopies to identify dysplastic, *low*- or *high*-grade lesions or incipient neoplasia
    - ✓ is a precancerous condition $\Rightarrow$ risk of adenocarcinoma

HIATAL HERNIA

- **Definition**: herniation of the upper portion of the stomach through the diaphragm into the chest
- **Classification**:
  1. **Hiatal hernia type I, by SLIDING**: *sliding of the gastroesophageal junction into the chest through the esophageal hiatus* – is the **most common form**, caused by:
     - congenital short esophagus
     - abdominal trauma
     - weakening of the diaphragm at the eso-gastric junction (e.g., increased intra-abdominal pressure in obesity, third trimester of pregnancy)
     - complication: reflux esophagitis due to *frequent association with GERD*
  2. **Hiatal hernia type II, PARAESOPHAGEAL** = herniation/prolapse of the *stomach* into the chest through a weakened phreno-esophageal ligament, *but with intra-abdominal preservation of the gastroesophageal junction*
     - complication: risk of acute hernia incarceration with ischemia and necrosis (rare)

Observation!
In type III hiatal hernia, both the gastroesophageal junction as well as the stomach migrate into the chest.
Type IV is type III in which other abdominal viscera (colon, spleen) migrate into the chest.

GASTRO-DUODENAL DISORDERS

GASTRITIS

1. ACUTE gastritis

- **Definition**: acute inflammation of the gastric mucosa manifested by:
  - **hyperemia**, edema and *moderate* infiltrate with *neutrophils* in the lamina propria - in *mild* forms
  - **superficial erosions**, *abundant* infiltrate with *neutrophils* and punctate hemorrhages of the mucosa - in *severe* forms, responsible for acute *erosive* gastritis
- **Etiology**:
  - massive exposure to chemical irritants: caffeine, alcohol, NSAIDs or biological: **Helicobacter pylori**, herpes virus
  - acute ischemia of the gastric mucosa: severe infections, polytrauma, shock, surgical interventions, severe stress, burns (vasoconstriction + hypoxia of the gastric mucosa)
  - after chemo- or radiotherapy
  - in portal hypertension from liver cirrhosis - *portal gastropathy*
- **Complications:**
  - severe hemorrhage
  - deep ulceration with risk of perforation

2. CHRONIC gastritis

- **Definition**: chronic inflammation of the gastric mucosa manifested by inflammatory infiltrate with *monocyte-macrophages* and *lympho-plasmacytes* and over time, atrophy of the gastric mucosa
- **Classification** (Tab. 2.):
  - chronic **active, infectious** gastritis - type B (frequent)
  - chronic **autoimmune, atrophic** gastritis - type A (rare)

Table 2. Presentation of the 2 types of chronic gastritis.

	Chronic ACTIVE gastritis type B	**Chronic ATROPHIC gastritis type A**
Frequency	95% of cases	5% of cases
Localisation	Gastric antrum	Gastric body/fundus
Pathogenesis	Infectious	Autoimmune
Acid production	Increased (but may decrease in evolution)	Decreased
Serology	Anti-H.pylori antibodies	Anti-gastric parietal cell antibodies (common) and anti-IF (rare)
Complications	Peptic ulcer Adenocarcinoma, B-cell lymphoma	Gastric atrophy, pernicious anemia Adenocarcinoma, carcinoid tumors
Associations	Smoking, alcoholism, chronic use of NSAIDs, responsible for gastropathy*	Autoimmune diseases: type I diabetes, Hashimoto's thyroiditis, Graves-Basedow disease

*Gastropathy is defined as changes in epithelial cells with their regeneration, in the absence of inflammation.

a) ACTIVE (INFECTIOUS) chronic gastritis – type B

- **Characteristics**:
  - the most common form
  - the infection has a high prevalence in developing countries (80-90% compared to only 20-50% in developed countries) being associated with low socio-economic status, being acquired in childhood under poor hygiene conditions (and very rarely in adulthood)
  - located in the **gastric antrum**
  - **infectious** pathogenesis proven by colonization with *Helicobacter pylori*
  - over time, in some patients, **antral** gastritis can evolve into **multifocal atrophic gastritis or pangastritis** characterized by:
    - ✓ concomitant damage to the gastric body and fundus
    - ✓ reduced acid secretion
    - ✓ intestinal metaplasia with increased risk of gastric cancer
- **Helicobacter pylori** is a Gram-negative, spiral, flagellated bacteria that:
  - is **typically** located in the **gastric antrum** where it causes ***chronic inflammation*** mediated by the *local* release of **IL-8, IL-1** with **chronic gastritis**
  - only 15% of infected people develop peptic ulcer in the presence of risk factors: smoking, increased bacterial virulence (85% remain asymptomatic throughout life)
  - its **virulence** is determined by its ***structural*** and ***secretory characteristics***:

- ✓ presence of **flagella** ⇒ ensures mobility and penetrability at the level of the antral mucus layer
- ✓ secretion of **adhesins** ⇒ molecules that increase the adhesion of the bacteria to the gastric epithelial cells
- ✓ **urease** with the generation (from endogenous urea) of ammonia and CO_2 ⇒ with 2 effects: i) alkalization of the antral mucosa that allows the survival of the bacteria under acidic gastric pH conditions and ii) direct cytotoxic effect
- ✓ **secretion of enzymes**: *proteases, mucinase* that alter the mucus layer and *phospholipases* that damage the gastric epithelial cells ⇒ through both effects the risk of developing *peptic ulcer* increases
- ✓ **secretion of exotoxins**: *cytotoxin associated with gene A* (CagA) and *vacuolating cytotoxin A* (VacA) which have been associated with: i) increased inflammatory response (mediated by IL-8) and ii) exaggerated immune response ⇒ risk of developing *gastric adenocarcinoma* and *MALT (Mucosa Associated Lymphoid Tissue) type gastric B-cell lymphoma*

b) ATROPHIC (AUTOIMUNE) chronic gastritis – type A

- **Characteristics:**
  - rare form
  - localized in the **body and fundus of the stomach**
  - **autoimmune** pathogenesis proven by:
    - ✓ presence of **diffuse lymphocytic infiltrate**: *T lymphocytes have a toxic effect on parietal cells* (secreting hydrochloric acid and intrinsic factor) and *chief cells* (secreting pepsinogen); the destruction of the 2 cell types will lead to:
      - **IF deficiency** ⇒ decreased *absorption of vitamin B_{12}* ⇒ pernicious anemia
      - **hypo-/achlorhydria** which will stimulate:
        - G cell hyperplasia ⇒ *hypergastrinemia*
        - "enterochromaffin-like" endocrine cell hyperplasia ⇒ risk of neuroendocrine or carcinoid tumors (rare)
    - ✓ presence in the patients' serum of **IgG antibodies against gastric parietal cells** (non-specific, but frequent) and **anti-intrinsic factor** (specific, but rarer)
    - ✓ **association with other autoimmune diseases**: Hashimoto's thyroiditis, type I diabetes, rheumatoid arthritis, Addison's disease

PEPTIC ULCER

DEFINITION: chronic, recurrent condition that appears as a **well-defined ulceration** (loss of substance) in the gastric and duodenal mucosa that **goes beyond the muscularis mucosa** in depth (as opposed to erosions that are limited to the mucosa)

PATHOGENESIS: the appearance of ulceration is the consequence of an imbalance characterized by:

- **increase in luminal aggression factors** - the main mechanism in **duodenal ulcer**
- **decrease in protective factors** of the gastro-duodenal mucosa - the main mechanism in **gastric ulcer**

A. DUODENAL ULCER

- **Definition**: chronic, recurrent condition that appears as a well-defined ulceration, most frequently in the ***duodenal bulb***
  The disease affects approximately 10% of the adult population and is 2-3 times more common than gastric ulcer.
- **Etiopathogenesis**:
  1. **Chronic infection with Helicobacter pylori - is the main cause of ulcers in developing countries** (where the prevalence of the infection is high)
  2. Gastric acid hypersecretion
  3. Acid overload of the duodenal bulb
  4. Associated factors/comorbidities that favor ulcers

1. Chronic infection with HELICOBACTER PYLORI

- is currently present in 50-75% of patients (in the past it was 100%), the explanation being related to the decrease in the prevalence of ulcers associated with H. pylori infection and the increase in the prevalence of ulcers associated with the use of NSAIDs
- is responsible for the *recurrence of painful episodes*
- eradication of the infection is *mandatory* for ulcer healing

2. Gastric acid hypersecretion - is determined by:

a) Increase in the mass of parietal cells:

- primary, due to genetic predisposition
- secondary, through hypergastrinemia (gastrin has a trophic effect on parietal cells)

b) Hypergastrinemia – results from the alteration of the negative feedback mechanism of gastrin secretion inhibition by gastric acidity, colonization with H. pylori causing alkalization of the antral area

c) Vagal hypertonicity – increases acid secretion through 2 mechanisms:

- directly, by stimulating M3 muscarinic receptors
- indirectly, by stimulating G cells with gastrin release

3. ACID overload of the DUODENUM:

- is determined by the increase in the evacuation speed of gastric chyme, with the overcoming of the buffering capacity of gastric chyme acidity by alkaline intestinal juices (duodenal and pancreatic juice)
- is responsible for *gastric metaplasia* in the duodenal mucosa which will favor bacterial grafting (H. pylori has selective tropism for the gastric mucosa)

4. Factors ASSOCIATED with the increased incidence of duodenal ulcers are:

a) Genetic factors – ulcers occur more frequently in:

- first-degree relatives of ulcer patients (familial forms of ulcers)
- subjects with blood type 0

b) Smoking – is responsible for the **decrease in response to treatment** by:

- favoring the chronicity of H. pylori infection
- inhibition of pancreatic bicarbonate secretion

c) Comorbidities associated with peptic ulcers:
- *COPD*: hypercapnia determines chlorhydro-peptic hypersecretion
- *Liver cirrhosis*: stasis in the portal circulation determines hypoxia + altered mucosal trophic function + local hypercapnia
- *Zollinger-Ellison syndrome*: hypergastrinemia determines chlorhydro-peptic hypersecretion
- *Chronic pancreatitis*: decreased bicarbonate-rich exocrine secretion is responsible for reducing the buffering capacity of the acidic gastric chyme
- *Chronic kidney disease and hyperparathyroidism*: associated hypercalcemia stimulates gastrin secretion

d) Psychological factors: chronic stress and anxiety

B. GASTRIC ULCER

- **Definition**: chronic, recurrent condition that appears as a well-defined ulceration, most frequently located on the ***lesser curvature of the stomach***
- **Pathogenesis**: the major role is played by the **decrease in the resistance of the gastric mucosa** to **chlorhydro-peptic aggression** due to the **increased use of non-steroidal anti-inflammatory drugs** (NSAIDs) – the **main cause of ulcers in the elderly population in developed countries.**

PROTECTIVE FACTORS OF THE GASTRIC MUCOSA - Brief physiological overview:

a) Tight junctions between gastric epithelial cells that create a "barrier" of gastric mucosa to which is added the increased cell regeneration

b) Mucus and bicarbonate secretion
Gastric mucus creates an adherent gel film that:
- retains HCO_3^- ions ensuring a pH of 6-7 at the surface of the gastric epithelium (protection against the corrosive action of hydrochloric acid)
- also offers protection against the proteolytic action of pepsin
- mixes with the gastric contents having a lubricating role

c) Prostaglandin (PG) secretion that has a cytoprotective role through:
- vasodilating effect – trophic role at the level of gastric epithelial cells
- increased mucus and HCO_3^- secretion

d) Optimal blood flow at the level of the gastric mucosa

Decreased GASTRIC MUCOSA RESISTANCE in ulcer:

- **Causes**:

a) Alteration of the protective mucus layer by:
- reduction of mucus secretion in chronic gastritis
- depolymerization of glycoprotein subunits due to H. pylori infection

b) Damage to the gastric mucosal "barrier" - determined by:
- **Increased consumption of nonsteroidal anti-inflammatory drugs (NSAIDs)** - causes *direct toxic effects* on the gastric mucosa & *inhibition of cyclooxygenase* (COX) with decreased PG synthesis with a vasodilating and cytoprotective effect on the mucosa $\Rightarrow$ risk of hemorrhages
- **Helicobacter pylori infection** - causes:
  - damage to *foveolar cells* - decreased mucus secretion
  - *direct damage to epithelial cells* by the enzymes and cytotoxins released

Observation!
There are 2 isoforms of COX: i) *COX-1 is the constitutive isoform*, ubiquitously present physiologically and ii) *COX-2 is the inducible isoform* at the level of areas of inflammation due to the release of cytokines.
NSAIDs (aspirin, naproxen, ibuprofen, diclofenac) inhibit both COX isoforms. Selective COX-2 inhibitors (e.g., celecoxib, nimesulide) have a lower risk of gastric mucosal damage compared to NSAIDs.
The association of H. pylori infection with NSAID use has a synergistic effect in the development of peptic ulcers, especially in the elderly.

- **Duodenogastric bile reflux**
  Cause: pyloric sphincter dysfunction which can be:
    - primary, due to a genetic defect
    - secondary, due to hypergastrinemia
  Effects: bile contains substances that can damage the gastric mucosa:
    - bile salts, with a tensioactive effect
    - lysolecithin, with a cytotoxic effect

- **Consequences**: damage to the integrity of the gastric mucosa "barrier" allows the **retrodiffusion of H^+ ions from the lumen into the gastric wall** with 2 effects:
  a) The appearance of GASTRIC ULCER - through 2 mechanisms:
    i) Increased chlorhydro-peptic secretion induced by:
    - stimulation of chief cells with the release of *pepsin*, responsible for mucosal erosions, vascular lesions with hemorrhages
    - release of *histamine* from enterochromaffin cells in the damaged gastric mucosa

    ii) Increased permeability of the gastric mucosa capillaries by:
    - *direct* effect of H^+ retrodiffusion
    - *indirect* effect of histamine release that produces vasodilation, hyperpermeability and mucosal edema

  b) Development of CHRONIC GASTRITIS - which:
    - favors the **persistence of gastric ulcer** by further decreasing gastric mucosal resistance
    - causes a **reduction in parietal cell mass** and a **decrease** in **chlorhydro-peptic secretion** over time, which explains the **normal/low gastric acidity** in patients with **gastric ulcer**
    - evolves over time towards **multifocal atrophic gastritis / pangastritis**, with intestinal metaplasia and an increased risk of gastric adenocarcinoma

COMPLICATIONS of peptic ulcer:

a) Hemorrhage – common in *posterior* duodenal ulcer, by erosion of the pancreaticoduodenal artery or of a branch
- *Acute*:
  - Upper digestive bleeding $\Rightarrow$ hematemesis or melena
  - Hypovolemic shock
- *Chronic*: occult bleeding in stool $\Rightarrow$ iron deficiency anemia

b) Perforation – common in gastric ulcer & *anterior* duodenal ulcer with risk of **peritonitis**

c) Penetration of neighboring organs, especially in the pancreas, with risk of **acute pancreatitis**; is important to recognize given the evolutionary severity

d) Pyloric stenosis – currently, rare as a consequence of gastric ulcer/cancer, but more frequently secondary to Crohn's disease or compression by cancer of the head of the pancreas
- *Causes*:
  - ✓ pyloric edema and spasm - in the acute flare
  - ✓ retractile scar - in the case of healing with fibrosis
- *Consequences*:
  - ✓ Strong gastric contractions → epigastric pain
  - ✓ Gastric distension and stasis → feeling of fullness
  - ✓ Severe vomiting (large volume, often painless) → dehydration + metabolic alkalosis
  - ✓ Anorexia with weight loss

e) Malignancy - 1% of gastric ulcers, NOT duodenal ulcers

C. STRESS ULCER

- **Definition**: form of *ischemic ulcer* characterized by the presence of *multiple gastro-duodenal ulcerations*
- **Etiology**:
  - polytrauma, burns
  - major post-surgical interventions
  - septic conditions
  - shock states with severe hypotension

Observation!
Stress ulcer was initially described as Curling ulcer in severe burns on the background of hypovolemia/hypoxia with necrosis of gastric mucosa cells and major risk of hemorrhage and perforation.

- **Pathogenesis**:
  - *intense sympatho-adrenergic stimulation* with vasoconstriction and ischemia of the gastric and duodenal mucosa

A particular form of stress ulcer is CUSHING ULCER:
- **Etiology**: head trauma, neurosurgical interventions
- **Pathogenesis**:
  - *central* stimulation of the vagal nuclei (secondary to intracranial hypertension) with induction of chlorhydro-peptic hypersecretion
  - increased release of cortisol from the adrenal cortex level with damage to the gastric mucosal barrier

4. ZOLLINGER-ELLISON SYNDROME

- **Definition**: *gastrin-secreting tumor* (gastrinoma) with pancreatic or gastroduodenal localization responsible for *multiple gastroduodenal ulcers*
- **Pathogenesis**: *hypergastrinemia* is responsible for chlorhydro-peptic hypersecretion
- **Positive diagnosis:**
  - *Clinical*: ulcer-like pain, diarrhea with steatorrhea (inactivation of intestinal enzymes in conditions of decreased intestinal pH and consequent lipid malabsorption)
  - *Paraclinical*: increased serum gastrin levels + hyperacidity

DISORDERS OF THE LIVER

Bile pigment metabolism – BRIEF PHYSIOLOGICAL OVERVIEW

a) UNCONJUGATED (indirect) bilirubin – UB (IB)

- is the **product of heme catabolism** at the level of the *reticuloendothelial system* as follows:
  - 85% of the daily production of UB comes from Hb released by *physiological hemolysis* of senescent erythrocytes
  - 15% of the daily production of UB comes from:
    - ✓ *catabolism of other heminic compounds* (myoglobin, cytochromes)
    - ✓ *inefficient erythropoiesis* (Hb released at the medullary level by hemolysis of abnormal precursors of the erythrocyte series)
- has 4 isomers:
  - *the natural isomer is insoluble in water* (intramolecular hydrogen bonds give it a hydrophobic character, but it is lipophilic)
  - the other 3 isomers are *photoisomers* (formed in the case of exposure to light), are *soluble in water* and can be eliminated via the urinary (and biliary) route without being conjugated
- UB (IB) circulates in plasma **bound to albumin** and **cannot be excreted in urine**; therefore, **jaundice with unconjugated hyperbilirubinemia** is called **acholuric jaundice** (absence of bilirubinuria). Exception: the situation in which the transformation of the natural hydrophobic isomer into photoisomers is favored by exposure to blue light (with λ=460-490 nm)
- bilirubin is a **toxic compound** and in order to be eliminated from the body it is **metabolized at the hepatocyte level**, being converted into a *water-soluble* compound in a process that goes through **3 stages:**
  1. **Uptake**: takes place at the *sinusoidal* pole of the hepatocyte with the help of *membrane proteins transporting organic anions* (OAT, Organic Anion Transporter) followed by the binding to *cytoplasmic* proteins called *ligandins (Y and Z)* which ensure the transport of UB at the endoplasmic reticulum level
  2. **Conjugation**: takes place in the *endoplasmic reticulum of the hepatocyte* under the action of *uridinediphosphate-glucuronyl-transferase* (UGT) - which transfers glucuronic acid to UB with the formation of bilirubin-monoglucuronide and then diglucuronide
     The resulting conjugated bilirubin (CB) is a *mixture of diglucuronide (85%) and monoglucuronide (15%).*
  3. **Biliary excretion**: occurs at the *biliary* pole of the hepatocyte; CB is actively secreted in the bile canaliculi and reaches the intestine along with bile. Inhibition of this stage leads to a decrease in CB excretion in bile and its regurgitation into the blood of the sinusoidal capillaries.

b) CONJUGATED (direct) bilirubin – CB (DB)

- is **water-soluble** and *can be excreted in the urine* under conditions of regurgitation into plasma from the hepatocyte level; therefore, **jaundice with conjugated hyperbilirubinemia** is called **choluric jaundice** (presence of bilirubinuria)
- is hydrolyzed at the *terminal ileum* level under the action of *bacterial enzymes* and transformed into **urobilinogen (Ubg)** of which a part will be reduced to **stercobilinogen**

c) Metabolism OF BILE PIGMENTS

1. At the intestinal level

- normally, **80-85% of Ubg is eliminated through feces** under the oxidized form (brown color) of ***stercobilin*** (50-250 mg/day)
- pathologically, there are:
  - conditions associated with **decreased Ubg elimination and discoloration of feces** in the case of:
    - ✓ decreased excretion of CB in the intestine from *mechanical and hepatocellular jaundice*
    - ✓ suppression of commensal intestinal flora, e.g. by administering poorly absorbable antibiotics

  - conditions associated with **increased Ubg elimination and hypercoloration (pleiochromia) of feces** in the case of hyperproduction of bilirubin and, respectively, of Ubg, in *hemolytic jaundice*
- normally, the remaining **10-15% of Ubg is reabsorbed through the portal circulation and reaches the liver**, being uptaken by hepatocytes and reexcreted in bile within the ***enterohepatic circuit of bile pigments***

2. At the renal level
- **a small part (1%) of Ubg reabsorbed in the portal circulation passes into the systemic circulation** and is excreted in the urine, producing a ***physiological urobilinogenuria*** (2 – 4 mg/day)
- pathologically, there are:
  - conditions associated with **decreased urobilinogenuria** in the case of:
    - ✓ decreased excretion of CB in the intestine from *mechanical and hepatocellular jaundice*
    - ✓ suppression of intestinal flora, e.g. by administering poorly absorbable antibiotics
  - conditions associated with **increased urobilinogenuria** in the case of:
    - ✓ hyperproduction of bilirubin and, respectively, Ubg in *hemolytic jaundice*
    - ✓ hepatocyte dysfunction, in *hepatocellular jaundice*

JAUNDICE

DEFINITION: yellow pigmentation of the sclera and skin determined by the accumulation of bilirubin (elastin has a high affinity for bilirubin) in conditions of **increased bilirubinemia > 2.5-3 mg/dL** (between 1.5 - 2.5 mg/dL, subicterus)

CLASSIFICATION:

I. Jaundice due to the predominant increase in UNCONJUGATED BILIRUBIN (UB) – ACHOLURIC

II. Jaundice due to the predominant increase in CONJUGATED BILIRUBIN (CB) - CHOLURIC

I. Jaundice due to the predominant increase in UNCONJUGATED BILIRUBIN (UB)

A. Jaundice due to UB HYPERPRODUCTION

1. HEMOLYTIC or PREHEPATIC jaundice

Characteristics:
- **hyperproduction of UB** secondary to **pathological hemolysis**:
  - **intravascular**: hemolytic anemias from: sickle cell anemia, thalassemia, presence of antierythrocyte antibodies, transfusion accidents
  - **extravascular**: hemolytic anemias due to membrane and enzymatic defects, hematoma resorption
  - through **ineffective erythropoiesis**: pernicious anemia, thalassemias
- hyperproduction of UB exceeds the liver's metabolic capacity ⇒
  - **unconjugated / indirect hyperbilirubinemia**
- because UB is insoluble in water and bound to albumin, it cannot be excreted in the urine ⇒ **absence of bilirubinuria** - *acholuric jaundice*
- at the hepatic level, the production of UB increases which will be excreted with bile into the intestine ⇒ **intestinal hyperproduction of Ubg** with 2 consequences:
  - **hypercoloration/pleiochromia of feces** (through increased production of stercobilin)
  - **increased urobilinogenuria** (through increased production of urobilin)

2. NUCLEAR jaundice (KERNICTERUS, Kern = nucleus, Germ)

- a particular form of hemolytic jaundice that occurs in newborns with **fetal erythroblastosis**, caused by *Rh incompatibility*, mother Rh (-) – fetus Rh (+)
- **Pathogenesis** – 2 mechanisms:
  a) **hyperproduction of bilirubin** through increased hemolysis and
  b) deficiency of uptake and conjugation at hepatocyte level due to **functional immaturity of the liver**

  The child is born with **marked indirect hyperbilirubinemia** which at high values (> 20 mg/dL), *forces the blood-brain barrier* with **preferential storage of UB at the basal ganglia level** (UB is toxic to both neurons and glial cells, causing acute mitochondrial damage and inhibition of axonal and dendritic growth) with **lesions responsible for acute and chronic encephalopathy**, respectively
- **Treatment: exposure of the newborn to blue light** in order to convert the natural hydrophobic isomer of UB into *water-soluble photoisomers* with urinary/biliary elimination (without prior conjugation)

B. Jaundice due to decreased UB CONJUGATION

1. PRIMARY hepatic conjugation deficiency – occurs in:

a) Gilbert's syndrome

- **Pathogenesis: *primary* defect in conjugation** of UB with glucuronic acid due to **decreased uridine diphosphate-glucuronyl-transferase (UGT) activity** determined by mutations in the UGT gene
- is the **most common familial form of unconjugated hyperbilirubinemia** (affects 2 – 7% of the population)
- is **asymptomatic,** being detected: **i) occasionally, as mild isolated hyperbilirubinemia** (< 6 mg/dL) or **ii) as episodes of benign (intermittent) jaundice** triggered by: viral infections, alcohol consumption, fatigue/stress, physical exertion/dehydration
- reticulocyte count is normal (excluding hemolysis), treatment is not necessary

b) Crigler-Najjar Syndrome

- **Pathogenesis: *primary* UB conjugation defect** due to a **genetic deficiency of UGT**
- is a rare condition, with 2 types:
  - **type I - the severe, lethal form** characterized by **total UGT deficiency** with *severe hyperbilirubinemia* (20 - 40 mg%) present at birth and *lack of response to enzyme induction with phenobarbital* $\Rightarrow$ *death by kernicterus* in the second year of life, in the absence of liver transplantation
  - **type II - the mild, non-lethal form** characterized by **partial UGT deficiency** with *moderate hyperbilirubinemia* (7 - 20 mg%), with onset in adolescence, good prognosis and *favorable response to enzyme induction with phenobarbital*

2. SECONDARY hepatic conjugation deficiency – occurs in:

a) NEONATAL jaundice

- **Pathogenesis: secondary hepatic conjugation deficiency** determined by:
  - *immaturity of hepatic enzyme systems*
  - *hyperproduction of bilirubin* through *physiological hemolysis with increased UB≤12 mg%*

- is a *physiological transient jaundice*, with onset 48 hours after birth (reaches a maximum on day 5) and which disappears in 7-10 days in term infants (in approx. 21 days in premature infants)
- UGT activation can be induced by medication by *administering phenobarbital*

b) Acquired UGT deficiency

- **Pathogenesis**: conjugation deficiency by *drug inhibition of the enzyme* (secondary to *chloramphenicol* administration) or sometimes, secondary to *breastfeeding* because breast milk contains a progesterone metabolite (3-alpha, 20-beta pregnanediol) that inhibits UGT

II. Jaundice due to the predominant increase in CONJUGATED BILIRUBIN (CB)

A. Jaundice due to the PRIMARY decrease in the excretory function

1. Dubin-Johnson Syndrome

- **Pathogenesis: *primary* deficiency of CB excretion** determined by the mutation of a channel protein called **Multidrug Resistance Protein 2 (MRP2)** at the level of the **bile canaliculi** with a role in transporting CB into bile, which is associated with:
  - **predominantly conjugated hyperbilirubinemia, asymptomatic with chronic jaundice**
  - **excretion of heme metabolites → *significant* coproporphyrinuria**
  - **presence of deposits of black pigment** (melanin?) in hepatocytes

2. Rotor Syndrome

- **Pathogenesis: *primary* deficiency of CB excretion**, partially elucidated, that associates:
  - **predominantly conjugated hyperbilirubinemia asymptomatic with chronic jaundice**
  - **excretion of heme metabolites → *moderate* coproporphyrinuria**
  - ***absence* of deposits of black pigment** in hepatocytes

3. Recurrent jaundice in PREGNANCY

- **Pathogenesis**: it is a form of **intrahepatic cholestasis** that occurs in some pregnant women in the third trimester of pregnancy due to the existence of a *genetic hypersensitivity to estrogen and progesterone hormones* (highly increased during pregnancy) and which decrease the *bile-excreting function of the hepatocyte* with:
  - *mild* conjugated hyperbilirubinemia (2-6 mg%) → jaundice and pruritus
  - ± increase in cholesterol and alkaline phosphatase (cholestasis marker)

Observation!

The term "recurrent" refers to the risk of jaundice recurring in subsequent pregnancies.

B. Jaundice due to the SECONDARY (acquired) decrease in the excretory function

1. HEPATOCELLULAR jaundice – INTRAHEPATIC cholestasis

- **Causes**: **hepatocyte damage** in:
  - *acute viral hepatitis*: hepatitis A, B ± D, C, Epstein-Barr v., cytomegalovirus
  - *acute toxic hepatitis*: acetaminophen, steroids, rifampicin, nutritional supplements
  - *acute/chronic ethanolic hepatitis*
  - *non-alcoholic fatty liver disease* (NAFLD) and *non-alcoholic steatohepatitis* (NASH)
  - *liver cirrhosis* (decompensated)

- *cholestatic diseases*: primary biliary cholangitis, sclerosing cholangitis
- *infiltrative diseases*: amyloidosis, sarcoidosis, with iron (hemochromatosis) or copper (Wilson's disease)
- *septic shock*

- **Consequences**:
  - **decreased excretion capacity for CB into the biliary canaliculi** associated with **moderate deficit of uptake and conjugation** (but the most affected stage is the biliary excretion of CB) is responsible for:
    - **Regurgitation of CB into the blood of the sinusoidal capillaries** with:
      - **MIXED hyperbilirubinemia, predominantly conjugated**
      - **bilirubinuria** = *choluric jaundice* (yellow-brown urine)
    - **Variable decrease in the amount of CB that reaches the intestine** $\Rightarrow$ intestinal production of Ubg decreases or NOT (depending on the severity of hepatocellular lesions) with **normally colored or discolored feces**
  - although intestinal absorption of Ubg is reduced due to the decrease in its production, in the presence of hepatocellular lesions we witness a deficiency in the functioning of the enterohepatic circuit and thus, a greater amount of Ubg will remain in the plasma and will reach the urine with **increased urobilinogenuria**
  - **predominant** increase in plasma of the **enzymes of the hepatocytolysis syndrome**: **aminotransferases and LDH** and to a lesser extent of the enzymes of the cholestasis syndrome: γ-glutamyl-transpeptidase (GGT) and alkaline phosphatase (ALP)

2. MECHANICAL jaundice – EXTRAHEPATIC cholestasis

- **Causes**: **obstruction of bile excretion in the bile ducts** in:
  - *biliary or choledochal lithiasis*
  - *carcinoma of the head of the pancreas, ampulla of Vater, bile ducts (cholangiocarcinoma)*
  - *acute or chronic pancreatitis*
  - *choledochal strictures*
- **Consequences**:
  - **Regurgitation of bile from the bile canaliculi into the sinusoidal capillaries** with:
    - **predominantly conjugated hyperbilirubinemia**
    - **bilirubinuria** = *choluric jaundice* (dark brown urine)
    - **predominant** increase in plasma of **cholestasis syndrome enzymes** (GGT, ALP) and to a lesser extent of hepatocytolysis syndrome enzymes (AST, ALT, LDH)
    - **increase in cholesterol and bile salts** $\Rightarrow$ bile salts are deposited in the skin, being responsible for the appearance of *pruritus*
  - **Reduction of bile excretion in the intestine** with:
    - decrease in urobilinogen production with:
      - ✓ **discolored feces**
      - ✓ **ABSENCE of urobilinogenuria**
    - *lack of bile salts* (with tensioactive effect) in the intestine is responsible for:
      - ✓ **lipid malabsorption** with **steatorrhea**
      - ✓ **malabsorption of fat-soluble vitamins** with **vitamin K deficiency**, prolonged prothrombin time and **risk of hemorrhagic syndrome**

HEPATIC CIRRHOSIS

DEFINITION: the final evolutionary stage of chronic liver diseases characterized by **irreversible alteration of the liver cytoarchitecture**, characterized by **4 elements**:

1. **NECROSIS of hepatocytes** with progressive destruction of the liver parenchyma
2. **DIFFUSE HEPATIC FIBROSIS** - **PRIMORDIAL mandatory** element for diagnosis
3. **Compensatory NODULAR REGENERATION** of the remaining healthy parenchyma
4. **DISTORTION OF THE HEPATIC VASCULARIZATION** with the appearance of **portal hypertension (PHT)**

ETIOLOGY:

- **chronic ethanolic hepatitis** on the background of chronic alcoholism (the cirrhogenic risk occurs with chronic consumption of a daily amount of alcohol of over 30 g/day in men, in women the required amount being lower). It is the **most common cause of liver cirrhosis in most developed countries** (50-70% of cases in Europe)
- **chronic viral hepatitis** (type C, B, D) especially in association with daily alcohol consumption which accelerates the progression to cirrhosis (having an additive effect with hepatic viruses)
- **Metabolic dysfunction–associated steatotic liver disease (MASLD)** and its progressive inflammatory form, **metabolic dysfunction–associated steatohepatitis (MASH)**, are currently among the leading causes of chronic liver disease in developed countries. MASLD is defined by the presence of hepatic steatosis in individuals with at least one cardiometabolic risk factor, such as obesity, dyslipidemia, arterial hypertension, type 2 diabetes mellitus, or insulin resistance, in the absence of other major causes of liver disease. MASLD is considered the hepatic manifestation of metabolic syndrome.
- MASH represents the progressive form of MASLD and is characterized by hepatic steatosis associated with hepatocellular injury and inflammation, which promote the development of fibrosis. Both MASLD and MASH may progress to advanced fibrosis and cirrhosis; however, progression is significantly more frequent in MASH, with approximately 10–30% of patients developing cirrhosis and/or hepatocellular carcinoma. These conditions are regarded as stages along a dynamic disease continuum rather than distinct entities, and disease regression may occur with improvement of metabolic risk factors.
- **chronic biliary obstruction** leading to **biliary cirrhosis**:
  - **primary**, in *primary biliary cholangitis* and *sclerosing cholangitis* (autoimmune diseases)
  - **secondary**, in *chronic biliary lithiasis*
- **venous congestion** from: *right heart failure, constrictive pericarditis* leading to **cardiac cirrhosis**
- **metabolic causes**: *hereditary hemochromatosis* and *Wilson's disease*
- **drug/toxic causes**: methotrexate, amiodarone, isoniazid, propyl-thiouracil (usually, cessation of exposure stops the evolution of liver lesions)

PATHOGENESIS:

1. **Damage to HEPATOCYTES** under the action of *toxic* factors *(alcohol!)*, *viral, lipid accumulation (steatosis)*, causes the release of the cellular contents (by necrosis) and increased production of reactive oxygen species with 2 consequences:
   a) **activation of hepatic macrophages** (Kupffer cells) that release chemokines (CCL-2, Chemokine Ligand-2) that attract monocytes from the circulation with their transformation into pro-inflammatory macrophages by interacting with the chemokine receptor 2 (CCR-2, Chemokine Receptor-2); both activated macrophages will release pro-inflammatory cytokines (TNF-α and IL-1) and growth factors (TGF-β, transforming growth factor β; PDGF, platelet-derived growth factor)
   b) **activation of stellate cells** (inactive lipocytes with a role in storing vitamin A) with their transformation into active myofibroblasts that proliferate under the action of growth factors, triggering the **process of hepatic FIBROSIS**.

2. **Diffuse hepatic FIBROSIS**
   Activated myofibroblasts cause:
   - ✓ **excessive synthesis of collagen fibers** and their **increased storage in the spaces of Disse** (the spaces located between the discontinuous walls of the hepatic sinusoidal capillaries and the cords of hepatocytes and which have a major role in the nutritional exchanges of hepatocytes) leads to the **remodeling of the sinusoidal capillaries** by the loss of fenestrations and the appearance of a basement membrane (the so-called "capillarization of the sinusoids") with 3 consequences: i) impairment of the exchanges between hepatocytes and capillary blood, ii) favoring of hepatocyte necrosis and iii) increased resistance to blood flow with the onset of PHT
   - ✓ **increased density of the extracellular matrix** and its **continuous remodeling** via matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs, tissue inhibitors of metalloproteinases) – mediators also secreted by activated macrophages
   - ✓ **chemotactic effect for inflammatory cells and CHRONIC INFLAMMATION** with flare-ups of activation
   - ✓ **contraction of sinusoidal capillaries** with **worsening PORTAL HYPERTENSION**

Observation !
Early fibrosis is considered reversible in viral hepatitis types B and C under the conditions of disappearance of exposure to the etiological agent by administering antiviral therapy, when catabolism of collagen fibers is possible (recent collagen, in reduced quantity). **Advanced fibrosis from established cirrhosis is irreversible.**

3. **NODULAR regeneration**
   – appearance of *parenchymal nodules* made up of healthy hepatocytes that proliferate and are delimited by fibrosis bands, with dimensions ranging from under 3 mm (*micronodules*) to over 3 mm (*macronodules*).

Remember!
The presence of a nodular transformation of the liver parenchyma but **WITHOUT fibrosis DOES NOT allow confirmation** of the diagnosis of liver cirrhosis.

4. **Disorganization of the LOBULAR and VASCULAR ARCHITECTURE of the liver** with:
   - *deficient irrigation of healthy hepatocytes* and the installation of **HEPATOCELLULAR DYSFUNCTION**
   - *alteration of hepatic vascularization* and the onset of **PORTAL HYPERTENSION (PHT)**

5. INVOLVEMENT OF THE GUT-LIVER AXIS
   - has a key role in: i) the *progression of chronic liver diseases* and ii) the *occurrence of complications in cirrhosis*
   - **stasis in the portal circulation** determines the compromise of the intestinal barrier function and the increase in intestinal permeability with the **translocation of bacteria and bacterial components (e.g., lipopolysaccharides, LPS) at liver level** via the portal circulation, which causes the perpetuation of **CHRONIC INFLAMMATION and the process of HEPATIC FIBROGENESIS** (via hepatic Toll-like receptors for LPS, lipopolysaccharide-Toll-like receptor 4 LPS - TLR4)
   - **stasis at the microcirculation level** is responsible for **portal GASTROPATHY and COLOPATHY** (punctate hemorrhages, parietal erosions with risk of bleeding)

Observation!
Blocking of bacterial translocation by suppressing the flora with poorly absorbable antibiotics (e.g., rifamixin) reduces PHT and the risk of spontaneous bacterial peritonitis in advanced cirrhosis.

The CONSEQUENCES of liver cirrhosis are:

A. **Progressive hepatocellular insufficiency** with the impairment of all intermediate metabolisms.

Manifestations of hepatocellular insufficiency:
   - general, nonspecific: asthenia, fatigue, weight loss
   - digestive: nausea/vomiting, jaundice, hepatomegaly
   - extradigestive:
     - ✓ palmar erythema, vascular stars
     - ✓ testicular atrophy and gynecomastia in men
     - ✓ menstrual disorders in women
     - ✓ hemorrhagic syndrome
     - ✓ peripheral edema
     - ✓ muscle atrophy

B. Portal hypertension (PHT)
- **Definition:** ***increased pressure in the portal vein above 10 mmHg*** (normally, 5-8 mmHg)
- **Etiology**: **increased resistance to flow in the portal circulation** following a localized **obstructive process** at the following levels:
  - ✓ **Prehepatic**: *portal vein thrombosis* (congenital anomalies of the portal vein, prothrombotic conditions - e.g., factor V Leiden, myeloproliferative disorders)
  - ✓ **Intrahepatic**: distortion of vascularization by fibrosis in ***liver cirrhosis*** **- the main cause!**
  - ✓ **Posthepatic**: obstruction of the inferior vena cava, constrictive pericarditis, right heart failure

- **Pathogenesis** – increased resistance to flow is based on 2 components:
  1. MECHANICAL component, represented by **hepatic vascular distortion** through:
  - ✓ capillarization and stenosis of sinusoids
  - ✓ fibrosis and compression of the centrilobular vein through fibrosis bands and regeneration nodules

  2. DYNAMIC component, responsible for **intrahepatic vasoconstriction** through:
  - ✓ contraction of smooth muscle in the arteriolar walls
  - ✓ contraction of activated stellate cells and myofibroblasts
  - ✓ alteration of the balance between *vasodilators* (NO, PG) and *vasoconstrictors* (endothelin, AII, vasopressin) with the predominance of vasoconstriction

This increased resistance leads to PHT with 2 consequences:
i) **development of collateral circulation**/opening of portosystemic anastomoses and
ii) **establishment of a hyperdynamic circulation** (produced by nitric oxide, cannabinoids and glucagon) with peripheral and splanchnic vasodilation

- **Manifestations of PTH:**

1. Development of PORTO-CAVAL SHUNTS
- has the role of *draining blood from the portal circulation into the vena cava system*, bypassing the hepatic obstacle
- anastomoses can come from the following branches of the portal system:
  - ✓ the short gastric veins anastomose with the esophageal submucosal plexus that drains through the azygos vein into the superior vena cava (SVC) ⇒ development of **esophageal varices**
  - ✓ the inferior mesenteric vein develops anastomoses with the veins of the rectal plexus that drains into the inferior vena cava (IVC) ⇒ onset of **hemorrhoids**
  - ✓ the periumbilical veins can anastomose with the epigastric vein that drains through the IVC or with the mammary veins that drain through the SVC ⇒ appearance of the **periumbilical circulation under the form of "caput medusae"**

2. SPLENOMEGALY
- is the result of chronic congestion, fibrosis and splenic siderosis
- if massive, it is associated with **hypersplenism**, defined by increased activity of sequestration and destruction of blood cells with thrombocytopenia (rarely, pancytopenia)

3. ASCITES
- **Definition**: *excessive accumulation of albumin-rich fluid, but usually < 3g/dL (TRANSUDATE)*, in the *peritoneal cavity* (which becomes clinically evident in the case of accumulation of ≥ 500 ml of fluid)

- **Pathogenesis**:
  a) **LOCAL mechanisms** - play a **<u>central role</u>** in pathogenesis:
  - **increased hepatic lymph flow** is the **main mechanism of ascites production** and is caused by:

- ✓ ***increased hydrostatic pressure in sinusoidal capillaries*** with increased extravasation of protein-rich fluid from their level into the spaces of Disse
- ✓ increased hepatic lymph flow that exceeds the drainage capacity of the thoracic duct (maximum 800 - 1000 mL/day) and can reach 20 L/day in cirrhotics

– **excess lymph** (rich in proteins) exits through the liver capsule into the peritoneal cavity ⇒ ***ascites***

b) **SYSTEMIC mechanisms** - contribute to the **worsening of ascites fluid formation**:

– **hypoalbuminemia**
- ✓ is not a critical factor in the production of ascites (there is no level of albuminemia at which ascites formation begins) but can worsen pre-existing ascites
- ✓ is based on the following mechanisms:
  - o *loss of albumins from the intravascular space into the ascites fluid*
  - o *hemodilution secondary to water-salt retention*
  - o *decreased albumin synthesis in severe lesions of the liver parenchyma*

– **splanchnic vasodilation** is compensatory in portal HT to reduce pressure in the hepatic artery and leads to a decrease in *effective arterial volume* with the activation of the mechanisms responsible for:
- ✓ **water-salt retention** - triggered by *decreased renal perfusion* and leading to:
  - o *decreased GFR + increased proximal reabsorption of Na^+ and water*
  - o *activation of the RAA system* ⇒ hyperaldosteronism (this increase is also contributed by reduced hepatic degradation of the hormone in severe hepatocyte lesions) ⇒ *increased distal reabsorption of Na^+ and water*
- ✓ **increased ADH release** ⇒ ↑ distal reabsorption of water with dilutional hyponatremia
- ✓ renal vasoconstriction ⇒ development of hepatorenal syndrome

Observation!
In the case of resistant ascites and in order to reduce the risk of bleeding from esophageal varices, a transjugular intrahepatic porto-systemic shunt (TIPS) can be chosen, in the ABSENCE of portal encephalopathy.

4. HEPATIC (PORTOSYSTEMIC) ENCEPHALOPATHY (HE)

- **Definition: chronic neuropsychiatric syndrome** in which **toxic substances produced in the intestine pass into the systemic circulation** and **accumulate in the CNS** where they exert **toxic effects** as a result of the *presence of spontaneous portocaval shunts* (or induced by TIPS) in **liver cirrhosis**.

Observation!
Acute encephalopathy occurs in severe alterations of hepatocyte function in **fulminant hepatitis** with **acute liver failure**.

- **Pathogenesis**:

a) TOXINS involved in the pathogenesis of encephalopathy are:

1. Ammonia:

– *Sources:*
- intestinal decomposition of urea under the action of a bacterial urease

- bacterial deamination of amino acids from food or blood proteins (in case of gastrointestinal bleeding)

– *Metabolism:*

Normal - *ammonia detoxification* is carried out in:

- *liver*: by transforming NH_3 into urea (ureogenetic cycle), mainly
- *skeletal muscles, CNS*: by forming glutamine (from glutamic acid under the action of glutamine synthetase)
- *kidneys*: NH_3 is generated by deamination of glutamine (renal ammonogenesis process)

In hepatic encephalopathy:

– **NH_3 detoxification is NO longer carried out** in:

- **liver** - its bypass by collateral circulation + decreased ureagenetic function
- **muscle** - skeletal muscles are atrophied in cirrhotics (effect of TNFα and myostatin)
- **kidneys** - NH_3 excretion decreases in conditions of dehydration and hypokalemia activates the process of renal ammoniogenesis (with increased serum NH_3 levels)
- **the detoxification task falls exclusively on the CNS** $\Rightarrow$ detoxification occurs at the level of **astrocytes** and when generated in large quantities in the cytosol, glutamine: i) favors the establishment of *cerebral edema* (by osmotic mechanism) and ii) is transported for metabolism at the mitochondrial level where it causes *irreversible mitochondrial damage*

2. Activation of GABA-dependent neurotransmission (gamma-aminobutyric acid) with an inhibitory role

3. **Endogenous benzodiazepines**: their role is proven by the improvement of symptoms after the administration of benzodiazepine receptor antagonists (e.g., flumazenil) in some patients
4. **False mediators**: *octopamine*
   – is formed by *bacterial degradation at the intestinal level of aromatic amino acids (phenylalanine, tyrosine)*
   – mechanism of action = *alters the transmission of nerve impulses at the level of central noradrenergic synapses* (it replaces normal mediators and blocks their synthesis in the nerve cell)
5. **Other toxins**: *mercaptans* - their presence in the breath of patients with HE is responsible for the "fetor hepaticus" - and *free fatty acids*

Observation!

In patients with HE, an abnormal increase in the permeability of the blood-brain barrier is observed $\Rightarrow$ responsible for the increased susceptibility of the CNS to the action of toxins and some drugs.

b) FACTORS THAT PRECIPITATE the onset of encephalopathy:

– increased endogenous NH_3 production: high-protein diets & gastrointestinal bleeding
– constipation: favors the development of intestinal flora
– infections: respiratory, urinary, spontaneous bacterial peritonitis
– hydroelectrolytic depletion: diarrhea, vomiting, massive paracentesis, treatment with diuretics

- drugs that depress the CNS
- surgical portosystemic shunts (TIPS, Transjugular Intrahepatic Portosystemic Shunt)
- occurrence of hepatocarcinoma

HEPATO-RENAL syndrome

- **Definition**: form of **severe acute <u>functional</u> renal failure** with sudden onset and poor prognosis that occurs in patients with **advanced liver cirrhosis, portal hypertension, jaundice and ascites** and is characterized by:
  i) decreased diuresis (decreased glomerular filtration)
  ii) preservation of the urine concentration capacity (normal tubular function) and
  iii) normal histological appearance of the kidney on biopsy
- **Pathogenesis**:
  - is characterized by **SEVERE RENAL VASOCONSTRICTION** on the background of **normal kidneys**, in which the reduction of glomerular filtration and the increase of tubular reabsorption of Na^+ and H_2O are determined by:
    - ✓ *extreme peripheral vasodilation* (proinflammatory mediators and bacterial endotoxins - which are normally detoxified by the liver - reach the systemic circulation due to the porto-caval shunts where they exert an intense vasodilating effect directly or indirectly, e.g., bacterial endotoxins increase the expression of inducible nitric oxide synthase - iNOS) $\Rightarrow$ *decrease in effective arterial volume*
    - ✓ *decrease in renal perfusion pressure* $\Rightarrow$ *activation of the RAA system* (plasma renin is increased), of the *sympathetic nervous system* and the *release of ADH* $\Rightarrow$ water-salt retention with dilutional hyponatremia
  - the contributing factor to the impairment of renal hemodynamics is:
    - ✓ *decreased renal synthesis of vasodilating PG* – a fact supported by the precipitation of the syndrome by the administration of NSAIDs

PANCREATIC DISORDERS

ACUTE PANCREATITIS

DEFINITION: acute pancreatic inflammation characterized by **autodigestion of the pancreas due to *local* activation of endogenous enzymes** and whose severity can vary from mild and self-limited forms to extremely severe forms, with extensive pancreatic and peripancreatic necrosis and systemic inflammatory response

ETIOLOGY:

1. ALCOHOLISM – massive alcohol ingestion causes:
- direct toxic effect $\Rightarrow$ local activation of trypsinogen into trypsin
- decreased tone of the sphincter of Oddi $\Rightarrow$ duodeno-pancreatic reflux
- stimulation of secretin release $\Rightarrow$ stimulation of local secretion of pancreatic proenzymes (zymogens)

2. BILIARY LITHIASIS – gallstones can cause:
- blockage of the sphincter of Oddi at the level of the ampulla of Vater responsible for *bilio-pancreatic reflux* $\Rightarrow$ bile brings lecithin into the pancreatic duct which will form lysolecithin with cytotoxic effect

- incompetence of the sphincter of Oddi responsible for *duodeno-pancreatic reflux* ⇒ duodenal juice brings active proteolytic enzymes into the pancreatic duct with the activation of trypsinogen

Remember!
Alcoholism and **gallstones** are responsible for **80% of cases.**

3. **IATROGENIC**, after: i) endoscopic retrograde cholangiopancreatography (1-5% of ERCP cases) by sudden increase in pressure in the pancreatic ducts upon injection of contrast medium and ii) neighboring surgical interventions
4. **IDIOPATHIC** (8-10% of cases)
5. **OTHER CAUSES** (rarely):
   a) **VIRAL INFECTIONS**: mumps virus (epidemic parotitis, mumps), Coxackie B virus, adenoviruses, Epstein-Barr virus, HIV
   b) **METABOLIC causes**:
      - ✓ **Hypertriglyceridemia (e.g., hyperlipoproteinemias types I and V):** in the presence of an excess of circulating TG, pancreatic lipase (released in small quantities from the pancreatic acini) breaks down TG into FFA and glycerol; FFA have a local irritant action causing inflammation and damage to the membrane of pancreatic acinar cells with the release of new enzymes
      - ✓ **Hypercalcemia**, e.g., from hyperparathyroidism, multiple myeloma – causes:
        - precipitation of Ca^{2+} in the pancreatic ducts with their obstruction
        - activation of trypsinogen → trypsin in the pancreatic ducts with pancreatic autodigestion
   c) **MECHANICAL causes**: pancreatic tumors or trauma
   d) **MEDICATIONS**: immunosuppressants, thiazide diuretics, sulfonamides

PATHOGENESIS: **activation of TRYPSINE in pancreatic acini** under the action of:
- lysosomal enzymes (cathepsin B)
- increased intracellular Ca^{2+} concentration
- decreased activity of intracellular pancreatic trypsin inhibitor

Consequences of pancreatic enzyme activation:
a) LOCAL effects of pancreatic enzyme activation:
- **trypsin** causes *activation of other pancreatic enzymes*: chymotrypsin, elastase, phospholipase A2
- **chymotrypsin** causes a proteolytic effect (hydrolysis of peptide bonds) ⇒ **edema and vascular lesions**
- **elastase** causes: digestion of elastin from vascular walls with *hemorrhages* ⇒ **retroperitoneal hematoma**
- **phospholipase A2** transforms lecithin into lysolecithin which is cytotoxic and leads to *necrosis of acini and peripancreatic fat* (steatonecrosis) ⇒ **pancreatic abscess**
- **pancreatic lipase** will amplify the process of pancreatic and peripancreatic autodigestion with the appearance of *areas of necrosis* ⇒ **acute hemorrhagic pancreatitis**
- trypsin and chymotrypsin are also responsible for the activation of the systems of:

i) kinins / complement system ⇒ *vasodilation, hyperpermeability, edema*
ii) coagulation / fibrinolysis ⇒ microcirculation impairment with *local thrombosis/ hemorrhage*

b) SYSTEMIC effects of pancreatic enzyme activation:

- **passage of activated pancreatic enzymes into circulation ⇒ vasodilation, hypotension, risk of hypovolemic shock**
- **release of proinflammatory cytokines** (IL-1, IL-6, TNFalpha, PAF) ⇒ **systemic inflammatory response syndrome** and the risk of **multiple organ failure** (acute respiratory distress syndrome, acute kidney injury) or **DIC**

CHRONIC PANCREATITIS

DEFINITION: **chronic inflammation of the pancreatic parenchyma** with progressive destruction of the acini, stenosis and dilation of the ducts and a process of ***glandular fibrosclerosis*** that evolves towards **exocrine pancreatic failure**

ETIOLOGY:

- **chronic alcoholism in 80% of cases**
- chronic kidney disease
- hypercalcemia
- cystic fibrosis
- chronic obstruction of the pancreatic duct/excretory ducts (carcinoma, cysts, strictures)
- hereditary (episodes of pancreatitis with onset in childhood)
- autoimmune (antipancreatic antibodies)

Observation!
The research activity of the last decade has identified an important genetic component responsible for the diversity of the evolutionary modalities of chronic pancreatitis both in the case of the alcoholic etiology and especially in the case of other etiologies

PATHOGENESIS:

The pathogenesis involves:

- prolonged activation of trypsinogen ⇒ trypsin (and, respectively, of the other enzymes) and/or
- inactivation deficiency of pancreatic enzymes (in the context of the increase in intracellular calcium concentration induced by alcohol)

In the context of chronic local activation of enzymes, *precipitation of proteins with Ca^{2+} ions* is favored with:

i) obstruction of *small pancreatic ducts* by **protein plugs** and
ii) obstruction of *LARGE pancreatic ducts* by **calculi**

⇒ ductal hypertension and progressive damage to the pancreatic parenchyma

The result is a process of **fibrosclerosis** or **fibro-cystic degeneration** characterized by:

- ✓ necrosis of some acini and replacement with fibrous tissue
- ✓ dilation of other acini with cyst formation

Consequences of FIBROSCLEROSIS of the pancreatic parenchyma:

1. **EXOCRINE pancreatic failure** - decreased secretion of enzymes and bicarbonate occurs when the mass of the functional parenchyma decreases to approx. 10% of normal and leads to:
   a) **Lipase deficiency ⇒ lipid malabsorption ⇒ STEATORRHEA**
   b) **Deficiency of proteolytic enzymes (trypsin, chymotrypsin) ⇒ malabsorption of proteins** with:
      - **AZOTORRHEA** (increased fecal nitrogen) and **CREATORRHEA** (increased excretion of undigested muscle fibers)
      - **decreased protein synthesis** with:
        - ✓ **decreased muscle mass** and body **emaciation**
        - ✓ **hypoproteinemia** with hypoalbuminemia ⇒ **edema** (by the decrease in plasma oncotic pressure, Pop)
      - ***putrefaction dyspepsia***: proteins undergo a putrefactive process under the action of intestinal flora → alkaline fecal pH, bloating, flatulence
   c) **Decreased bicarbonate content in the intestinal juice** ⇒ *decreased activity of intestinal and pancreatic enzymes* (whose optimal pH of action is alkaline)
   d) **Decreased absorption of vitamin B_{12}** - due to impairment of proteolytic degradation of factor R from the vitamin B_{12}-factor R complex with prevention of the formation of the vitamin B_{12}-intrinsic factor complex ⇒ ***megaloblastic anemia***

2. ENDOCRINE pancreatic failure - occurs when destruction of islet beta cells causes *decreased insulin release* ⇒ **decreased glucose tolerance, diabetes mellitus**

GALLBLADDER DISORDERS

BILIARY LITHIASIS

DEFINITION: a common condition in Western countries (prevalence 2-3 times higher in women than in men) consisting of the *precipitation of bile constituents in the gallbladder* with the formation of:

- **cholesterol** calculi - in 85% of cases
- **pigment** calculi, mainly composed of **calcium bilirubinate** (and a little cholesterol) - in the remaining cases

PATHOGENESIS:

- **abnormalities in the composition of bile** that lead to its insolubility by changing the balance between the main constituent elements: *cholesterol, phospholipids and bile salts* in the sense of *increased cholesterol concentration in bile* (compared to the concentration of phospholipids and bile salts), respectively the ***supersaturation of bile in cholesterol***
- **biliary stasis** (hypomotility) that favors the ***concentration of bile*** and ***hypersecretion of mucus***

- **chronic inflammation** that favors the ***crystallization process*** by: i) increasing the absorption of water and bile salts with reduced cholesterol solubility and ii) mucus hypersecretion

1. CHOLESTEROL calculi

- **Causes** of **cholesterol precipitation** in the bile are:
  - increased dietary intake (which explains the beneficial effect in preventing stone formation of ezetimibe - the inhibitor of intestinal cholesterol absorption)
  - increased cholesterol secretion in bile:
    - ✓ results from increased synthesis in hepatocytes – via activation of hydroxy-methyl-glutaryl-CoA/HMG-CoA reductase, e.g., by estrogens (which explains the increased prevalence of lithiasis in women)
    - ✓ present in patients with metabolic syndrome and insulin resistance
  - decreased hepatic secretion of bile salts by:
    - ✓ decreased reabsorption of bile salts at the terminal ileum - in Crohn disease, ileal resections
  - genetic factors, responsible for cases of gallstones present in members of the same family and in monozygotic twins

2. PIGMENT calculi

- **Causes** of increased **unconjugated bilirubin** in bile are:
  - **increased hemolysis** in:
    - ✓ hemolytic anemias: spherocytosis, sickle cell anemia, thalassemias when UB synthesis exceeds the liver's conjugation capacity
    - ✓ liver cirrhosis, with splenomegaly and hypersplenism (plus decreased conjugation)
  - **decreased conjugation capacity** in: Gilbert's syndrome (increased monoglucuronide excretion)
  - **conjugated bilirubin (CB) deconjugation**:
    - ✓ non-enzymatic - against the background of prolonged biliary stasis
    - ✓ enzymatic - under the action of bacterial enzymes against the background of bacterial colonization of the biliary tree
- **Consequence**: increased unconjugated bilirubin (UB) at the bile level determines its precipitation with calcium ions with the appearance of **calcium bilirubinate stones** of 2 types:
  - ✓ **black**, which additionally contain salts of calcium carbonate and phosphate
  - ✓ **brown**, which additionally contain salts of fatty acids (stearate, palmitate)

COMPLICATIONS of biliary lithiasis:

- **Biliary colic**: blockage of the bile ducts by migrated stones causes strong peristaltic contractions that produce pain in the right hypochondrium with irradiation in the epigastric and posterior thoracic region, accompanied by vomiting
- **Acute cholecystitis** with risk of perforation: the above symptoms + fever with leukocytosis
- **Obstructive jaundice** with risk of acute biliary pancreatitis
- **Mucosal metaplasia** with risk of carcinomatosis (rare)

INTESTINAL DISEASES

DIARRHEA

DEFINITION: frequent elimination of feces - more than 3 soft stools/day, which can be:
- *acute* - duration < 2 weeks
- *persistent* - duration 2-4 weeks
- *chronic* - duration > 4 weeks

CLASSIFICATION:
A. **Secretory** diarrhea
B. **Osmotic** diarrhea
C. Diarrhea due to **altered peristalsis**
D. **Inflammatory** (exudative) diarrhea - see Inflammatory bowel disease (IBD)

A. SECRETORY diarrhea

- **Definition**: diarrhea caused by ***malabsorption of water and electrolytes*** by *stimulating their active secretion and inhibiting their reabsorption*, which will cause the elimination of *isotonic* (sometimes hypotonic) feces; diarrhea persists despite fasting.

Absorption of water and electrolytes in the small intestine – Brief physiological overview:
- **Paracellular pathway**: consists of the passage of water & electrolytes at the junctions between the cells of the intestinal mucosa
- **Transcellular pathway**: involves crossing the enterocyte membrane with the help of some transporters/channels/ion pumps:
  - ✓ the *anionic* exchange transporter Cl^- - HCO_3^-, responsible for the net secretion of HCO_3^- into the intestinal lumen $\Rightarrow$ which explains the fact that chronic diarrhea frequently causes *metabolic acidosis*
  - ✓ the *cationic* exchange transporter Na^+ - H^+ (NHE, Natrium-Hydrogen Exchanger)
  - ✓ the Na^+-dependent glucose transporter (SGLT, Sodium-dependent GLucose Transporter)
  - ✓ the Na^+-K^+-dependent ATPase
  - ✓ the Cl^- channel of the CFTR type (Cystic Fibrosis Transmembrane Conductance Regulator)

- **Etiology**:
  1. ***Infectious***: Vibrio cholerae (enterotoxins), E.coli (thermolabile and thermostable toxin), Clostridium difficile (frequently after aggressive antibiotic therapy), ***rotaviruses*** (these are the most common current cause of severe diarrhea in children), noroviruses
  2. ***Hormonal***: hypersecretion of *vasoactive intestinal peptide* (VIP) in VIP-omas, *serotonin* in carcinoid syndromes, *gastrin* in gastrinomas
  3. ***Drug-induced***: digoxin, colchicine, metformin, misoprostol
  4. ***Bile acid-induced***: secondary to malabsorption from: Crohn's disease, ileal resection, post-cholecystectomy
- **Pathogenesis**:
  - **Stimulation of ion secretion**, e.g. chlorine, in the intestinal lumen
  - **Inhibition of sodium and water reabsorption** due to the **increase in cAMP** in intestinal epithelial cells

  Classic example: diarrhea in **cholera**, which occurs through 2 mechanisms:

- stimulation of Cl^- ion secretion into the lumen via the cystic fibrosis transmembrane channel (CFTR)
- inhibition of the Na^+-H^+ cationic transporter ⇒ decreased absorption of Na^+ ions and secondarily of water with severe rapid dehydration

Observation!
Since the Na^+-glucose coupled transporter is not affected, in *mild* forms of the disease correction of the hydro-electrolyte imbalance can be achieved by oral administration of glucose-containing saline.

B. OSMOTIC diarrhea

- **Definition**: diarrhea caused by the *osmotic effect of unabsorbed compounds* with the elimination of *hypertonic* fecal matter and which ceases upon fasting or after cessation of the intake of the osmoactive compound
- **Etiology**:
  - consumption of laxatives and antacids containing **magnesium**
  - ingestion of osmoactive food compounds: *lactose* (in lactase deficiency), fructose, sorbitol, lactulose
  - maldigestion of ingested foods with their increased elimination (see the syndrome of carbohydrates, lipids, proteins malabsorption)
- **Pathogenesis**: **accumulation of poorly absorbable, osmoactive** substances in the lumen with the attraction of water

C. Diarrhea due to the ALTERATION OF PERISTALTISM

- **Etiology**:
  - vagotomy, diabetic neuropathy - frequently, *inhibition* of peristalsis
  - postgastrectomy dumping syndrome, carcinoid syndrome (hypersecretion of serotonin) - *acceleration* of peristalsis
  - irritable bowel syndrome (irritable colon/spastic colon) - their *alternation*
- **Pathogenesis** – is based on 2 mechanisms:
  - **Inhibition of peristalsis** with intestinal stasis and bacterial proliferation
  - **Acceleration of peristalsis** with:
    - ✓ decrease in food-mucosa contact time and fluid overload of the colon
    - ✓ premature emptying of the colon

MALABSORPTION SYNDROMES

DEFINITION: increased elimination of *food principles* through feces due to altered *digestion, absorption or both*

A. CARBOHYDRATES Malabsorption

- **Pathogenesis** – carbohydrate malabsorption is responsible for:
  1. **The presence of unabsorbed carbohydrates in the <u>duodenum/jejunum</u>** determines:
     - Increased osmolarity of intestinal contents ⇒ water attraction into the lumen ⇒ **osmotic diarrhea**
     - Decreased reabsorption of sodium and water ⇒ **secretory diarrhea**
     - Increased loss of bicarbonate ⇒ **metabolic acidosis**

2. **Fermentation of unabsorbed carbohydrates in the terminal ileum/right colon** under the action of bacterial flora determines **fermentation dyspepsia** with:
   - *hyperproduction of short-chain organic acids* (acetic, propionic, butyric, lactic) ⇒ *acidification of feces* ⇒ **buttock erythema** (especially in children)
   - *hyperproduction of gases* (CO_2, H_2) with *abdominal distension* ⇒ **meteorism and flatulence**
3. **Decrease in liver and muscle glycogen reserves** in chronic forms

LACTASE DEFICIENCY malabsorption

- **Definition**: common form of carbohydrate malabsorption characterized by **intolerance to milk/milk derivatives** which is caused by **lactase deficiency** (disaccharidase responsible for lactose hydrolysis at the level of the intestinal epithelium microvilli)
- **Classification**:
  - **primary** deficiency: congenital
  - **secondary** deficiency: bacterial/viral enteritis, celiac disease, inflammatory bowel disease, giardiasis
- **Pathogenesis** - lactase deficiency causes:
  a) **Osmotic and secretory diarrhea** = water attraction and decreased sodium reabsorption, determined by the *presence of lactose in the **small intestine***
  b) **Fermentation dyspepsia**, determined by *bacterial fermentation of unabsorbed lactose in the **large intestine*** with:
     - hyperproduction of acids ⇒ acidic fecal pH
     - hyperproduction of gases ⇒ abdominal distension with *cramps and flatulence*

B. LIPID malabsorption

- **Pathogenesis –** lipid malabsorption is responsible for:
  a. **Weight loss** due to deficient caloric intake
  b. **Steatorrhea** due to increased lipid elimination (> 7g/day) + presence of FA in feces
  c. **Formation of hydroxylated FA** with irritating effect and blocking of water + electrolyte reabsorption in the colon, responsible for **secretory diarrhea**
  d. **Calcium malabsorption** (FA form insoluble salts with calcium) and **increased oxalate absorption** that precipitates at the renal level ⇒ **renal lithiasis**
  e. **Malabsorption of fat-soluble vitamins**:
     - **Vitamin A** ⇒ *hyperkeratosis, hemeralopia*
     - **Vitamin D** ⇒ worsening of hypocalcemia through secondary hyperparathyroidism with risk of **rickets** (children) and **osteomalacia** (adults)
     - **Vitamin K** ⇒ deficiency of activation of vitamin K-dependent coagulation factors ⇒ **hemorrhagic syndrome**

C. PROTEIN malabsorption

- **Pathogenesis –** protein malabsorption is responsible for:
  1. **Decrease in muscle mass** with **body emaciation** - determined by:
     - decrease in hepatic protein synthesis secondary to amino acid absorption deficiency

- increase in protein loss in the intestinal lumen - *exudative enteropathy* secondary to: intestinal inflammation (Crohn's disease), celiac disease and lymphatic obstructions (lymphangiectasia)
- increase in protein catabolism (in conditions associated with carbohydrate malabsorption)

2. **Hypoproteinemia** - with decreased plasma oncotic pressure and *edema*
3. **Putrefaction diarrhea** - by bacterial decomposition of unabsorbed proteins in the **transverse/left colon** ⇒ hyperproduction of NH_3 ⇒ *alkalization of feces*

D. POSTGASTRECTOMY Malabsorption

- **Etiopathogenesis** – malabsorption associated with subtotal gastrectomy performed through 2 surgical procedures:
  1. ***Gastroduodenal anastomosis (Billroth I)*** with loss of the stomach's reservoir function, accelerated gastric evacuation and **postgastrectomy "dumping" syndrome**, with 2 forms:
     a) EARLY dumping syndrome – characterized by:
     - onset 15 – 30 minutes postprandial
     - etiology: rapid emptying of hyperosmolar content into the intestine which causes:
       - ✓ an osmotic gradient with water attraction into the lumen and distension of the intestinal loops immediately postprandial with **abdominal symptoms**
       - ✓ release of vasoactive substances with **vasomotor symptoms**
     - symptoms/signs:
       - ✓ abdominal: abdominal cramps, nausea and vomiting, diarrhea
       - ✓ vasomotor: arterial hypotension and tachycardia (due to hypovolemia) with vertigo, palpitations, sweating

     b) LATE dumping syndrome – characterized by:
     - onset 1 – 3 hours postprandial
     - etiology: rapid absorption of food principles causes hyperglycemia which will lead to a significant secretion of insulin ⇒ **hypoglycemia** with the feeling of overwhelming hunger
     - symptoms/signs:
       - ✓ increased sympathetic stimulation: palpitations, sweating (diaphoresis), pallor
       - ✓ neuronal suffering: difficulty concentrating, fatigue
  2. ***Gastrojejunal anastomosis (Billroth II)*** with closure and shunting of the duodenum which causes:

- stasis in the afferent loop with bacterial proliferation = **blind (afferent) loop syndrome**
- inadequate mixing of gastric chyme entering the *jejunum* with bile/pancreatic juice flowing into the *duodenum*
- deficiency of pancreatic secretion and gallbladder emptying due to the release of low amounts of secretin and, respectively, cholecystokinin-pancreozymin (CCK-PZ) at the duodenal level

E. Malabsorption from BACTERIAL PROLIFERATION in the small intestine

- **Definition**: pathological bacterial colonization of the **small intestine** (from 10^3 normally, up to 10^{11} germs/ml)
- **Etiology**:
  a) **Hypo-/achlorhydria** from: pernicious anemia, long-term treatment with proton pump inhibitors
  b) **Alteration of intestinal motility** from: scleroderma, diabetic neuropathy
  c) **Structural abnormalities** from: postgastrectomy (Billroth II operation), strictures, jejunal diverticulosis or entero-colic fistulas (Crohn's disease),
- **Pathogenesis**: the presence of bacteria leads to:
  - **alteration of bile salt metabolism** because bacteria determine the **deconjugation of bile salts**
  - the deconjugated form will be reabsorbed in the proximal intestine leading to a **decrease in the concentration of bile salts in the jejunum** (where they are necessary for the micellization process) with:
    - ✓ *lipid* malabsorption with **steatorrhea**
    - ✓ malabsorption of *fat-soluble vitamins A, D, K* (see consequences above)
    - ✓ unabsorbed FAs form insoluble salts with calcium with **hypocalcemia and oxaluria**
  - **metabolism of vitamin B_{12}** and/or its **decreased absorption** (by preventing its binding to IF) $\Rightarrow$ **megaloblastic anemia** (but which rarely leads to neurological disorders)
  - **damage to the intestinal mucosa by bacterial toxins** and direct invasion of the intestinal wall leads to **atrophy of intestinal villi** (in severe forms)

F. Malabsorption from EXOCRINE PANCREATIC FAILURE

- **Definition**: decreased function of the **exocrine** pancreas that occurs in conditions of significant parenchymal destruction from: *chronic pancreatitis, pancreatic cancer, cystic fibrosis*
- **Pathogenesis**: the following mechanisms are at the basis of malabsorption:
  - severe deficiency of pancreatic enzymes:
    - ✓ lipase (mainly) $\Rightarrow$ steatorrhea
    - ✓ amylase
    - ✓ trypsin, chymotrypsin
  - HCO_3^- deficiency in the duodenum/jejunum $\Rightarrow$ decreased activity of pancreatic and intestinal enzymes due to the acidic pH

G. Malabsorption from GLUTEN ENTEROPATHY (celiac disease, non-tropical sprue)

- **Definition**: chronic enteropathy present in about 1% of the population that affects the **proximal small intestine (jejunum!)** and is characterized by:
  - gluten intolerance (gliadin) $\Rightarrow$ malabsorption sdr.
  - chronic intestinal inflammation $\Rightarrow$ atrophy of the intestinal mucosa
  - the changes being reversible by eliminating gluten from the diet and reappearing upon its reintroduction.

- **Pathogenesis**: gluten enteropathy is an **autoimmune disease** triggered by the presence of **gliadin** in the intestine (a fragment of indigestible gluten - resistant to the action of gastric, pancreatic and intestinal proteases) in people with a genetic predisposition

a) IMMUNE mechanism with a triggering role in the disease:

The pathogenetic sequence of the immune mechanism involves the following stages:

1. Damage to intestinal epithelial cells by gliadin and penetration of **gliadin** (plus other food antigens and microorganisms) into the lamina propria, release of **IL-15** by intestinal epithelial cells, triggering the immune/inflammatory response
2. Deamidation of **gliadin** under the action of **tissue transglutaminase** (TGt) released by damaged enterocytes
3. Presentation of deamidated gliadin (in association with HLA DQ2 and DQ8 antigens on the surface of antigen-presenting cells - APC) to:
   - ✓ **CD8+ cytotoxic T lymphocytes with their activation and proliferation, damage to the intestinal epithelium** and the persistence of access of toxic gliadin molecules at the intraepithelial level
   - ✓ **CD4+ T helper lymphocytes** that will:
     - induce the **synthesis of IgA class antibodies** (by B lymphocytes transformed into plasma cells) of the type: **anti-gliadin, anti-TGt** and **anti-endomysium** with a diagnostic role
     - produce **cytokines** (e.g., IFNγ) with the worsening of **intestinal mucosal lesions** present at intestinal biopsy

b) Predisposing/associated GENETIC factors:

1. Increased incidence of the disease in first degree relatives of patients with celiac disease and disease concordance in monozygotic twins
2. Presence of HLA-DQ8 and DQ2 antigens (with a role in the immunological recognition of gliadin)
3. Association with other autoimmune diseases: ***dermatitis herpetiformis*** - most frequently, through cross-reacting anti-epidermal transglutaminase antibodies, type I diabetes, autoimmune thyroiditis, Sjögren's syndrome, etc.

c) Mechanisms of malabsorption:

1. Biochemical/cellular functional abnormalities:

- decreased esterification of FA into triglycerides $\Rightarrow$ lipid malabsorption $\Rightarrow$ **steatorrhea**
- decreased activity of intestinal disaccharidases (lactase) $\Rightarrow$ **lactose intolerance**

2. Abnormalities of intestinal function regulation:

- decreased release of *secretin* and *CCK-PZ* $\Rightarrow$ decreased biliary and pancreatic secretion $\Rightarrow$ lipid malabsorption $\Rightarrow$ worsening of steatorrhea

3. Diarrhea by dual mechanism:

- **secretory**: by *decreased absorption of water & electrolytes* at the level of the damaged mucosa of the small intestine
- **osmotic**: by *attracting water into the lumen* due to decreased absorption of the *osmoactive compound (lactose)*

4. Fermentation dyspepsia at the level of the **large intestine,** manifested for unabsorbed **carbohydrates** (lactose) and **lipids** with:

- hyperproduction of lactic acid and fatty acids ⇒ acidic fecal pH
- gas hyperproduction ⇒ abdominal distension with *cramps and flatulence*

- **POSITIVE DIAGNOSIS** - ***intestinal mucosal biopsy*** that reveals:
  - lymphocytic infiltrate in the lamina propria of the intestinal mucosa
  - atrophy of intestinal villi (partial, subtotal, total villous atrophy)
  - crypt hyperplasia

INFLAMMATORY BOWEL DISEASE (IBD)

DEFINITION: the term *inflammatory bowel disease* (IBD) defines *two chronic recurrent diseases* with *unpredictable evolution* that variably affect the *small and large intestine*:

- **Crohn's disease** or **regional enteritis/terminal ileitis** - can extend to *any level of the digestive tract* and the inflammation is *transmural*
- **ulcerative colitis** or **ulcerative-hemorrhagic colitis (UHC)** - affects the *colon* and *rectum* and the inflammation is localized to the *mucosa*

ETIOLOGY: multifactorial, with the involvement of the following factors:

1. GENETIC factors - explain only about 1/5 of IBD cases

Their involvement is suggested by:

- ethnic differences (the risk is higher in whites and Ashkenazi Jews)
- familial aggregation (in 20% of cases, 1 in 5-6 patients)
- incidence of the disease in monozygotic twins (20-50% concordance in Crohn's disease and 10% in UHC)
- association with genetic syndromes (Turner syndrome)

Genetic factors have been studied in terms of mutations in genes responsible for:

a) Triggering the chronic inflammation process:

In IBD, gene *mutations/polymorphisms* responsible for triggering intestinal inflammation have been identified. The most intensively studied is the mutation of the **NOD2 gene** (Nucleotide Oligomerization Domain 2) which encodes the synthesis of a protein with the role of *intracellular sensor of bacterial peptides* (peptidoglycan in the cell wall). NOD2 is present in intestinal epithelial cells and macrophages. NOD2 gene mutations are frequently associated with Crohn's disease with ileal location, with onset at a young age and predisposition for the formation of strictures.

b) Impairment of the autophagy process:

Autophagy is important in cellular homeostasis by degrading and recycling damaged organelles and by ensuring antimicrobial resistance. In IBD, *mutations in genes specific to proteins responsible for the autophagy process* have been identified – being associated with an increased risk of Crohn's disease.

Impairment of the autophagy process is responsible for:

- ✓ decreased degradation of pathogenic microorganisms
- ✓ altered activity of dendritic cells (antigen presenting cells)
- ✓ decreased production of antibacterial peptides called defensins by Paneth cells

Consequence: *alteration of the intestinal barrier function* and *translocation of bacteria from the lumen to the intestinal wall*

2. ENVIRONMENTAL factors:

a) **Smoking**: increases the risk and severity (recurrence rate) in Crohn's disease but has the opposite effect in ulcerative colitis which, paradoxically, has a late onset and is less severe in smokers (and quitting smoking leads to relapses)
b) **Consumption of NSAIDs**: increases the risk of Crohn's disease and recurrences in those in whom the disease has been diagnosed
c) The **"hygiene" hypothesis**: the poor population in overcrowded territories is at reduced risk of developing Crohn's disease. In the context of increased hygiene in industrialized countries, we witness a reduction in exposure (from childhood) to infectious agents (microbes, helminths), which prevents the normal maturation of the immune system with the deficient development of regulatory T lymphocytes and the triggering of an *abnormal immune response to the passage of bacteria through the damaged epithelial barrier*. This hypothesis (partially accepted today) is supported by the fact that the onset of the disease frequently follows an episode of acute gastroenteritis.
d) **Dietary factors**: increased consumption of unsaturated fats and refined sweets and the deficiency of vegetable fibers in the diet favor the occurrence of IBD
e) **Psychological factors**: chronic psychological stress and depression favor recurrences of IBD

Observation!
Appendectomy (performed before the age of 20), paradoxically, protects against the development of ulcerative colitis and has a favorable effect on its evolution, but apparently increases the risk of developing Crohn's disease.

3. Intestinal DYSBIOSIS: in patients with IBD there has been reported:

a) a reduction in the diversity of commensal microbial flora (normally, there are 300-400 distinct species)
b) increase in the concentration of *E.coli and Bacteroides* and decrease in that of *bifidobacteria*
c) increase in the number of bacteria (E.coli) adherent to the intestinal epithelium (ileum!) with invasion of the mucosa in Crohn's disease

- **Pathogenesis**:

The central pathogenic element in IBD consists in the **triggering of an abnormal immune response in the intestinal mucosa to bacterial antigens** (and food antigens?) against the background of ***intestinal dysbiosis*** and ***alteration of the intestinal epithelial barrier***.
The immune response in IBD has the following characteristics:

✓ is triggered by the presentation of bacterial antigens to CD4 lymphocytes by dendritic cells
✓ is mediated by **Th1** lymphocytes and the **Th17 subset** in Crohn's disease
✓ is mediated by **Th2** lymphocytes in ulcerative colitis
✓ is associated with a **chronic inflammatory reaction** mediated by **TNFα, IL-1** and **IL-6**

The pathogenetic sequence of the **chronic immune-inflammatory response** includes the following stages:

1. **Interaction of bacteria with Toll-like receptors** on the **surface of enterocytes and *intracellular* NOD proteins and processing of bacterial antigens at the level of dendritic cells**
2. **Migration of dendritic cells to Peyer's patches** and **mesenteric lymph nodes**, presentation of antigens to **naive T lymphocytes** and their differentiation into **effector and regulatory T lymphocytes**:

   a) The pathological immune response in CROHN'S DISEASE consists of:
   - **proliferation of <u>Th1 lymphocytes</u>** that will secrete:
     - ✓ **IFN-γ** responsible for the **activation of macrophages**
     - ✓ **TNF-α** responsible for: i) **apoptosis of intestinal mucosa cells**, ii) increased expression of **adhesion molecules** at the vascular endothelium level and **chronic intestinal inflammatory reaction** and iii) **systemic symptoms** (loss of appetite, asthenia, fever, bone disorders)
   - **proliferation of the <u>Th17 helper</u> T lymphocyte subclass** that secrete **IL-23**, with **chemotactic effect** and **activation of neutrophils** with the release of lysosomal enzymes and cytotoxic effect, responsible for **ulcerations of the intestinal mucosa**.

   b) The pathological immune response in ULCERATIVE COLITIS consists of:
   - **proliferation of <u>Th2 lymphocytes</u>** that will secrete:
     - ✓ **IL-13** with a lesional effect at the intestinal epithelium level
     - ✓ **IL-4** responsible for the differentiation of B lymphocytes into Ab-secreting plasma cells and
     - ✓ **IL-5** responsible for the activation of eosinophils.

3. Cytokines released by activated cytotoxic-effector T lymphocytes stimulate macrophages to secrete **TNF-alpha, IL-1 and IL-6** ⇒ a **vicious cycle of chronic inflammation** maintenance

Observation!

The central role of the chronic inflammatory/immune response in the pathogenesis of these diseases is proven by the fact that the treatment is based on therapies aimed at diminishing these responses, namely: *anti-inflammatory medication - corticosteroid therapy* (prednisone, prednisolone), *immunosuppressive therapy* (azathioprine, 6-mercaptopurine, methotrexate) and in recent years, *biological therapy* (monoclonal antibodies: i) anti-TNFalpha - infliximab, certolizumab, adalimumab, ii) anti-adhesion molecules - natalizumab, vedolizumab and iii) anti-IL12/IL-23 - ustekinumab).

- **Manifestations and complications** (Tab. 3):

Table 3. Manifestations and complications of inflammatory bowel diseases.

	Crohn disease	**Ulcerative colitis**
Primary location	Terminal ileum ± colon (right!)	Rectum + colon (left!)
Inflammation	Granulomatous	Ulcerative and exudative
Lesions	Transmural	Limited to the mucosa
Affectation	Deep and segmental (with portions of healthy bowel between lesions – "cobblestone" appearance)	Superficial and continuous ("pseudopolyp" appearance)
Onset	Insidious, progressive	Acute, severe

Diarrhea	Watery (with steatorrhea in the ileal location) - abundant stools and diffuse/localized abdominal pain	With mucus and blood (+ independent rectal bleeding) - frequent stools in small quantities & tenesmus
Local complications	*Common:* deep ulcerations, fissures, fibrosis with strictures, fistulas into neighboring organs (rectum, bladder, vagina), abdominal abscesses, perianal lesions	*Rare:* superficial ulceration, toxic megacolon, intestinal perforation
Malabsorption syndrome	Yes	No
Postsurgical recurrence	Yes	No
Dysplasia/Malignancy	Yes, in case of colon affectation	Yes

IRRITABLE BOWEL SYNDROME (IBS)

DEFINITION: Irritable Bowel Syndrome (IBS) is the most common **<u>functional</u>** digestive disorder characterized by **abdominal pain** or **discomfort at least 1 day/week, in the last 3 months** + ≥ **2 of the following**:

- ✓ ***IMPROVEMENT of symptoms after defecation***
- ✓ onset of symptoms with change in the ***ASPECT of the stool***
- ✓ onset of symptoms with change in the ***FREQUENCY of defecation***

The syndrome is associated with **diarrhea, constipation or their alternation (mixed)** & other symptoms:

- ***dyspeptic sdr.*** (bloating, flatulence, feeling of imminent defecation, incomplete evacuation, increased mucus excretion)
- ***headache, depression, anxiety, asthenia/lethargy*** against the background of psychosocial/emotional stress

in the **ABSENCE of organic lesions on colonoscopy**

The disease affects 10-20% of the population of developed countries, is more common in women (W:M ratio = 2:1) age of diagnosis between 20-40 years.

PATHOGENESIS - *multifactorial, incompletely elucidated* - 3 major mechanisms:

1. **Dysfunction of the GUT-BRAIN AXIS** favoring:
   - **a) Alteration of intestinal MOTILITY** with:
     - ✓ increased motility / accelerated transit ⇒ clinical form with diarrhea predominance
     - ✓ localized spasticity ⇒ clinical form with constipation predominance
     - ✓ their alternation ⇒ mixed clinical form
   - **b) Visceral HYPERSENSITIVITY** due to altered communication between the enteric (intrinsic) nervous system and the CNS
   - **c) Exaggerated response of the CNS to STRESS**

- ✓ triggering of symptoms after negative events ⇒ against the background of anxiety, depression

2. **Intestinal DYSBIOSIS = the quantitative and/or qualitative modification of the composition of the intestinal bacterial flora** and/or the instability of its composition thereby influencing the response to stress has led to the expansion of the concept of **dysfunction of the MICROBIOME-GUT-BRAIN AXIS**
3. **PATHOLOGICAL IMMUNE-INFLAMMATORY response** in irritable colon with **postinfectious onset**, characterized by:
   a) Moderate CHRONIC INFLAMMATION with:
   - **proliferation of enterochromaffin cells** and **increased release of serotonin** (5-HT) responsible for **visceral hypersensitivity** and **increased intestinal motility** ⇒ clinical form with predominance of diarrhea
   - activation flare-ups objectified by **increased plasma levels of proinflammatory cytokines** (IL-1, IL-6, TNFα)

   b) Local PATHOLOGICAL IMMUNE RESPONSE demonstrated by:
   - infiltrate with activated T lymphocytes and mast cells in the colonic mucosa (proportional to the severity of the painful flare-up)

DIVERTICULOSIS

DEFINITION:
- saccular herniation of the colonic mucosa through the muscular layer at the site of penetration of a blood vessel
- most frequently located in the **sigmoid colon**

PATHOGENESIS:

1. Structural factors = connective tissue abnormalities:
- **primary/genetic** (Ehlers-Danlos sdr., Marfan sdr., people aged ≤ 40 years)
- **secondary** to aging (collagen deposition, thickening of the circular muscles) + fiber-deficient diet + sedentary lifestyle

2. Functional factors = increased intraluminal pressure:
- cause: *increased contractions of the colon muscles* (especially the left/descending and sigmoid) due to a fiber-deficient diet, responsible for a reduced volume of feces
- effect: *abdominal pain relieved by defecation*

CLINICAL manifestations:
- asymptomatic
- irritable bowel syndrome:
  - ✓ alternating diarrhea/constipation
  - ✓ discomfort in the lower abdomen (bloating, flatulence)

COMPLICATIONS:

1. **Diverticulitis** - inflammation of the diverticular wall due to *retention of a fecaloma* ± *overlapping bacterial infection* is responsible for:
   - intramural/pericolic abscess manifested by: pain in the left iliac fossa, nausea and vomiting, low fever, leukocytosis with neutrophilia
   - perforation ⇒ peritonitis
   - penetration with fistulization into neighboring organs: urinary bladder, small intestine
2. **Diverticular hemorrhage** - the main cause of lower digestive bleeding at ages ≥ 60 years, painless, most frequently by erosion of a blood vessel by a fecaloma

5. PATHOPHYSIOLOGY OF THE RENAL SYSTEM

STRUCTURE & FUNCTION OF THE GLOMERULAR MEMBRANE – Brief physiological overview

The glomerular filtration membrane is made up of **3 layers:**

1. ENDOTHELIAL layer *(lamina fenestrata):*
- forms a *loose mesh network* (70-100 nm)
- is an **effective barrier** for the **formed elements of the blood**
- is covered by *glycocalyx* containing **negatively charged** proteoglycans and glycosaminoglycans with the role of **repelling plasma albumins** (which dissociate as anions)

2. Glomerular BASEMENT MEMBRANE (GBM):
- is made up of **3 layers**: *lamina rara interna, lamina densa and lamina rara externa* (in electron microscopy)
- contains 4 major components (*laminin, type IV collagen, nidogen* and a *heparan sulfate proteoglycan* = agrin - the latter conferring the *negative* electrical charge of the GBM)
- creates an effective barrier for **proteins with large MW** (> 70 kDa, > 8 nm diameter) and for **albumins** (MW = 67 kDa, diameter approx. 3.6 nm) which are **negatively charged**

3. EPITHELIAL layer:
- consists of **podocytes** with cytoplasmic extensions (*foot processes*) with which they bind to the GBM via adhesion molecules
  - between the foot processes there are *tunnel-like spaces* called **filtration slits** (20-30 nm)
  - the filtration slits are covered by a **thin diaphragm** with a complex structure - made up of numerous proteins - mainly **nephrin** (but also podocin, P-cadherin, CD2AP protein, etc.) and which is considered the main barrier to the loss of **proteins with small MW**
  - on the surface of the foot processes there are acidic glycoproteins (sialoglycoproteins) which give them an intense **negative charge**, thus having a role as a barrier for **albumin**

Observation!
The **mechanical and electrostatic barrier function** of the glomerular filtration membrane prevents the loss of **formed elements** and **proteins** in the urine. Therefore, in conditions of its alteration, such as in mild/incipient glomerular nephropathies, isolated urinary changes characterized by **asymptomatic hematuria** and/or **proteinuria** occur.

GLOMERULAR NEPHROPATHIES (GLOMERULOPATHIES)

DEFINITION: **acute or chronic renal diseases**, characterized by:
i) **SYMMETRIC** renal damage
ii) **exclusive or predominant damage** to the renal **GLOMERULI**
iii) concomitant damage to the renal **INTERSTITIUM**
iv) **HEMODYNAMIC consequences** responsible for the worsening of the glomerular dysfunction

MANIFESTATIONS – depending on the predominantly affected cells, glomerulopathies present as:
- **NEPHROTIC syndrome** – predominant damage to **podocytes**
- **NEPHRITIC syndrome** – predominant damage to **endothelial and mesangial cells**
- **MIXED, NEPHROTIC & NEPHRITIC syndrome** – damage to all three cellular components
- Isolated proteinuria, hematuria (or both)

CLASSIFICATION:

- **PRIMARY glomerular nephropathies** → the kidney is the only organ affected
- **SECONDARY glomerular nephropathies** → renal damage is:
  i) a component of a ***systemic disease*** → e.g., **HT and diabetes mellitus** = the **most common causes of chronic kidney disease (CKD)**, SLE, vasculitis
  ii) consequence of an ***infection***
  iii) ***toxic/drug induced***

PATHOGENESIS

The pathogenesis of glomerular lesions usually involves the intervention of 2 types of mechanisms:

- **primary** mechanisms → responsible for the **INITIATION** of glomerular lesions
- **secondary** mechanisms → responsible for the **PROGRESSION** of glomerular lesions

I. Mechanisms of INITIATION of glomerular lesions

Glomerular lesions are triggered by **IMMUNE mechanisms** in both primary as well as most secondary glomerulopathies, with the participation of **humoral and cellular immunity** (Tab. 4).

The ***humoral immune response*** is controlled by **Th2 lymphocytes** and leads to the formation of **Ig deposits and complement activation at the glomerular level**, while the ***cellular immune response*** is controlled by **Th1 lymphocytes** and leads to the **infiltration of the glomeruli with mononuclear cells (lymphocytes and macrophages).**

Table 4. Immune mechanisms responsible for the **initiation** of glomerular lesions.

Mechanisms dependent on humoral immunity
Formation of antibodies against glomerular Ag (type II HS) with the *generation of in situ immune complexes* in which the antigens (Ag) are:
Intrinsic Ag
From the glomerular BM (collagen IV) → **GN via anti-GBM antibodies = Goodpasture syndrome** characterized by **glomerulonephritis ± pulmonary vasculitis** **From podocytes → membranous GN**
Extrinsic Ag that were "planted" glomerularly
Endogenous: DNA and nuclear proteins, Ig, immune complexes, protein aggregates **Exogenous: Bacterial, viral, parasitic Ag; spirochetes; drugs (bisphosphonates, antiangiogenics etc.)**
Formation of cytotoxic antibodies with *direct damage production* (type II HS)
Anti-mesangial Ag antibodies → destruction of mesangial cells
Anti-endothelial Ag antibodies → endothelial damage and capillary thrombosis
Anti-epithelial Ag antibodies → epithelial lesions, foot processes fusion, podocyte detachment from GBM
Deposition of circulating immune complexes (type III HS) containing Ag:
Endogenous: nuclear DNA (GN from SLE), tumor cells (GN from lung, colon

cancers) **Exogenous: bacterial (poststreptococcal GN), viral (GN from hepatitis B, C), parasitic (malaria), spirochetes (syphilis)**
Mechanisms dependent on cellular immunity (HS type IV)
Activation of T lymphocytes and NK cells → leads to the progression of glomerular nephropathies by maintaining chronic inflammation.

(Ag – antigens, GN – glomerulonephritis, HS – hypersensitivity, GBM - glomerular basement membrane)

Observation!

Depending on their size and electrical charge, Ag and immune complexes (IC) can be deposited at 3 levels:

- **subendothelial** (between endothelial cells and the GBM)
- **subepithelial** (between the GBM and podocytes)
- **mesangial**

Cationic Ag cross the BM (polyanionic) and tend to be deposited at subepithelial level, anionic Ag at the subendothelial level, and neutral molecules at the mesangial level.

IC formed *in situ* are frequently located at the **subepithelial** level (between GBM and podocytes), while **circulating IC** diffuse passively at the **subendothelial** (between endothelial cells and GBM) and **mesangial** levels but can also be deposited at the subepithelial level.

Local formation/deposition of immune complexes at the glomerular level will induce glomerular lesions **associated or not with an INFLAMMATORY reaction** (release of inflammatory mediators of cellular and plasma origin – Tab. 5) with variable consequences, as follows:

- **formation/deposition** of immune complexes at the **subepithelial** level and the **absence of an acute inflammatory reaction** (which would favor healing), will determine **persistent proteinuria** for months or even years → GLOMERULOPATHY clinically manifested by ***nephrotic syndrome***.
- **formation/deposition** of immune complexes at the **subendothelial**, **GBM** or **mesangial** level is associated with an **important acute inflammatory reaction** → GLOMERULONEPHRITIS (GN) clinically manifested by ***nephritic syndrome***

Observation!

In nephritic sdr., inflammation can act as a "double-edged sword": if controlled, it allows for accelerated healing (acute GN), if exacerbated or persistent, it will worsen glomerular lesions (chronic GN).

Table 5. Consequences of activation of immune mechanisms at the glomerular level.

1. Activation of the COMPLEMENT system with the generation of: ○ **Anaphylatoxins - C3a and C5a → vasodilation and capillary hyperpermeability** and **chemotactic effect (C5a)** → attraction of leukocytes to the glomerular level with hypercellularity ○ **Membrane attack complex (C5b-9)** → damage to the GBM with **proteinuria** and **hematuria**
2. Activation of PHAGOCYTES with the release of: ○ **Arachidonic acid mediators** → responsible for hemodynamic disorders ○ **Lysosomal enzymes (collagenase)** → GBM damage ○ **Proinflammatory cytokines (IL-1 and TNF)** → increased leukocyte adhesion ○ **Reactive oxygen species (ROS)** → aggravation of cellular damage

3. Activation of MESANGIAL cells with the release of:
- **Proinflammatory cytokines** → amplification of the inflammatory reaction **– exudative GN**
- **Growth factors** responsible for:
  - ✓ *GBM thickening* **– membranous GN**
  - ✓ *proliferation of the cellular component* **– proliferative GN**
  - ✓ *extracellular matrix synthesis* **– sclerosing GN**
  - ✓ *collagen deposition* **– fibrosing GN**

4. Activation of platelet aggregation/coagulation with fibrin deposition responsible for:
- **Capillary thrombosis**
- **Fibrin deposition in Bowman's capsule** with stimulation of epithelial proliferation and crescent formation → **Rapidly progressive GN**

II. Mechanisms of PROGRESSION of glomerular lesions

Immune mechanisms and pro-inflammatory mediators are responsible for the *initiation of glomerular lesions* which, if persistent, lead to the development of **chronic** glomerular nephropathies. Identification of the factors responsible for the *progression of glomerulopathies* is of major therapeutic importance (Tab. 6).

Table 6. Mechanisms responsible for the progression of glomerular lesions.

1. Development of a focal or diffuse glomerulosclerosis process	
Cause: ▪ **compensatory hypertrophy of the remaining glomeruli** to compensate for the loss of nephrons ▪ **glomerular membrane damage** with **proteinuria**	**Consequences:** ▪ **hemodynamic changes** with **intraglomerular hypertension ± systemic hypertension** ▪ **protein hyperfiltration** with **their accumulation in the mesangial space** ▪ **mesangial cell hyperplasia and extracellular matrix deposition** with **focal or diffuse glomerulosclerosis**
2. Tubulointerstitial fibrosis	
Causes: ▪ **ischemia of the tubular system** located distal to the sclerosed glomeruli ▪ **proteinuria** that directly determines the **activation of tubular cells** that will secrete: – proinflammatory cytokines ⇒ **inflammation of the adjacent interstitium** – growth factors ⇒ **cell proliferation**	**Consequences:** ▪ **tubulointerstitial fibrosis**

GLOMERULAR SYNDROMES

I. NEPHROTIC SYNDROME

DEFINITION: **complex of signs/symptoms** with ***progressive*** onset and ***slow remission***, determined by the alteration of the barrier function of the glomerular membrane, with predominant damage to *podocytes*, in the **ABSENCE of inflammation** – it is a **glomerulopathy and a podocytopathy.**

CLASSIFICATION:

A. PRIMARY nephrotic syndrome (idiopathic) - represents **95%** of cases in **children** and **60%** of cases in **adults**.

Clinical forms:

The most common glomerular nephropathies that are accompanied by nephrotic syndrome (NS) are:

1. **Glomerulopathy with minimal changes (lipoid nephrosis)**
2. **Focal and segmental glomerulosclerosis**
3. **Membranous glomerulopathy**

Table 7. Clinical forms of **primary** nephrotic syndrome.

Clinical form	Particularities
1. Minimal change glomerulopathy	– **most common cause of NS in children** (boys, under 10 years old) – **pathogenesis: podocyte alteration** – **good response to corticosteroid therapy and favorable prognosis** (less than 5% develop CKD in 25 years).
2. Focal and segmental glomerulosclerosis	– **pathogenesis: presence of a circulating factor** (cytokine 1 cardiotrophin-like?) responsible for **massive non-selective proteinuria** – **reduced response to corticosteroid therapy, requires immunosuppressive therapy and has a reserved prognosis** (over 50% develop CKD within 10 years).
3. Membranous glomerulopathy	– **most common cause of NS in adults** (men between 40 - 50 years old) – **pathogenesis: autoAb (IgG) anti-podocyte Ag** – **reduced response to corticosteroid therapy, requires immunosuppressive therapy and has a reserved prognosis** (40% - slow evolution towards CKD)

B. Pure SECONDARY nephrotic syndrome - is a *secondary* glomerular damage within a systemic disease:

- **Metabolic diseases: diabetes mellitus*** (diabetic nephropathy), amyloidosis

C. SECONDARY mixed nephrotic and nephritic syndrome is found in:

- **Immune diseases: SLE**, rheumatoid arthritis, Schonlein-Henoch purpura, cryoglobulinemias
- **Infections: i) bacterial** - e.g., GN associated with bacterial endocarditis and **ii) viral** - hepatitis B and C v., Epstein-Barr v., herpes v.
- **Drug-induced**: NSAIDs, lithium, bisphosphonates, antibiotics, interferon, penicillamine or **drugs**: heroin
- **Neoplasias**: GN associated with solid tumors (stomach, colon, breast, bronchial), leukemias, lymphomas, multiple myeloma

***Diabetic nephropathy**

- **Particularity: diabetic kidney disease is the most common cause of secondary nephrotic syndrome in adults**, and is also the **main cause of chronic kidney disease in clinical practice**.
- **Pathogenesis**: glomerular lesions in diabetic nephropathy can determine, depending on the severity: **persistent albuminuria, nephrotic syndrome and chronic kidney disease, in advanced stages.**

Glomerular nephropathy is a form of **diabetic microangiopathy** (along with diabetic retinopathy) that typically develops after 10-20 years of DM evolution and is based on 2 major mechanisms:

- ***hemodynamic changes***, characteristic of the initial stages, in which intraglomerular hypertension (vasodilation of the afferent arteriole and vasoconstriction of the efferent one) determines an **increase in the glomerular filtration rate**; hyperfiltration leads to *podocyte damage* (apoptosis and detachment with early podocyteuria), *proteinuria* and *increased protein storage at the mesangial level* with increased extracellular matrix and glomerular size - **glomerular hypertrophy**;
- ***metabolic changes***, in which hyperglycemia is responsible for the production of intra-/ extracellular and plasma advanced glycation end products (AGE), which cause **non-enzymatic glycation** of: **i) GBM proteins** - with alteration of its electrostatic (reduction of negative electrical charges) and mechanical barrier properties; **ii) mesangial proteins** - with *diffuse mesangial proliferation* and **iii) vascular proteins** with ischemia by narrowing of the vascular lumen (vascular sclerosis), changes that will lead over time to the installation of the **glomerulosclerosis** process.

The **characteristic lesions** of diabetic nephropathy consist of the **triad**:

- ***GBM thickening***
- ***mesangium expansion***
- ***intercapillary nodular glomerulosclerosis*** (Kimmelstiel-Wilson)

THE **POSITIVE DIAGNOSIS of nephrotic syndrome** includes:

1. **Proteinuria > 3.5 g/day**
2. **Hypoalbuminemia < 3 g/dL**
3. **Edema (localized, but also possibly generalized – anasarca)**
4. **Hyperlipemia + lipiduria**
5. **Hypercoagulability**

1. Proteinuria

It is characterized by the **elimination of proteins with a high molecular weight**, in an amount of **> 3.5 g/day** (up to 15 g/day) and can be:

- ***selective*** in **pure, functional NS**, characterized by **minimal glomerular lesions** with ***exclusive loss of albumins (albuminuria)***
- ***non-selective*** in **impure, organic NS** characterized by **advanced glomerular lesions** with **loss of albumins and globulins** associated with HT and microscopic hematuria

Consequences of proteinuria:

- **hypoalbuminemia** (< 3 g/dL) and **edema**
- **dysproteinemia** with: **decreased albumin and γ-globulins (IgG) and increased α-2 and β globulins**
- **urinary loss of functional proteins** responsible for the consequences presented in Tab. 8.

Table 8. Consequences of **proteinuria** in nephrotic syndrome.

Proteins excreted in urine	Functional consequence
IgG and complement components (f. B and D) with opsonization role	**Increased susceptibility to infections** with encapsulated bacteria (staphylococci, pneumococci) & risk of peritonitis with Gram-negative bacteria
Transferrin **Erythropoietin**	**Microcytic hypochromic anemia** resistant to iron treatment
Antithrombin III **(± increased hepatic synthesis of coagulation factors, which are globulins)**	**Hypercoagulability and risk of thrombotic accidents:** renal vein thrombosis, deep vein thrombosis (with risk of pulmonary embolism)
Drug transport proteins	Increased free plasma fraction of drugs leading to **toxic drug manifestations even at therapeutic concentrations**
Trace elements (zinc, copper) transport proteins	**Increased susceptibility to infections** by decreased phagocytosis and cellular immunity
25-hydroxy cholecalciferol transport protein	**Decreased serum levels of 1,25 dihydroxy-cholecalciferol** (active vit. D) **and hypocalcemia**. ***Observation!*** However, in patients with NS, stimulation of parathyroid hormone secretion with secondary hyperparathyroidism and osteomalacia is not found, these changes being characteristic of advanced CKD.
Transcortin, the corticosteroid-binding protein	Impairment of the distribution of **exogenously administered corticosteroids**, favoring the faster onset and at lower doses of iatrogenic Cushing's syndrome
Thyroxine-binding globulin	Decreased serum T4 hormone levels → **hypothyroidism** in some patients

2. Hypoalbuminemia

It is based on 3 mechanisms (combined in varying degrees):

a) Massive loss of protein via the kidneys: proteinuria > 3.5 g/day

b) Increased renal protein catabolism: increased glomerular filtration causes a marked increase in albumin reabsorption (but also some globulins) at the proximal convoluted tubule (PCT) level which is accompanied by increased catabolism.

c) Inadequate hepatic synthesis of albumins - although **increased compared to normal**, it cannot compensate for the increased tubular loss/catabolism.

3. Edema

It is the **characteristic symptom** in patients with nephrotic syndrome, *localized* at the level of the face and calves (painless, soft, fluffy, pitting - leaving a pit or a well) or *generalized* (anasarca) in severe forms.

- **PATHOGENIC mechanism**: the determining factor of edema is **hypoalbuminemia** with a **decrease in plasma oncotic pressure → 2 consequences:**
  - *water passage from the vessel into the interstitium* (edema)
  - *decrease in effective arterial volume* (hypovolemia) which triggers increased renal sodium and water reabsorption through:
    - ✓ activation of the *RAA system* primarily stimulates distal tubular sodium reabsorption
    - ✓ increased *sympatho-adrenergic stimulation* with renal vasoconstriction, potentiated by angiotensin II, which leads to a marked decrease in GFR and increased proximal tubular sodium and water reabsorption
    - ✓ increased *ADH release* with increased water reabsorption at the distal and collecting tubules level

4. Hyperlipemia and lipiduria

They are frequently encountered in patients with nephrotic syndrome. Hyperlipemia is the consequence of both ***increased production*** and ***decreased catabolism*** of serum lipoproteins.

It manifests itself through:

a) Alteration of LDL and cholesterol metabolism:

- ✓ **Increased LDL synthesis** - including small and dense LDL with the highest atherogenic risk - by **increasing hepatic cholesterol synthesis** due to *increased HMG-CoA-reductase activity*
- ✓ **Deficit of LDL catabolism** due to functional deficiency of LDL receptors - by *increasing the expression of the enzyme PCSK9* (proprotein convertase subtilisin/kexin type 9) which decreases LDL metabolism via internalization and degradation of LDL receptors

b) Alteration of VLDL and triglyceride metabolism:

- ✓ **Deficit of VLDL (and chylomicrons) catabolism** determined by inhibition of lipoprotein lipase (by angiopoietin-like 4 protein released by adipose, muscle, skeletal and heart tissue in the presence of increased levels of FFA)

c) Increased Lp(a) synthesis

Consequences of hyperlipemia:

- increased atherogenic risk
- increased incidence of coronary heart disease

5. Hypercoagulability

It has a multifactorial etiology, being determined by **4 processes:**

a) Increased hepatic synthesis of coagulation factors secondary to increased hepatic synthesis of globulins (stimulated by hypoalbuminemia):
- ✓ **hyperfibrinogenemia**
- ✓ moderate increase in the concentration of **factors II, V, VII, VIII, X**

b) Increased platelet aggregation due to increased sensitivity of platelets to TxA2

c) Decrease in soluble inhibitors of coagulation factors:
- ✓ urinary loss of antithrombin III
- ✓ decreased concentration/activity of proteins C and S

d) Fibrinolysis deficiency:
- ✓ increased α1-antiplasmin

Consequences of hypercoagulability:
- predisposition for spontaneous venous thrombosis
- risk of pulmonary embolism

II. NEPHRITIC SYNDROME

DEFINITION: **complex of signs/symptoms** with ***rapid*** **onset**, characterized by **immune-mediated glomerular damage** (humoral and cellular), predominantly of *mesangial and endothelial cells* (possibly also of epithelial ones), associated with **significant acute inflammation** – it is a **glomerulonephritis**.

CLINICAL FORMS:

The most common glomerular nephropathies that are accompanied by nephritic syndrome are (Tab. 9.):

1. **Acute poststreptococcal GN**
2. **Rapidly progressive GN**
3. **IgA nephropathy (Berger's disease)**

Table 9. Clinical forms of nephritic syndrome.

Clinical form	Particularities
1. Poststreptococcal glomerulonephritis **Non-streptococcal infectious glomerulonephritis** is more common today, being caused by: i) bacteria (staphylococcus, pneumococcus), ii) viruses (hepatitis B and C, chickenpox, Epstein-Barr, mumps) and iii) parasites (toxoplasmosis, trichinosis, malaria)	– **the most common form of nephritic syndrome in children and young people** – **prototype of acute diffuse GN** triggered 1-3 weeks (latency period is necessary for Ab synthesis and IC formation) after a respiratory (e.g. pharyngitis) or cutaneous (impetigo) infection with *group A hemolytic streptococcus* – **glomerular deposition of CIC** containing bacterial Ag, with local complement activation and *decreased serum C3* – **the prognosis is excellent in young people** (spontaneous remission within 6-8 weeks in 95% of cases)

2. Rapidly progressive "crescent" glomerulonephritis - deposition in the space bounded by Bowman's capsule of inflammatory cells and fibrin	– **the most <u>severe</u> form of glomerular damage** – **pathogenesis: 3 types** according to the immune mechanism: ✓ **type I** caused by **anti-glomerular BM antibodies** (type II HS, Goodpasture sdr.) ✓ **type II** caused by the **deposition of circulating immune complexes at the glomerular level** (type III HS) **with the formation of crescents** that obstruct Bowman's space ✓ **type III** (pauci-immune) in which **antibodies against the cytoplasm of neutrophils (ANCA)** are present, being associated with systemic vasculitis – in the absence of treatment (plasmapheresis, corticosteroids), **accelerated evolution (months) towards CKD with renal failure and death**
3. IgA nephropathy (Berger's disease)	– **the most <u>common</u> form of <u>chronic GN</u>** and **cause of <u>recurrent hematuria</u> in young adults** – onset *synchronous* with a mucosal infection (respiratory!) that induces IgA hypersecretion – **pathogenesis: formation of CIC with IgA and their deposition at the mesangial level** with: i) complement activation - C3 deposits, ii) mesangial hyperproliferation, iii) segmental glomerulosclerosis and in advanced forms, iv) tubular atrophy and interstitial fibrosis – **good prognosis** (25% evolve towards CKD)

- **Positive diagnosis** of nephritic syndrome includes:
  1. Microscopic or macroscopic **hematuria**
  2. **Proteinuria below 3 g/day**
  3. Transient **oliguria**
  4. **Azotemia** - increased serum urea and creatinine
  5. **HT**
  6. **Edema** - initially *periorbital* (morning, white, soft, puffy - leaves a pit, painless), later characterized by declivity; peripheral or generalized (anasarca)

1. Hematuria

It is characterized by:

- presence of **more than 3 red blood cells/microscopic field**
- **erythrocyte dysmorphism**: more than 30% of erythrocytes are dysmorphic (pale and small cells, acanthocytes) due to mechanical or osmotic stress exerted when erythrocytes pass through the nephron
- **association with erythrocyte casts** by incorporation of erythrocytes into the matrix of the Tamm-Horsfall protein (urinary glycoprotein normally secreted by the cells of the ascending limb of the Henle loop and the distal tubules)
- **specific color of the urine** ("tea or cola colored") determined by the degradation of erythrocytes by stagnation in urine
- **association with the presence of PMN neutrophils in urine**

2. Proteinuria

- is of **glomerular type, moderate (1-3 g/day) and selective - albumins are lost (100%),** without globulins
- **cause:** ***hyperpermeability of glomerular capillaries*** induced by the *inflammatory process*
- **consequences**: serum albumin concentration and plasma oncotic pressure remain approximately normal

3. Oliguria

- **cause**: *decrease in filtration surface area and glomerular filtration rate (GFR)*
- **consequences**:
  - ✓ *nitrogen retention*
  - ✓ triggering of *water-salt retention* mechanisms with *hypertension* and *edema*

Observation!

Most glomerular lesions can produce a mixed, nephritic and nephrotic syndrome in the evolution, requiring **renal biopsy** for the diagnosis of certainty.

CHRONIC GLOMERULONEPHRITIS

DEFINITION: the EVOLUTIONARY stage of glomerular diseases manifested by nephritic and/or nephrotic syndrome with SLOW progression towards chronic kidney disease

Frequently:

- **onset is insidious**, without obvious history of acute glomerular disease
- discovered during the evaluation of a patient with **HT, proteinuria or azotemia**

POSITIVE DIAGNOSIS of certainty - **renal biopsy** showing:

- Small kidneys with thin cortex
- Glomerular hyaline obliteration
- Arteriolosclerosis
- Tubulo-interstitial fibrosis

Observation!

The severity at onset and the persistence of proteinuria are used as a predictor of the development/progression of chronic kidney disease. The increase in the amount of proteins in the glomerular filtrate stimulates their endocytosis at the level of tubular cells and triggers an inflammatory-fibrotic process that leads to the loss of nephrons. Therefore, reducing proteinuria with medications such as angiotensin-converting enzyme inhibitors exerts a renoprotective effect.

TUBULO-INTERSTITIAL NEPHROPATHIES

DEFINITION: acute or chronic renal disorders, determined by the **predominant damage of the tubules and/or the renal interstitium**, as follows:

1. **Predominant TUBULAR damage**, of ***ischemic*** or ***toxic*** etiology, leads to **acute tubular necrosis**
2. **Predominant INTERSTITIAL damage**, of varied etiology - ***drug-induced, infectious, immune-mediated, from malignant hemopathies*** or ***metabolic causes***, determines **tubulo-interstitial nephritis**

ACUTE TUBULAR NECROSIS (ATN)

DEFINITION: pathological entity characterized by the **acute, potentially reversible decrease in renal excretory function**, being the main cause of **acute kidney injury** (previous name, *acute renal failure*)

ETIOPATHOGENIC CLASSIFICATION:

1. ISCHEMIC ATN is determined by the pathological conditions that lead to the decrease/ interruption of renal blood flow = **prolonged renal ischemia** from ***all shock states***:

- **septic shock** - the main cause in critical patients in the ICU!
- **hypovolemic shock** in the case of:
  - hemorrhage
  - severe hypovolemia (diarrhea, vomiting, excessive diuretics administration)
  - severe burns
  - acute pancreatitis (retroperitoneal fluid retention)
  - polytrauma
  - post-cardiovascular surgery
- **hepatorenal syndrome**
- **pre-eclampsia and eclampsia**
- **vascular nephropathies with diffuse renal damage**: malignant hypertension, thrombotic microangiopathies

2. NEPHROTOXIC ATN is caused by exposure to:

a. Endogenous toxins:

i. ***pigments*** - form *pigment casts* with an increased iron content, toxic to the tubular epithelium:
   - ✓ *hemoglobin* released during pathological hemolysis, transfusion accidents, malaria
   - ✓ *myoglobin* released via rhabdomyolysis (crush syndrome) from trauma or electric shock, excessive physical exertion (marathon runners), convulsions (status epilepticus), muscle toxins (statins, snake venom)

ii. ***immunoglobulin light chains*** in: multiple myeloma

iii. ***uric acid*** in: gout, tumor lysis syndrome

b. Exogenous toxins, represented by:

i. ***contrast agents*** - the **main cause in hospitalized elderly patients undergoing invasive investigations**; Contrast nephropathy is dose-dependent (being associated with the administration of high doses in prolonged interventions - angiographies with or

without angioplasty) and occurs frequently in patients with heart failure, chronic kidney disease, diabetic nephropathy, consumption of nephrotoxins (ACE inhibitors, NSAIDs)

ii. ***nephrotoxic drugs***: antibiotics (aminoglycosides, tetracyclines), chemotherapeutics (cisplatin), antifungals (amphotericin B), NSAIDs

iii. ***other toxins***: ***solvents*** (CCl4), ***heavy metal salts*** (HgCl2).

Observation!

PCT is the tubular segment the *most sensitive to ischemia* and, respectively, to *nephrotoxins* due to the following favoring factors:

- *large tubular reabsorption surface*
- *tubular concentration capacity of toxins*
- *presence of active secretion systems for organic substances (including nephrotoxic drugs)*
- *high rate of metabolism and O_2 consumption*

The risk of ATN occurrence at **PCT** level is higher:

- *in the elderly*
- *in patients with pre-existing renal diseases, diabetes mellitus or recent exposure to other nephrotoxic agents*
- *in the presence of volume depletion*

PATHOGENESIS:

At the basis of ATN, ischemic and toxic, are 2 distinct mechanisms, ***vascular*** and ***tubular***, which mutually potentiate each other in determining the severity of the disease:

1. VASCULAR (HEMODYNAMIC) changes consist of **intense VASOCONSTRICTION of INTRARENAL MICROCIRCULATION with progressive TUBULAR HYPOXIA** determined by:

- **increased production of ET-1**, **TxA2** (vasoconstricting) and **decreased production of NO and PGI2** (vasodilating) secondary to *endothelial dysfunction* = vascular endothelial damage induced by ischemia;
- **sympathetic-adrenergic (S-A) stimulation** mediated by activation of baroreceptor reflexes in high pressure areas, carotid sinus and aortic arch (by hypovolemia/arterial hypotension) with vasoconstriction of the *afferent* arteriole responsible for: i) decreased GFR, ii) increased tubular reabsorption of water and sodium in the PCT and iii) renin release;
- **activation of the RAA system** - **AII** with vasoconstricting effect at the *efferent* arteriole level and vasodilating at the afferent arteriole level, responsible for the transient increase in GFR and sodium load at the macula densa level with the *triggering of the tubulo-glomerular feed-back mechanism* = adenosine release with vasoconstriction of the afferent arteriole;
- **increased leukocyte adhesion to the damaged endothelium** with their activation = release of *proinflammatory cytokines*.

Consequences:

- **decrease in renal blood flow (RBF) by 30-50%**
- **redistribution of RBF from the cortical to the medullary area** (normally, 90% of RBF is distributed to the cortex where 80% of nephrons are located and only 10% to the medullary area)
- marked decrease in GFR with oliguria
- alteration of tubular function (PCT)

2. Damage to TUBULAR EPITHELIAL CELLS which display increased sensitivity towards ischemia and toxins (as compared to glomerular ones) with **depletion of cellular ATP reserves** and **induction of necrotic or apoptotic cell death**, secondary to:

- **increased cytosolic calcium concentration;**
- **increased production of intracellular proteases** (such as calpain) with *cytoskeleton proteolysis* and cell wall collapse
- **tubular obstruction** by damaged, desquamated or necrotic epithelial cells with the *formation of cellular (epithelial) casts* in the tubular lumen

Consequences: OLIGURIA caused by:

- **increased pressure in the tubular lumen** (through obstruction) is transmitted retrogradely into Bowman's capsule with a **secondary decrease in glomerular filtration**
- **focal tubular lesions** determine **total and non-selective retrodiffusion of primary urine**
- **increased peritubular pressure** with **interstitial edema** and amplification of the tubular lesion

EVOLUTION:

1. **Reversible tubular lesion** → allows **regeneration of tubular cells** (reepithelialization) under the action of **growth factors** produced by tubular and inflammatory cells (*tubular epithelial cells have a high capacity for regeneration* unlike glomerular ischemia which does not heal with regeneration but with scarring, leading to *glomerulosclerosis*). Recovery of renal function typically occurs within 7-21 days; the exception is septic shock - where it is delayed
2. **Irreversible tubular injury** → causes **acute cortical necrosis** in all conditions associated with *prolonged* cortical ischemia leading to rupture of the basement membrane called *tubulorexis*

TUBULOINTERSTITIAL NEPHRITIS (TIN)

DEFINITION: renal diseases with varied etiopathogenesis, characterized by **INFLAMMATION OF THE TUBULES** and especially of the **RENAL INTERSTITIUM**

ETIOPATHOGENIC CLASSIFICATION:

1. ACUTE TIN

Characteristics:

- inflammatory infiltrate with eosinophils or neutrophils
- interstitial edema
- possibility of progression towards ATN

Etiology: Tab. 10.

In approx. **70%** of cases, acute TIN is the consequence of **drug-induced hypersensitivity reactions**, being associated with hypereosinophilia and eosinophiluria and renal biopsy reveals interstitial cellular infiltrate with eosinophils. In 2nd place (**15%** of cases) are infections (most frequently bacterial), when the interstitial cellular infiltrate is rich in neutrophils. In both situations, varying degrees of tubular necrosis may occur.

Table 10. Causes of **acute** tubulo-interstitial nephritis.

Drug/toxic induced (70% of cases)

Antibiotics: penicillins, cephalosporins, quinolones (ciprofloxacin), erythromycin, rifampicin, sulfonamides
Analgesics: NSAIDs
Diuretics: thiazides, furosemide, triamterene
Others: proton pump inhibitors, allopurinol, carbamazepine, cimetidine
Infectious (15% of cases)
Bacteria: E.coli, Proteus, Klebsiella, Staphylococcus saprophyticus/epidermidis, Enterococcus, Pseudomonas → acute and chronic **pyelonephritis** (the latter being favored by reflux nephropathy)
Viruses: Epstein-Barr, herpes, hepatitis C, AIDS, cytomegalovirus, adenovirus, measles
Other infectious agents: Leptospira, Mycobacterium, Mycoplasma, Rickettsia, Chlamydia
Idiopathic (8% of cases)
Tubulointerstitial nephritis with uveitis – sdr. with unexplained etiology, especially in children, manifested by *acute TIN, uveitis, anemia, increased ESR and weight loss* (5% of cases)
From systemic diseases/collagenosis - SLE (2% of cases)

2. CHRONIC TIN

Characteristics:

- inflammatory infiltrate with mononuclears: macrophages, lymphocytes
- interstitial fibrosis
- tubular atrophy
- possibility of progression towards CKD

Etiology: Tab. 11.

Table 11. Causes of **chronic** tubulointerstitial nephritis.

Drug/toxic induced - all causes of acute NTI
Antibiotics: penicillins, cephalosporins, quinolones (ciprofloxacin), erythromycin, rifampicin, sulfonamides
Analgesics: phenacetin (classic), currently NSAIDs
Aristolochic acid: nephropathy induced by Chinese nutritional supplements for weight control
Metals: cadmium, lead, titanium
Irradiation
Infectious
Viruses: AIDS virus, Epstein-Barr virus
From systemic diseases
Diabetes mellitus
Arterial hypertension
SLE, vasculitis
Sarcoidosis
From malignant hemopathies
Nephropathy in multiple myeloma (Ig light chain excretion)
Sickle cell nephropathy

Due to metabolic causes
Uric nephropathy
Hypercalcemic nephropathy (nephrocalcinosis)
Oxalic nephropathy

POSITIVE DIAGNOSIS of **chronic** tubulo-interstitial nephritis includes:

1. Tubular proteinuria characterized by:
- *mild* proteinuria: less than 1 g/day
- increased elimination of *proteins with low MW* (< 25 KD): e.g., beta 2-microglobulin
- results from the *prevention of normal reabsorption/catabolism in the proximal tubule of glomerularly filtered proteins*
- undetectable with strips (which detect only albuminuria)

2. Absence of hypoproteinemia and edema

3. Absence of severe hypertension

4. Presence of leukocyturia and leukocyte casts in the infectious etiology (± bacteriuria, in acute forms)

5. Polyuria, nicturia - by alteration of the urine concentration function

6. Loss of sodium, potassium, calcium, bicarbonates, phosphates, amino acids - by selective defects of the tubular reabsorption/secretion function

7. Possibly, metabolic acidosis via the alteration of the urine acidification function

PYELONEPHRITIS

DEFINITION: inflammation of the **parenchyma** (renal tubules and interstitium) and of the **renal pelvis**

ETIOPATHOGENIC CLASSIFICATION:

1. **Acute pyelonephritis (APN)** - is determined by **bacterial infection** in the **upper urinary tract**
2. **Chronic pyelonephritis (CPN)** - has a **complex** etiology, in which the **bacterial infection** is associated with **predisposing factors** that favor the **recurrence** of exacerbation episodes

1. ACUTE Pyelonephritis (APN)

- **Etiology:**
  - **over 80% of cases - *Gram-negative bacteria*: E. coli!, Proteus, Klebsiella, Enterobacter, Pseudomonas**
  - *Gram-positive bacteria*: Staphylococcus aureus, Streptococcus faecalis
  - *mycobacteria, fungi and viruses* in immunocompromised subjects
- **PREDISPOSING factors:**
  - **age > 40 years**
  - **F gender**
  - **instrumental maneuvers**: bladder catheter, endoscopy
  - **pregnancy** - via:

  - ✓ relaxation of the bladder and ureteral smooth muscles due to increased progesterone levels
  - ✓ partial obstruction due to the enlarged uterus
- – **diabetes mellitus** - by:
  - ✓ increased susceptibility to infections
  - ✓ neurogenic bladder
- – **spinal cord injury** (atonic bladder)
- – **immunosuppression, immunodeficiency**

- **Pathogenesis:**
  - – there are **2 ways** by which bacteria enter the kidneys:
    - ✓ from the urinary tract → *ascending infection* - **the most common!**
    - ✓ from the blood → *hematogenous infection* (e.g., septicemia, infective endocarditis)
  - – leukocyte infiltration of the renal pelvis, calyces and medulla → **inflammatory sites** in the pelvis, calyces and medulla, **renal edema** and **purulent urine**
  - – in severe infections there are: *medullary and cortical abscesses, papillary necrosis* → **acute tubular necrosis** (ATN)
  - – if APN is recurrent, healing occurs with *fibrosis formation, tubular atrophy and scar lesions* → **chronic pyelonephritis** (CPN)
- **CLINICAL manifestations:**
  - – *altered general condition*: fever, chills, headache, lower back pain
  - – *symptoms of bladder irritation*: dysuria, pollakiuria, pyuria
  - – *urine examination*: leukocyturia with leukocytic casts, bacteriuria
- **Prognosis**: favorable under antibiotic treatment

2. CHRONIC PYELONEPHRITIS (CPN)

- **Definition: persistent or recurrent chronic inflammation**, which causes **scarring**
- **Etiopathogenic forms:**
  - – **Reflux nephropathy** (congenital vesico-ureteral reflux) → clinical form of CPN in children with progression towards CKD
  - – **Obstructive CPN** (chronic obstruction in the urinary tract) → clinical form of CPN in adults with progression towards CKD
- **PREDISPOSING factors**:
  - – **renal obstruction and stasis**: renal lithiasis, prostate adenoma, etc.
  - – **vesico-ureteral reflux**: incompetence of the vesico-ureteral valve that allows the reflux of urine from the ureter into the renal pelvis and which can be:
    - ✓ congenital (in children)
    - ✓ acquired (in adults): neurogenic bladder, atonic bladder
  - – **intrarenal reflux**: reflux of urine from the renal pelvis into the renal parenchyma
- **Pathogenesis**: the chronic inflammatory process causes *sclerosis of the renal interstitium, tubular atrophy, scar lesions and deformations of the calyces and pelvis* responsible for:
  - – impairment of the capacity to concentrate and dilute urine
  - – evolution towards CKD in the presence of *obstructive uropathy* or *diabetes mellitus*
- **CLINICAL manifestations**:
  - – are the consequences of damage to the **medullary segments** of the renal tubules (Henle's loop and the collecting duct):

  - ✓ loss of the capacity to concentrate and dilute urine → *polyuria* + *isosthenuria*
  - ✓ impairment of the mechanism of urine acidification → *alkaline* urine
- are the consequences of damage to the **cortical segments** of the renal tubules (proximal tubule!):
  - ✓ decreased tubular reabsorption of Na^+ and glucose → *osmotic diuresis* + *glycosuria*
- late association of **glomerular damage** with:
  - ✓ *proteinuria*
  - ✓ *severe HT* contributing to the progression of CPN
    ⇒ evolution towards CKD (10-20% of CKD causes)

VASCULAR NEPHROPATHIES

DEFINITION: vascular damage is encountered **SECONDARY** to both ***glomerular and tubular RENAL diseases***, as well as in ***SYSTEMIC*** diseases such as vasculitis and HT (which is both a cause and a consequence of renal lesions)

1. RENAL ARTERY stenosis

- **Definition**: **unilateral or bilateral obstruction** of the renal artery with decreased RBF and chronic renovascular HT
- **Etiology:**
  - *atheromatosis of a renal artery with subsequent thrombosis* (70% of cases) → in the *elderly with diabetes*
  - *fibromuscular dysplasia of the tunica media* (30% of cases) → in *young women*
- **Pathogenesis:** activation of the RAA system on the affected kidney side, with *sodium retention*
- **Consequences:**
  - **minor** water-salt retention in conditions in which only one kidney is affected, the functional kidney taking over its function as well
  - risk of **acute pulmonary edema** in patients with a single functioning kidney

2. BENIGN nephroangiosclerosis

- **Definition: sclerosis of arterioles and small renal arteries** with *focal* ischemia of the parenchyma vascularized by the narrowed vessels
- **FAVORING factors**: genetic defects, advanced age, the presence of diabetes mellitus and their combination, even in the absence of arterial hypertension
- **Pathogenesis**: *narrowing of the lumen of small arteries and arterioles* by *hyaline deposits* secondary to protein extravasation at the level of the damaged endothelium
- **Consequences:**
  - *arteriolosclerosis with glomerulosclerosis*
  - *sites of tubular atrophy and interstitial fibrosis*

3. Nephrosclerosis in MALIGNANT HYPERTENSION

- **Definition**: *accelerated nephrosclerosis associated with malignant HT*
- **Etiology**: pre-existing benign HT (only in 5% of cases), chronic kidney disease (e.g., glomerulonephritis, reflux nephropathy)
- **Pathogenesis**:

- *increased permeability of small arteries and arterioles for fibrinogen* and other proteins
- *endothelial cell necrosis*
- *intravascular thrombosis* secondary to platelet adhesion to the damaged endothelium
- characteristic lesion: **fibrinoid necrosis of vascular walls**

- **Consequences**: ischemia of afferent arterioles leads to **activation of the RAA system** with worsening of the intrarenal vasoconstriction and of hypertension through hydro-saline retention.

ACUTE KIDNEY INJURY (AKI)

DEFINITION: SUDDEN (hours-days), **COMPLETE but POTENTIALLY REVERSIBLE** (days-weeks) **decrease in the renal functions** of:

- **EXCRETION** with **decreased GFR** responsible for:
  - the rapid increase in nitrogen catabolism products (creatinine, urea, uric acid): ***nitrogen retention, azotemia***
  - ***oligo-anuria***
- **REGULATION OF THE INTERNAL ENVIRONMENT HOMEOSTASIS** with:
  - impairment of the hydro-electrolyte balance
  - impairment of the phospho-calcium balance
  - impairment of the acid-base balance

and which usually occurs in **healthy kidneys**.

Observation!

The term **"acute kidney injury"** (AKI) has now replaced the term "acute renal failure" because it better defines the *variety of renal damage* that can be included in this category, from minimal changes in renal function to severe renal failure.

CLASSIFICATION:

1. PRERENAL AKI or Prerenal AZOTEMIA (functional)

- **Definition: decreased GFR** caused by **decreased renal perfusion pressure** in conditions of **renal ISCHEMIA**
- **Causes:**

a. Hypovolemia - caused by fluid losses:
- **cutaneous**: profuse sweating
- **renal**: diabetes insipidus, poorly controlled diabetes mellitus, diuretic abuse
- **digestive**: vomiting, diarrhea, fistulas
- **hemorrhage**: trauma, digestive hemorrhage, postpartum

b. Fluid retention in:
- acute pancreatitis: in the retroperitoneal space
- intestinal occlusion: in obstructed loops
- severe burns: plasma in blisters

c. Circulatory shock states with severe hypotension

Remember!

AKI can also occur in patients with chronic kidney disease (CKD), under additional stress on the background of a kidney with borderline function.

2. RENAL AKI or Renal AZOTEMIA (intrinsic)

- **Definition: decreased GFR** caused by **alteration of the renal structure**
- **Causes:**

a. **ISCHEMIC tubular necrosis** → renal ischemia - all causes of **prerenal azotemia**
b. **TOXIC tubular necrosis** → nephrotoxic effects of **pigments** (hemoglobin, myoglobin), nephrotoxic **drugs** (aminoglycosides, NSAIDs, ACE inhibitors), **contrast agents, organic solvents**, fungi, etc.
c. **Acute VASCULAR nephropathies**: renal infarction, bilateral cortical necrosis
d. **Acute PARENCHYMAL nephropathies**: acute glomerulonephritis, acute suppurative pyelonephritis
e. **DISSEMINATED INTRAVASCULAR COAGULATION (DIC) syndrome**

3. POSTRENAL AKI or Postrenal AZOTEMIA (obstructive)

- **Definition: decreased GFR** caused by **urinary tract obstruction** (mechanical cause) in: **bilateral obstructive uropathy** or **anatomically/functionally solitary kidney**
- **Causes:**

a. **Bilateral ureteral obstructions**: calculi, tumors, strictures
b. **Bladder obstructions**: tumor, neurogenic bladder
c. **Urethral obstructions**: prostate hypertrophy, strictures

PATHOGENESIS:

1. Decreased GFR induced by RENAL ISCHEMIA - is determined by:

- **Increased permeability of cell membranes for Ca^{2+}** → Ca^{2+} entry at the cellular level triggers:
  - **arteriole contraction** → decreased GFR by decreased renal blood flow (RBF)
  - **mesangial cell contraction** → further decrease in RBF and GFR
- **Activation of the RAA system → vasoconstriction of renal arterioles** with increased renal vascular resistance and **redistribution of RBF from the cortical towards the medullary area** under the action of AII.

2. The mechanisms of OLIGOANURIA - are **3**, being present, in different proportions, in all 3 etiopathogenic forms of AKI (prerenal, renal and postrenal):

- **Decreased GFR**
- **Tubular obstruction** - by the formation of **cellular casts** and/or by **interstitial edema** with decreased GFR by the retrograde increase of the *hydrostatic pressure* in the filtration space delimited by the Bowman capsule
- **Total and non-selective retrodiffusion of primary urine at the level of the renal tubules** via *tubular necrosis*.

EVOLUTIONARY PHASES:

A. ONSET phase (initial stage)

- **Characteristics:**
  - is the period from the *exposure* to the causative agent until the *onset* of tubular lesions
  - duration 1-2 days
  - symptoms of the *underlying condition* (the cause that led to AKI) predominate

- renal ***EXCRETION*** function is **altered** with:
  - ✓ **asymptomatic azotemia (nitrogen retention)**
  - ✓ **oliguria** (< 400 ml/day) in **50-60% of cases** = ***oliguric form*** **of onset of AKI - 2 types**:
    - FUNCTIONAL oliguria → in prerenal azotemia
    - ORGANIC oliguria → in renal azotemia
  - ✓ **normal diuresis** (> 800 ml/day) in **40-50% of cases** = ***non-oliguric form*** **of onset of AKI**, but with decreased urine concentration capacity
- **internal environment homeostasis REGULATION** function is **NOT affected**
- ***COMPLETE RECOVERY of renal function*** through therapeutic intervention aimed at restoring renal perfusion and/or eliminating toxins in this phase

- **Types of OLIGURIA:**

a) FUNCTIONAL Oliguria - in **PRERENAL** azotemia:

- oliguria is due to the **decrease in GFR** with a **disproportionate increase in blood urea** ("Blood Urea Nitrogen", BUN) as compared to creatinine (urea is tubularly reabsorbed in **the PCT, but not creatinine) → BUN:creatinine ratio > 20**
- **TUBULAR** function is **normal**:
  - ✓ **Na^+ conservation** capacity is **normal**
  - ✓ **urine concentration** capacity is **normal**

b) ORGANIC Oliguria - in **RENAL** azotemia:

- oliguria is due to the **impairment of tubular function** with *total and non-selective retrodiffusion* of primary urine and a **disproportionate increase in blood creatinine** (the excess of renally reabsorbed urea can be excreted via extrarenal pathways: digestive, cutaneous – as opposed to creatinine) → **BUN:creatinine ratio < 10**
- **TUBULAR** function is **affected**:
  - ✓ **Na^+ conservation** capacity is **low**
  - ✓ **urine concentration** capacity is **low**

B. OLIGO-ANURIC stage

- **Characteristic**: the renal functions of **EXCRETION and internal environment homeostasis REGULATION** are **severely altered**

a) Impairment of the EXCRETION FUNCTION (azotemia)

- **Definition**: azotemia consists in the accumulation of nitrogenous compounds represented by **creatinine + urea + uric acid**
- **Pathogenesis:**
  - **Decreased excretion due to the decreased GFR**
  - **Hyperproduction** determined by:
    - **increased cellular catabolism produced by the etiological factor** (most conditions that lead to acute kidney injury also manifest with increased cellular catabolism)
    - **cellular destruction** (the speed at which urea and creatinine increase is directly proportional to the severity of cellular destruction)

- **Consequences**: significant increases in nitrogenous catabolites become **symptomatic** and cause the **uremic syndrome**

b) Impairment of the REGULATORY FUNCTION

1. Impairment of the WATER BALANCE → <u>GLOBAL hyperhydration</u>

- **Causes:**
  - **water retention due to the decreased GFR**
  - **increased metabolic water** production determined by the **increased catabolism** → water production increases (from 300 ml/day → 1000 ml/day)
- **Pathogenesis**:
  - water retention associated with increased water production (through catabolism) determines → *hypotonic extracellular hyperhydration*
  - water will migrate from the **hypotonic extracellular** environment towards the **intracellular** environment → **global hyperhydration** known as ***water intoxication***
- **Consequences**:
  - accumulation of **water in cells** causes:
    - *neurological disorders*: cerebral edema, intracranial hypertension, convulsions
    - *digestive disorders*: anorexia, nausea, vomiting
  - accumulation of **water in the extracellular space** causes:
    - *cardiovascular disorders*: hypertension, risk of acute pulmonary edema

2. Impairment of the SODIUM BALANCE → hyponatremia

- **Causes:**
  - **dilutional mechanism** → accumulation of water due to the **impossibility of its elimination** and the increased production of **metabolic water**
  - **transmineralization** → entry of Na^+ into cells and exit of K^+ into the extracellular space caused by the inhibition of the Na^+/K^+-dependent ATPase by **uremic toxins**
  - **increased digestive losses of Na^+** (e.g., vomiting)

3. Impairment of the POTASSIUM BALANCE → HYPERkalemia

- **Causes**:
  - **decreased elimination** due to the **decreased GFR**
  - **transmineralization**
  - **increased release from cells as a consequence of tissue destruction** → in pathological hemolysis and rhabdomyolysis the increase in K^+ is very rapid and endangers the patient's life due to ECG changes that can occur when potassemia exceeds **8 mEq/l** (risk of intraventricular conduction disorders and cardiac arrest)

4. Impairment of the PHOSPHO-CALCIUM BALANCE → HYPERphosphatemia + HYPERmagnesemia + hypocalcemia

- **Causes**: **decreased GFR** leads to
  - decreased elimination of nonvolatile anions (HPO_4^-, SO_4^{2-}) → **HYPERphosphatemia**
  - decreased elimination of Mg^{2+} → **HYPERmagnesemia**
- **Consequences**:

- modification of the P/Ca ratio leads to the tendency for Ca^{2+} precipitation in tissues (especially bone) → **hypocalcemia**
- hypocalcemia stimulates PTH secretion → **secondary hyperPTH**

5. Impairment of the ACID-BASE BALANCE → METABOLIC ACIDOSIS

- **Cause: decreased elimination of non-volatile acids** (HPO_4^-, SO_4^{2-}) due to the decreased GFR - these will be buffered by plasma HCO_3^- → **pH < 7.35** which will trigger **compensatory reflex hyperventilation**.

C. DIURESIS RESUMPTION phase (polyuric stage) and RECOVERY OF RENAL FUNCTION

- **Characteristics**:

1. Restoration of GFR → resumption of diuresis with **polyuria** (> 3000 ml/day) and **water loss**
2. Persistence of tubular dysfunction → **significant losses of electrolytes (sodium, potassium)**
3. Urea and creatinine **gradually return to normal values** → creatinine may require 3-12 months to return to normal values
4. **Losses of water, Na^+ and K^+** can be significant with:
   - *intracellular dehydration*: sensation of thirst
   - *extracellular dehydration*: dry skin and mucous membranes, hypotension, collapse
   - *decrease in K^+*: risk of sudden death by ventricular fibrillation
5. **Progressive** regeneration of tubular epithelial cells with recovery of renal functions of concentration and dilution → in some patients renal function **CANNOT be recovered completely**.

CHRONIC KIDNEY DISEASE

DEFINITION: Chronic kidney disease (CKD) is a term used to describe the slow and potentially progressive deterioration of kidney function (from any cause) that is **confirmed** by a **decrease in glomerular filtration rate (GFR) < 60 ml/min/1.73 m²**, **persisting at least 3 months**.

Observation!
The term CKD has replaced the term "chronic renal failure" (CRF).

ETIOLOGY:

1. Glomerular nephropathies:
- **primary**: focal glomerulonephritis, rapidly progressive
- **secondary**: diabetic glomerulosclerosis, SLE, systemic sclerosis, accelerated HT, thrombotic thrombocytopenic purpura, sickle cell disease

2. Renal vascular nephropathies:
- hypertensive nephrosclerosis (common in black people of African descent)
- renovascular disease
- small and medium vessel vasculitis

3. Tubulo-interstitial nephropathies:

- chronic pyelonephritis: drug-induced (! nephrotoxic analgesics), by Chinese supplements
- reflux nephropathy
- renal papillary necrosis: diabetes mellitus, sickle cell disease, analgesic nephropathy
- multiple myeloma

4. Urinary tract obstructions:

- lithiasis
- prostate diseases
- retroperitoneal fibrosis
- schistosomiasis

5. Congenital and hereditary diseases:

- polycystic kidney disease
- congenital obstructive uropathy
- oxalosis
- cystinosis

Remember!

Diabetes mellitus and ***hypertension***, both risk factors for ATS (including renal ATS) are responsible for about **70% of CKD cases**. Given the increasing life expectancy and aging of the population at a global scale, the accelerated increase in the prevalence of diabetes and cardiovascular diseases, the global prevalence of CKD is continuously increasing (11-15%).

PATHOGENESIS

CKD is characterized, in evolution, by **3 major pathogenic processes**:

A. Decrease in the NUMBER OF FUNCTIONAL NEPHRONS

CKD begins with a **decrease in the number of functional nephrons** induced by chronic **kidney damage**, with the following observations:

- the severity of the natural progression towards CKD depends on the **initial pathology** (ischemic nephropathy has the highest rate of progression, while polycystic kidney disease has the lowest rate)
- although the number of nephrons is decreasing, the **GFR changes very little** due to the entry into action of *compensatory mechanisms at the level of intact nephrons*

B. COMPENSATORY changes at the level of INTACT NEPHRONS

The **INITIAL** decrease in the number of functional nephrons triggers **2 compensatory changes**:

1. Adaptive HYPERTROPHY and HYPERFUNCTION of INTACT NEPHRONS

It is usually triggered when the proportion of intact nephrons **falls below 75%** and includes **2 pathogenic mechanisms**:

a) Morphological hypertrophy of intact nephrons - mediated by *growth factors* whose secretion is induced by angiotensin II

b) Hyperperfusion and adaptive hyperfunction of intact nephrons - which consists of *glomerular hyperfiltration, increased reabsorption and tubular secretion*

- **Consequences:**

- maintenance of **GFR within relatively normal limits** and for a **long period of time**, despite the progressive decline in the number of functional nephrons
- **progression** of initial destructive lesions through **2 major pathogenic mechanisms**:
  1. **INCREASED glomerular FILTRATION of PROTEINS** - causes:
     - damage to mesangial cells (proteins accumulate in the mesangial area) with the onset of ***inflammation and glomerulosclerosis***
     - damage to tubular cells (proteins are reabsorbed at the tubular level) with the onset of ***inflammation and tubulo-interstitial fibrosis***
  2. **Increased LOCAL PRODUCTION of angiotensin II** - causes:
     - vasoconstriction of the efferent arteriole with *increased intraglomerular capillary pressure and filtration fraction* → worsening of proteinuria
     - worsening of *inflammation* by *stimulating the activity of local inflammatory cells* (especially macrophages)
     - worsening of *tubulo-interstitial fibrosis* by increased production of extracellular matrix by:
       i. stimulation of the production of *TGF-β*, a fibrogenic cytokine that increases collagen synthesis
       ii. differentiation of renal epithelial cells into *myofibroblasts* with a secretory phenotype

Remember!

a) The prognosis of CKD correlates with:
- arterial hypertension (especially if it is poorly controlled)
- proteinuria
- the degree of histological damage to the renal interstitium, NOT also with the objective changes at the glomerular level.

b) Inhibition of angiotensin II with angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists is indicated for the beneficial effect of slowing the progression of CKD.

2. COMPENSATORY POLYURIA at the level of INTACT NEPHRONS

It is usually triggered when the proportion of intact nephrons **falls below 50%** (in parallel with the hypertrophy and adaptive hyperfunction of intact nephrons) and includes **2 pathogenic mechanisms**:
- osmotic diuresis
- increase in the excreted fraction of sodium and water

a) OSMOTIC diuresis

- **Cause**: the osmotic overload per remaining nephron **retains water in the tubular lumen**
- **Consequence**: osmotic diuresis **increases the primary urine flow** at the level of **intact nephrons** and **decreases the contact time** of the tubular fluid with the reabsorption surface → **decrease in tubular reabsorption of Na^+ and water**

Observation!
- **In the normal subject**: the osmotic load of 600 mOsm/day is excreted by **100% of the nephrons**
- **In the patient with CKD**: the osmotic load of 600 mOsm/day can be excreted, **for example**, by **20% of the nephrons**, so that the **osmotic load per remaining nephron increases by 5 x and causes osmotic diuresis**.

b) The increase in the EXCRETED FRACTION of SODIUM and WATER - contributes to compensatory polyuria.

Observation!

- **In the normal subject**: the elimination of an osmotic load of **600 mOsm/day**, under conditions of a **GFR = 125 ml/min/1.73 m²**, which leads to the formation of **180 liters of primary urine/day**, can be achieved by a **volume of 2 liters of final urine** with an osmolarity of **300 mOsm/L** and corresponds to a *sodium and water excretion fraction* of **1% of the primary urine**
- **In the patient with CKD**: to eliminate the same osmolar load of **600 mOsm/day**, under conditions of a **GFR = 25 ml/min/1.73 m²**, only **40 liters of primary urine/day** are formed. To obtain the **2 liters of final urine** with an osmolarity of **300 mOsm/L**, it is necessary to increase the *excreted fraction of sodium and water* up to **5% of the primary urine** → a *fraction 5 times larger than normal* must be eliminated from the filtered volume, which can only be achieved by *decreasing the tubular reabsorption of sodium and water*

Compensatory polyuria in CKD has **3 characteristics**:

- **It is associated with isosthenuria** - the density of the final urine is identical to that of the primary urine (1008 - 1012) due to the *impairment of the concentration and dilution capacity of urine* by damage to the loop of Henle
- **It is stable** - *independent of water intake* and reflects the lack of response to ADH by damage to the DCT (distal convoluted tubule) and CD (collecting duct)
- **It is transient and reflects the percentage of functional nephrons**, being replaced in the phase of end-stage renal disease (no. of intact nephrons < 10%) with *oligo-anuria* (stage in which dialysis is mandatory).

C. IREVERSIBLE and PROGRESSIVE DAMAGE to FUNCTIONAL NEPHRONS

- Regardless of the initial damage, the **progression of CKD to end-stage renal disease (ESRD)** is the consequence of **unfavorable** aspects of **glomerular hyperperfusion and hyperfiltration** at the level of intact nephrons, which include:
  1. Intraglomerular hypertension
  2. Mesangial inflammation and glomerulosclerosis
  3. Renal interstitial inflammation and tubulointerstitial fibrosis
- With the progression of CKD, the decrease in GFR will determine the **decrease** in the **EXCRETION function** of uremic toxins with the onset of ***azotemia*** and then the ***uremic syndrome*** which is associated with the impairment of the **REGULATION** function and, respectively, the **ENDOCRINE** function of the kidney.

DIAGNOSTIC AND STAGEING CRITERIA

CKD is staged according to an international consensus (Kidney Disease Guidelines: Improving Global Outcomes – KDIGO, 2024) based on the **decrease in estimated glomerular filtration rate** (eGFR, using the prefix G) into **5 stages (G1-G5)** and **albuminuria** (using the prefix A) into **3 stages (A1-A3)** because both parameters correlate with the **progression of kidney damage** and **cardiovascular risk**

Observation!

1. Stage 3 of CKD has been divided into G3a and G3b considering the increase in the number of cardiovascular complications in advanced stages (e.g., a patient can be diagnosed as having disease stage G3bA3).

2. The KDIGO guideline defines CKD as abnormalities of kidney structure or function, present for a minimum of 3 months, with implications for health. The term „abnormalities of kidney structure or function" refers to:
   - Changes in **urinary sediment**: hematuria, proteinuria
   - Changes in **electrolytes** or other abnormalities due to **tubular dysfunction**
   - Structural changes detected on **renal biopsy/histopathological examination**
   - Changes detected by **imaging techniques**
   - History of **renal transplantation**

RENAL FUNCTIONS IMPAIRMENT IN CKD

A. Impairment of the EXCRETORY FUNCTION

- **Definition**: accumulation of uremic toxins due to **decreased GFR** with 2 consequences:

a) NITROGEN RETENTION or AZOTEMIA: **increase in plasma of nitrogenous catabolites – urea (BUN)** and **creatinine** as a result of the alteration of the excretory function defined by a decreased GFR < 90 ml/min/1.73 m^2

b) UREMIC SYNDROME or chronic UREMIA: **clinical-biological** syndrome determined by **severe** impairment of the **excretory** function defined by a severe decrease in GFR < 30 ml/min/1.73 m^2 → ***increase in plasma*** and ***accumulation in tissues*** of **uremic toxins**

UREMIC toxins - are substances of exogenous or endogenous origin that are eliminated from the body by glomerular filtration.

- **Exogenous source**: diet
- **Endogenous source**:
  - ✓ protein catabolism: urea, creatinine and uric acid
  - ✓ degradation products of bacterial flora that are absorbed in the intestine
  - ✓ β2-microglobulin
  - ✓ advanced glycation end-products (AGE)

Characteristics of the altered excretory function:

1) When the % of intact nephrons < 75%:
   - adaptive hyperfiltration ensures the elimination of the same amount of uremic toxins as a **normal kidney**
   - BUT the kidney has a **low capacity to adapt** to conditions associated with the **increase in uremic toxins** by:
     - increased cellular catabolism in hypercatabolic states
     - increased exogenous nitrogen intake - hyperproteic diet
     - increased absorption of toxins produced by intestinal bacteria

2) When the % of intact nephrons < 10%:
   - the kidney can **NO longer eliminate** the same amounts of uremic toxins as a normal kidney → dialysis or kidney transplantation is necessary
   - *decreased elimination of uremic toxins* causes:
     - **CELLULAR** alterations
     - **METABOLIC** alterations
     - **SYSTEMIC** manifestations

a) CELLULAR alterations in the UREMIC syndrome:

- **Cause**: partial inhibition of Na^+/K^+-dependent ATPase by uremic toxins
- **Consequences**:

- **transmineralization** - modification of the distribution of Na^+ and K^+ in the intracellular and extracellular space
- **ENDOGENOUS hypothermia** - impairment of the function of thermoregulation centers with a decrease in internal temperature (lower on average by 1°C compared to normal individuals)

b) METABOLIC alterations in the UREMIC syndrome – Tab. 12.

Table 12. **Metabolic** alterations in the uremic syndrome and the corresponding mechanisms.

Metabolism	Impairment	Pathophysiological mechanism
CARBOHYDRATE Metab. ☞ **Decreased G tolerance** ☞ **Hyperglycemia**	↓ Glycogenogenesis	Intracellular K^+ deficit (transmineralization)
	↑ Glycogenolysis	Hyperglucagonemia (decreased renal catabolism)
	↓ Glycolysis	Insulin resistance (uremic toxemia)
LIPID Metab. ☞ **MIXED Hyperlipidemia & Accelerated ATS**	↑ cholesterol / *oxidised* LDL-C ↑ triglycerides / VLDL	Increased hepatic synthesis of cholesterol and triglycerides and oxidation of LDL-C Decreased lipolytic activity in the liver (lipase) and in adipose tissue (lipoprotein lipase)
PROTEIN Metab. ☞ **Negative nitrogen balance**	Hypoproteinemia	Renal protein loss Decreased protein intake Protein malabsorption
	Loss of muscle mass	Increased cellular catabolism

c) SYSTEMIC manifestations of the UREMIC syndrome – Tab. 13.

Table 13. **Systemic** manifestations of uremic syndrome and the corresponding mechanisms.

System/ Organ	Manifestations	Pathophysiological mechanism
Blood	▪ **Normochromic, normocytic anemia**	▪ **Erythropoietin (EPO) deficiency** – *the main mechanism!* ▪ **Blood loss:** occult gastrointestinal losses or during hemodialysis sessions (absolute iron deficiency) ▪ **Bone marrow damage** by: i) direct effect of uremic toxins or ii) hyperPTH-induced fibrosis ▪ **Increased destruction of erythrocytes** – early hemolysis (60-90 days) aggravated by hemodialysis ▪ **Deficiency of vitamins necessary for erythropoiesis:** vitamin B_{12}, folate ▪ **Release of proinflammatory cytokines** – IL-6 which: o Reduce renal synthesis of EPO o Increase hepatic synthesis of hepcidin with: decreased serum iron (functional iron deficiency) and increased ferritin

	▪ **Infections**	▪ Decreased chemotaxis and phagocytosis capacity of PMN (decreased inflammatory response) ▪ Depressed cellular and humoral immune response
	▪ **Muco-cutaneous hemorrhagic syndrome** → purpura, epistaxis, prolonged wound bleeding, gastrointestinal bleeding, stroke	▪ Platelet dysfunction with: – *decreased platelet adhesion/aggregation* → impaired primary hemostasis – *decreased secretion of platelet factor 3* → impaired secondary hemostasis
Digestive	▪ **Nonspecific symptoms:** anorexia, nausea, vomiting, metallic taste, malnutrition ▪ **Delayed gastric emptying with risk of reflux esophagitis** ▪ **Uremic stomatitis** ▪ **Uremic halitosis** ("uremic faetor") ▪ **Uremic gastroenteritis** – ammonia production has **ulcerative** effects and explains: ○ **digestive bleeding** ○ **diarrhea + malnutrition** in patients with ESRD	▪ Direct effect of uremic toxins ▪ Urea is a highly diffusible substance and due to this fact it is secreted at the level of the gastric and oral mucosa where it will be degraded into **ammonia by bacterial ureases**. ***Observation!*** Although acute pancreatitis is more common among patients with CKD than in the general population, amylase levels can be up to 3 times higher without indicating pancreatic disease, due to reduced urinary amylase excretion.
Heart	▪ Uremic cardiomyopathy ▪ Ischemic syndrome ▪ Uremic pericarditis	▪ Negative inotropic effect of uremic toxins ▪ Worsening of coronary artery disease ▪ Hemorrhagic fluid effusion and risk of tamponade – severe, preterminal manifestation in uremia
Central NS	▪ Uremic encephalopathy – Confusion, drowsiness, delirium – Convulsions → coma	▪ Direct effect of uremic toxins ▪ Shrinkage of neurons as a result of increased plasma osmolarity (osmotic gradient between the extracellular fluid and the intracellular fluid)

Peripheral NS	▪ Carpal tunnel syndrome ▪ Uremic polyneuropathy ▪ Muscle cramps ▪ Hiccups (urea > 250 mg/dL)	▪ Compression of the median nerve (by secondary amyloidosis) ▪ Atrophy and demyelination of nerve fibers ▪ Interference of nerve transmission under the action of uremic toxins
Skin	▪ Delayed wound healing ▪ Dirty-yellow skin ▪ Dermatitis / Pruritus ▪ Nephrogenic systemic fibrosis	▪ Decreased collagen production ▪ Anemia and urochrome deposition ▪ Deposition of urea and calcium crystals in the skin ("uremic frost") ▪ Fibrosing skin disease that occurs as a result of the administration of gadolinium-based contrast agents to patients with GFR < 30ml/min (also prohibited in dialysis patients!).

B. Impairment of the REGULATORY FUNCTION of the HOMEOSTASIS of the INTERNAL ENVIRONMENT

1. WATER BALANCE

a) Initially: the water balance is in **equilibrium**, but **labile**:

- an exaggerated exogenous intake → **hyperhydration**
- an increased loss (e.g., vomiting, diarrhea, diuretic abuse) → **intense dehydration** and renal circulation disorders that can lead to ***acute renal injury in the context of CKD***

In the patient with CKD: the kidney cannot adapt to very large variations in water and Na^+ intake because the concentration and dilution capacity is **LOW**:

- the osmolarity of the final urine is *relatively constant* = ~ 300 mOsm/l similar to the primary urine (or deproteinized plasma) = **isoosmolarity**
- the density of the final urine is *relatively constant* = ~ 1.010 similar to the primary urine (or deproteinized plasma) = **isosthenuria**
- diuresis becomes **invariant** = independent of water intake or insensitive to ADH

b) Late: water retention with HT, edema, heart failure

Observation!

In the **normal subject**: the kidney can adapt to very large variations in water and Na^+ intake because the concentration and dilution capacity is **NORMAL**:

- final urine osmolarity *can vary* between 50 and 1200 mOsm/l
- final urine density *can vary* between 1.005 and 1.040
- diuresis *can vary* between 15 liters (maximum dilution in diabetes insipidus) and 0.5 liters (maximum concentration in severe dehydration)

2. SODIUM BALANCE

a) Initially: Na^+ balance is in **equilibrium** due to the **increased fraction of Na^+ excreted via the kidney** → ***mandatory loss of 20-40 mEq/day*** *which must be covered by increasing the exogenous Na^+ intake to prevent* ***hyponatremia*** *and* ***decreased volemia****!*

The mechanisms of renal Na^+ loss are:

1) **OSMOTIC diuresis** - increased primary urine flow at the level of intact nephrons causes decreased tubular reabsorption of Na^+ and water by decreasing the contact time of the tubular fluid with the reabsorption surface
2) **Impairment of Starling forces in the PERITUBULAR CAPILLARIES of the PCT** - causes decreased tubular reabsorption of Na^+ and water in the proximal tubule by the following mechanisms:
   - hypoalbuminemia causes *decreased oncotic pressure in the peritubular capillaries*
   - the presence of proteins in the primary urine causes *increased tubular oncotic pressure*
3) **Partial inhibitory effect of UREMIC TOXINS on the Na^+/K^+-dependent ATPase** - causes decreased Na^+ transport from the tubular cell towards the renal interstitium and peritubular capillaries

b) Late: Na^+ retention with HT, edema, heart failure

3. POTASSIUM BALANCE

a) Initially: K^+ balance is in **equilibrium** because the decrease in glomerular K^+ filtration is **compensated** by:

- **decreased K^+ reabsorption at the PCT level** - secondary to increased tubular flow through osmotic diuresis
- **increased K^+ secretion at the DT (distal tubule) level** - stimulated by aldosterone
- **increased K^+ secretion at the colon level** - stimulated by aldosterone

b) Late: K^+ retention with ***severe hyperkalemia*** requiring dialysis

Observation!

Normally, glomerularly filtered K^+ is almost entirely reabsorbed in the proximal tubule, so that the K^+ present in the final urine comes *exclusively* from distal tubular secretion. *In CKD*, the filtered amount is low, while the reabsorbed amount decreases, and the distally secreted amount increases and may even exceed the glomerularly filtered amount.

4. ACID-BASE BALANCE

- **Characteristic**: metabolic acidosis with increased anion gap
  - **decreased GFR** → decreased elimination of phosphate (HPO_4^{2-}) and sulfate (SO_4^{2-}) anions and consumption of plasma HCO_3^-
  - **tubular dysfunction** → impairment of renal mechanisms of tubular secretion of H^+ and restoration of plasma HCO_3^-

a) Initially: metabolic acidosis **compensated** by:

- **consumption of the bone buffer system** → worsening of bone demineralization
- **respiratory compensation with hyperventilation** (decreases $PaCO_2$) → Kussmaul dyspnea

b) Late: **decompensated** metabolic acidosis

5. PHOSPHO-CALCIUM BALANCE

- **Characteristics**:
  - decreased GFR → **hyperphosphatemia** that stimulates the release of the phosphaturic agent **FGF-23** (Fibroblast Growth Factor-23) by **osteocytes** with the **following consequences**:
    - decreased renal phosphate reabsorption with phosphaturia (in order to normalize phosphatemia)
    - inhibition of renal α1-hydroxylase with decreased active vitamin D with decreased intestinal phosphate absorption

Observation!
Elevated FGF23 levels are the strongest independent predictor of mortality in CKD.

  - with the progression of CKD, the **production of renal 1α-hydroxylase** decreases with a **decrease in the generation of the active form of vitamin D, 1,25 dihydroxycholecalciferol**, which causes a decrease in digestive absorption and renal reabsorption of calcium → **hypocalcemia** responsible for **<u>secondary</u> HYPERparathyroidism** with 2 consequences:
    - **increased *renal* calcium reabsorption** and phosphate excretion
    - **increased *bone* calcium reabsorption** through osteoclast activation and the appearance of combined lesions of fibrosis + bone cysts or **fibro-cystic osteitis**
  - prolonged hypocalcemia (vitamin D deficiency) and prolonged hyperphosphatemia (decreased GFR) cause over time **hyperplasia of the parathyroid glands** responsible for **tertiary HYPERparathyroidism** (autonomous = release of PTH independently of the level of calcemia) with **2** consequences:
    - **hypercalcemia** through increased bone calcium mobilization with **worsening of bone lesions**
    - **increased calcium x phosphorus product** (> 70 mg/dL) → promotes **soft tissue calcification** (calcium phosphate precipitation):
      - ✓ at the level of **arterial walls** (aorta, coronary a. and peripheral a.) → increased vascular stiffness and afterload, **worsening of left ventricular hypertrophy** and **accelerated ATS** which explains why CKD increases (over 15 times) **the risk of cardiovascular events: heart failure, myocardial infarction, stroke, peripheral vascular disease**
      - ✓ at the level of **heart valves** → their dysfunction

<u>C. Impairment of the ENDOCRINE FUNCTION</u>

1. Decreased secretion of ERYTHROPOIETIN (EPO)

- **Cause**: in CKD the kidney is NOT able to increase the secretion of EPO to values that achieve effective stimulation of the bone marrow
- **Consequence**: EPO deficiency causes **normocytic normochromic anemia**

2. Decreased generation of ACTIVE VITAMIN D (1,25-dihydroxycholecalciferol)

- **Cause**: inhibition of **1 α–hydroxylase** prevents the hydroxylation of 25-hydroxycalciferol (calcidiol) and the formation of **1,25-dihydroxycholecalciferol** (calcitriol), the active form of vitamin D
- **Consequences**:

– decreased intestinal absorption of Ca^{2+} → **hypocalcemia**
– bone demineralization → **osteomalacia**

3. Modification of RENIN secretion

- **Characteristic**: modification of renin synthesis can occur in both directions → increase or decrease in synthesis

a) Initially:

– **renin can be increased**: renal ischemia → renovascular hypertension
– in CKD → the kidney *loses the ability to adapt renin secretion to hemodynamic needs*

b) Late: **decrease in renin synthesis** caused by the destruction of renin-secreting cells

COMPLICATIONS OF CKD

1. CARDIOVASCULAR complications → major cause of mortality in CKD

a) Arterial Hypertension – by:
– activation of the RAAS
– water and salt retention

b) Accelerated ATS - by:
– HT
– dys/hyperlipidemia
– insulin resistance (uremic toxicity, systemic proinflammatory status, metabolic acidosis)
– vascular calcifications that can be complicated by *calciphylaxis* - the association of vascular calcifications with thrombosis of small vessels and which manifests itself through skin ulcerations (wounds, pressure ulcers) that do not heal and necrosis in the dermis

c) Heart failure - by:
– **primary decrease in contractility:**
  o ischemic cardiopathy
  o uremic cardiomyopathy
– **secondary decrease in contractility:**
  o *pressure overload*: HT
  o *volume overload*: hydrosaline retention, hyperdynamic state induced by severe anemia
– **decreased cardiac filling**: uremic pericarditis

2. PULMONARY complications

- **Kussmaul dyspnea** - compensatory reflex hyperventilation in metabolic acidosis
- **Cough reflex depression and increased viscous secretions** - cause:
  – bronchial obstructive syndrome
  – uremic pneumopathy
- **Pulmonary edema** - by volume overload in congestive HF ("uremic lung")

3. HEMATOLOGICAL and IMMUNOLOGICAL complications

a) Normocytic, normochromic anemia (possibly microcytic in the terminal stage) - by:

- **EPO deficiency -** the **main mechanism:**

  - absolute: decreased EPO production
  - relative: decreased bone marrow response to EPO – against the background of systemic inflammation that causes EPO resistance
- **UREMIC toxicity** responsible for:
  - pathological hemolysis
  - direct medullary inhibition
- **Blood loss** (occult gastrointestinal bleeding, dialysis)
- **Vitamin/mineral deficiency (vitamin B_{12}, folate, iron)** necessary for the erythropoiesis process
- **Increased HEPCIDIN level** due to increased hepatic synthesis under the action of IL-6

b) Suppression of the immune system with increased susceptibility to infections - through uremic toxicity

c) Hemorrhagic syndrome with increased risk of bleeding - through uremic toxicity

4. BONE complications: **spontaneous fractures, bone deformities/pain**

- **Characteristics**: bone complications are collectively known as ***mineral and bone disorders (MBD)*** in CKD (Chronic Kidney Disease – Mineral and Bone Disorder, CKD-MBD) which include:

  a) Changes in: calcemia, phosphatemia, serum levels of PTH, FGF23 and vitamin D metabolites

  b) Abnormalities of bone turnover (bone remodeling) - can coexist in variable proportions in the same patient:
  - **Osteomalacia** due to vitamin D deficiency
  - **Bone demineralization** due to metabolic acidosis
  - **Fibrocystic osteitis** due to secondary hyperPTH
  - **Osteosclerosis** - increased bone density typically observed in the spine (where the alternation of sclerotic bands/porous areas causes the radiographic appearance of a "rugger jersey"

  Vascular consequences: vascular calcifications accompanied by arterial stiffness

6. PATHOPHYSIOLOGY OF ACID-BASE DISORDERS

ACID-BASE BALANCE – Breef Physiology Overview

Definition: maintenance of the pH of the internal environment within normal limits through mechanisms that generate, buffer and eliminate acids and bases.

1. VOLATILE acids → 15,000 mmol CO_2/day - eliminated via *respiration*
- in the plasma CO_2 is hydrated with the formation of carbonic acid (dependent on the $PaCO_2$ level)
  - ✓ plasma $[H_2CO_3] = \alpha \times PaCO_2$
  - ✓ plasma $[H_2CO_3] = 0.03 \times 40$ mmHg = 1.2 mmol/L
- in cells that have *carbonic anhydrase* (CA), CO_2 hydration leads to the formation of HCO_3^- and H^+ (the CO_2 hydration rate is 5000 x higher than in plasma)

2. NON-VOLATILE acids → 1 mEq/kg body weight/day - eliminated via the *kidneys*
- *inorganic acids:*
  - ✓ **H_2SO_4** (sulfate anion, SO_4^{2-}): results from the oxidation of AA such as methionine, cysteine
  - ✓ **H_3PO_4** (phosphate anion, HPO_4^{2-}): results from the oxidation of phosphorylated compounds (nucleic acids)
  - ✓ **HCl** (chloride anion, Cl^-): results from the oxidation of AA such as arginine, lysine
- *organic acids:*
  - ✓ **lactic acid**: the result of anaerobic *glucose* metabolism
  - ✓ **ketoacids** (acetylacetic acid and beta-hydroxybutyric acid): the result of beta-oxidation of *fatty acids*

3. BASES
- ⇒ from AA metabolism (e.g., aspartate, glutamate)
- ⇒ from the metabolism of organic anions (e.g., citrate, lactate, acetate)

pH regulation mechanisms

I. BUFFER systems – represent the **first "line of defense" of pH**
- **Characteristics**: intervene **quickly**, but have **limited** efficiency in restoring the pH
- **Types**:
  - ✓ ***bicarbonate/carbonic acid buffer system (HCO_3^-/H_2CO_3) - the most important, being measurable***
  - ✓ *protein buffer system* (Pr^-/PrH)
  - ✓ *buffer system of phosphates* (Na_2HPO_4/NaH_2PO_4)

1. BICARBONATE / CARBONIC ACID (HCO_3^-/H_2CO_3) buffer system
- is the **main EXTRACELLULAR buffer system, its components being in equilibrium, according to the reaction:**

$$H^+HCO_3^- \leftrightarrow H_2CO_3 \xrightarrow[\text{carbonic anhydrase}]{} CO_2 + H_2O$$

  - the average bicarbonate concentration is **increased**: ~ 24 mmol/L
  - its components are **adjustable**:
    - ✓ **RESPIRATORY** component ($PaCO_2$ /H_2CO_3) by the **lung**
    - ✓ **METABOLIC** component (HCO_3^-) by the **kidneys**
- acts **very quickly** (sec → min)
- the parameters of the HCO_3^-/H_2CO_3 buffer system **represent the parameters of the acid-base balance** (Tab. 14)

2. PROTEIN buffer system
- **Characteristics**:
  - is the **main INTRACELLULAR buffer system**

– proteins are *amphoteric* → can function as acids (PrH) that release H^+ or as bases (Pr^-) that accept H^+

$$\mathbf{PrH \leftrightarrow Pr^- + H^+}$$

3. PHOSPHATE buffer system

- **Characteristics**:
  – is the main **URINARY buffer system**
  – Na_2HPO_4 (disodium phosphate) is filtered at the glomerular level and binds H^+ generating NaH_2PO_4 (monosodium phosphate) which is eliminated in the final urine as *titratable acidity*

$$\mathbf{Na_2HPO_4 + H^+ \rightarrow NaH_2PO_4 + Na^+}$$

4. TRANSCELLULAR H^+/K^+ EXCHANGE system

- **Characteristics**: ensures the **free transfer of H^+ and K^+** between the extracellular fluid (ECF) and the intracellular fluid (ICF)
  – **in acidosis**: excess H^+ from the ECF will enter the cells (ICF) in exchange for K^+ with 2 effects:
    - H^+ is **buffered** by intracellular proteins ($Pr^- \rightarrow PrH$)
    - K^+ exit from the cells causes **HYPERkalemia**
  – **in alkalosis**: in the presence of a deficit of H^+ in the ECF, protons exit the cells (ECF) in exchange for K^+ and:
    - H^+ is **released** by intracellular proteins ($PrH \rightarrow Pr-$)
    - K^+ entry into the cells causes **hypokalemia**

Table 14. Acid-base balance (ABB) parameters.

Parameter	NORMAL value	Significance
pH	7,35 - 7,45 (**tipically, 7,38 - 7,42**)	▪ **Reveals the state of the ABB** – **pH < 7,35 = acidosis** ✓ severe symptoms at pH < 7.25 ✓ death at pH < 7 – **pH > 7,45 = alkalosis** ✓ severe symptoms at pH > 7,55 ✓ death at pH > 7,65
$[HCO_3^-]$	22 - 26 mmol/L (mEq/L) **(average ~ 24)**	▪ It is the **METABOLIC** component of the buffer system (regulated by the intervention of the **kidney)** – **Primary ↓ in $[HCO_3^-]$ = metabolic acidosis** – **Primary ↑ in $[HCO_3^-]$ = metabolic alkalosis**
$PaCO_2$	35 - 45 mmHg **(average ~ 40)**	▪ It is the **RESPIRATORY** component of the buffer system (regulated by the intervention of the **lungs)** – **Primary ↑ in $PaCO_2$ = respiratory acidosis** – **Primary ↓ in $PaCO_2$ = respiratory alkalosis**
$[HCO_3^-]/[H_2CO_3]$ ratio ***or*** **$[HCO_3^-]/[0,03 \times PaCO_2]$ ratio**	18 - 22 **(average ~ 20)**	▪ It is the determining factor of **pH** according to the *Henderson-Hasselbalch equation:* $\mathrm{pH} = 6{,}1 + \log \frac{[HCO_3^-]}{[0{,}03 \times PaCO_2]}$ – **ratio < 18 = pH < 7,35** – **ratio > 22 = pH > 7,45**

Remember!

The interpretation of acid-base imbalances is always carried out in the ***clinical context*** and taking into account the calculation of compensation limits using specific formulas.

II. RESPIRATORY compensation – represents the **second "line of defense" of pH**

- **Role** - intervenes in the compensation of ***METABOLIC*** *acid-base imbalances (ABI)* in which:
  - *PRIMARY changes in plasma [HCO_3^-] are compensated by SECONDARY changes in the* ***same direction*** *of $PaCO_2$* → the [HCO_3^-]/[H_2CO_3] ratio is restored within normal limits of ~ 20
- **Characteristics** - compensation is:
  - *rapid* → occurs in minutes-hours, maximum in 12-24 hours
  - *incomplete* (pH is not corrected, but only adjusted) → metabolic ABI compensation is PARTIAL
- **Mechanism**: because H^+ ions cannot cross the blood-brain barrier, pH changes act on *peripheral chemoreceptors* in the carotid sinus and aortic arch and determine:
  - **in metabolic acidosis** (↓ pH): the increase in [H^+] increases the frequency of discharge of peripheral chemoreceptors followed by **pulmonary hyperventilation** with decreased $PaCO_2$
  - **in metabolic alkalosis** (↑ pH): decreased [H^+] decreases the discharge frequency of peripheral chemoreceptors followed by **pulmonary hypoventilation** with increased $PaCO_2$

III. RENAL compensation – represents the **third "line of defense" of pH**

- **Role** - intervenes in the compensation of ***RESPIRATORY*** *acid-base imbalances* in which:
  - *PRIMARY changes in $PaCO_2$ are compensated by SECONDARY changes in the* ***same direction*** *of plasma [HCO_3^-]* → the [HCO_3^-]/[H_2CO_3] ratio is restored within normal limits of ~ 20
- **Characteristics** - compensation is:
  - *slower* → onset within 12-24 hours, maximum in 3-5 days
  - *incomplete* (pH is not corrected, but only adjusted) → respiratory ABI compensation is PARTIAL
- **Mechanisms** - the kidney *compensates* for respiratory ABI and *corrects* metabolic ABI by:
  1. **Modification of proton secretion (H^+) coupled with**: a) **reabsorption** of HCO_3^- and b) **generation** of HCO_3^-
  2. **Modification of HCO_3^- secretion coupled with reabsorption of H^+**
  - in **acidosis** → the kidney eliminates **ACIDIC urine** by:
    - increased secretion of H^+ coupled with **reabsorption** and **generation** of newly formed HCO_3^-
    - decreased secretion of HCO_3^- coupled with H^+ reabsorption
  - in **alkalosis** → the kidney eliminates **ALKALINE urine** by:
    - decreased secretion of H^+ coupled with **reabsorption** and **generation** of newly formed HCO_3^-
    - increased secretion of HCO_3^- coupled with reabsorption of H^+

1. Modification of H^+ secretion:

a) H^+ secretion coupled with HCO_3^- REABSORBTION

- **Location** - at the level of the ***proximal convoluted tubule*** (due to a higher concentration of carbonic anhydrase in the PCT compared to the DCT)
- **Mechanism**:
  - in the *tubular fluid* (urine) - glomerularly filtered $Na^+HCO_3^-$ (sodium bicarbonate) dissociates into Na^+ and HCO_3^-. $HCO_3^- + H^+ \rightarrow H_2CO_3$ which dissociates into $H_2O + CO_2$ under the action of carbonic anhydrase (CA) at the brush border level
  - in the *tubular cell*: under the action of intracellular CA CO_2 and H_2O diffusing from the tubular fluid generate H_2CO_3 which dissociates into HCO_3^- and H^+: H^+ is secreted into the urine and resumes the cycle and HCO_3^- passes into the blood

b) H^+ secretion coupled with HCO_3^- GENERATION

- **Location** - at the level of the ***distal convoluted tubule and the collecting duct***, respectively, of the type A (α) intercalated cells, being the ***main process that ensures the elimination of H^+ from the body***
- **Mechanisms**: i) excretion of titratable acidity and ii) renal ammoniogenesis

  i) Excretion of titratable acidity
  - **Role** - ensures the elimination of **1/3** of H^+ (daily load of non-volatile acids)
  - **Mechanism**:

- in the *tubular fluid* - glomerularly filtered Na_2HPO_4 (disodium phosphate) dissociates into Na^+ and $NaHPO_4^-$. The latter binds H^+ generating NaH_2PO_4 (monosodium phosphate) which is eliminated in the final urine as *titratable acidity*
- in the *tubular cell* - under the action of CA H_2CO_3 is formed which dissociates into HCO_3^- and H^+:
  - H^+ is excreted in the urine
  - *Newly generated* HCO_3^- reaches the blood

ii) Renal ammoniogenesis

- Role - ensures the elimination of **2/3** of H^+ and is the ***main mechanism of H^+ excretion*** in states of ***chronic acidosis***
- Mechanism:
  - in the *tubular cell* - under the action of glutaminase, glutamine generates 2 NH_3 + **$2HCO_3^-$**:
    - ✓ NH_3 is passively secreted into the tubular lumen
    - ✓ *Newly generated* HCO_3^- reaches the blood
  - in the *tubular fluid* - NH_3 binds H^+ and generates the ammonium ion which is eliminated in the form of NH_4Cl (ammonium chloride)

2. Modification of HCO_3^- secretion coupled with reabsorption of H^+

- **Location** - at the level of *type B (β) intercalated cells* in the DCT + CD
- in the *tubular cell*: under the action of intracellular CA H_2CO_3 generates HCO_3^- and H^+:
  - HCO_3^- is secreted into the tubular fluid through the *Cl^-/ HCO_3^- exchanger*
  - H^+ is transported into the blood through the *H^+ pump*

Observation!

- **H^+ secretion** through the *H^+/K^+ pump* coupled with HCO_3^- generation from *type A intercalated cells* is regulated by **aldosterone, pH** and **potassium** levels
  - *have a stimulating effect*: increased aldosterone, decreased pH and decreased potassium levels ⇒ increased plasma [HCO_3^-]
  - *have an inhibitory effect*: decreased aldosterone, increased pH and increased potassium levels ⇒ decreased plasma [HCO_3^-]
- **HCO_3^- secretion** through the *Cl^-/HCO_3^- exchanger* coupled with H^+ reabsorption from *type B intercalated cells* is regulated by **pH** and **chloremia** and plays a role in regulating the **plasma Cl^-/HCO_3^- ratio** (plasma Cl^- and HCO_3^- concentrations vary *inversely proportional* in order to maintain plasma electroneutrality):
  - *have a stimulating effect*: increased pH, increased chloremia ⇒ decreased plasma [HCO_3^-]
  - *have an inhibitory effect*: decrease in pH, decrease in chloremia ⇒ increase in plasma [HCO_3^-]

METABOLIC ACIDOSIS

DEFINITION:

- primary decrease in [HCO_3^-] < 22 mmol/L
- arterial pH < 7.35

MECHANISMS OF pH RESTORATION:

1. ***Respiratory compensation through HYPERVENTILATION*** in view of the secondary decrease in $PaCO_2$ - is PARTIAL
2. ***Renal correction*** → **INCREASE in H^+ secretion coupled with REABSORBTION and GENERATION of HCO_3^-** with elimination of **acidic urine**
3. ***Activation of transcellular H^+/K^+ exchange*** → intracellular accumulation of H^+ + K^+ exit from cells with **HYPERkalemia**

4. ***Mobilization of bone buffer systems:***
   - it has the **main role** in *chronic metabolic acidosis*: e.g., chronic kidney disease
   - *mechanism*: H^+ penetrates the bone and dissolves the bone matrix with the release of Ca^{2+} ions that will buffer the excess H^+ at the level of the ECF
   - *consequences*: bone demineralization and renal lithiasis by increased elimination of Ca^{2+} in the urine

ETIOPATHOGENIC CLASSIFICATION according to the anion gap (AG) - Tab. 15:

A. Metabolic acidoses with **INCREASED anion gap (AG)**
B. Metabolic acidoses with **NORMAL anion gap (AG)**

The **anion gap** (AG) or undetermined anions represents the concentration of plasma anions that participate in the electroneutrality of the plasma (the sums of positive and negative charges must be equal) and which are **NOT determined during usual analyses**.
Undetermined anions include:
- *organic anions*: albumin, ketoacids, lactate, acidic metabolites
- *inorganic anions*: phosphate, sulfate

$$AG = [Na^+ + K^+] – [Cl^- + HCO_3^-] \qquad N.V. = 12 – 16 \text{ mmol/L}$$

Table 15. Etiopathogenic classification of metabolic acidoses.

A. Metabolic acidosis with INCREASED AG	**B. Metabolic acidosis with NORMAL AG**
1. Lactic acidosis	1. Acidosis due to increased *digestive* losses of HCO_3^-
2. Ketoacidosis	2. Acidosis due to increased *renal* losses of HCO_3^-
3. Toxic metabolic acidosis	3. Acidosis due to *decreased renal excretion* of H^+
4. Chronic kidney disease	4. Acidosis due to Cl^- loading

Observation!
The 2 forms of acidosis can coexist: for example, in cholera, the massive loss of HCO_3^- through diarrhea would typically cause metabolic acidosis with normal AG but in some conditions the acidosis associates an increased AG against the background of decreased volemia with tissue hypoperfusion and consequent lactic acidosis (to which is added the risk of acute kidney injury).

A. Metabolic acidoses with INCREASED AG

- **Definition**: **decreased [HCO_3^-]** is the consequence of ***bicarbonate consumption*** in order to buffer the ***increased amounts of nonvolatile acids in plasma***

- **Etiopathogenic forms:**

1. LACTIC acidosis

- **Characteristic**: the most common cause of acute metabolic acidosis - type A in hospitalized patients
- **Pathogenesis**:
  - activation of anaerobic glycolysis with increased lactic acid production
  - decreased lactic acid metabolism
- **Types of lactic acidosis** (Tab. 16):

Table 16. Types of lactic acidosis.

	Lactic acidosis type A (hypoxic)	**Lactic acidosis type B (non-hypoxic)**
Pathogenic mechanism	▪ increased lactic acid PRODUCTION in conditions of cellular HYPOXIA/ tissue HYPOPERFUSION	▪ decreased METABOLISM and/or ELIMINATION of lactic acid or via MITOCHONDRIAL DYSFUNCTION
Causes	▪ acute circulatory failure - all forms of shock ▪ respiratory failure - asphyxia, severe forms of COPD, asthma ▪ severe anemia ▪ pathological Hb compounds (carboxyHb, cyanHb) in CO and cyanide poisoning	▪ ketoacidosis: diabetes, alcoholism, starvation ▪ renal or hepatic failure ▪ toxic metabolic acidosis (acute overdose or chronic ingestion): ethanol, methanol, ethylene glycol and drug-induced: salicylates (aspirin), biguanides (metformin), paracetamol (the metabolite pyroglutamic acid accumulates secondary to glutathione depletion)

Observation!
Lactate has 2 optical isomers: L-lactate and D-lactate. Both types of lactic acidosis type A and B are based on the **increase of L-lactate** (the final product of anaerobic glycolysis in humans). D-lactate is produced only by bacterial flora (glucose fermentation), its increase in humans being the consequence of increased bacterial proliferation in the small intestine.

2. KETOACIDOSIS

- **Pathogenesis**: increased production of ***ketone bodies*** *(beta-hydroxybutyric acid and acetyl-acetic acid)* with their accumulation in plasma (**ketonemia**) and urinary elimination (**ketonuria**) determined by:
  - *decreased use of carbohydrates* as an energy substrate due to: insulin deficiency or decreased glycogen stores
  - *increased use of FFA* as an energy substrate → stimulation of FFA β-oxidation and hepatic ketogenesis

The excess of acetyl-CoA resulting from FFA beta-oxidation exceeds the metabolic capacity of the Krebs cycle. By condensation of 2 acetyl-CoA molecules, **acetyl-acetic acid** results (the first ketone body, 20%) and by dehydrogenation, **beta-hydroxybutyric acid** results (the main quantitative ketone body, 78%); a small part (2%) of aceto-acetate is converted by spontaneous decarboxylation into **acetone** (the third ketone body).

a) DIABETIC ketoacidosis → occurs in conditions of **high** blood glucose

- **Pathogenesis**:
  - is the **major complication of type 1 diabetes** caused by *intercurrent conditions* (respiratory infections, gastroenteritis, pancreatitis, myocardial infarction) or by *insulin discontinuation*
  - **absolute** insulin deficiency involves *decreased glucose utilization in peripheral tissues* with 2 consequences: i) *increased lipolysis* in adipose tissue with increased release of FA (which will be transported to the liver) and ii) *increased hepatic*

ketogenesis and blood ketone body levels (with exceeded buffering capacity and severe decrease in plasma HCO_3^-) → *metabolic acidosis*
- sometimes it can be associated with *lactic acidosis* (after initiating insulin therapy that induces the suppression of neoglucogenesis which will cause the decrease in lactate extraction from the circulation)

b) ALCOHOLIC ketoacidosis → occurs in conditions of **normal/low** blood glucose
- **Pathogenesis**:
  - ethanol is metabolized in the liver under the action of *alcohol dehydrogenase* and generates large amounts of NADH + H^+ which favors the metabolism of pyruvic acid to lactic acid with 2 consequences:
    - accumulation of lactic acid → *lactic acidosis*
    - decrease in pyruvic acid reduces neoglucogenesis → *hypoglycemia*
  - deficient nutrition in chronic alcoholism causes depletion of glycogen stores → *hypoglycemia*
  - hypoglycemia stimulates the release of counter-regulatory hormones: catecholamines, cortisol, glucagon, STH → *increased beta-oxidation of FFA* and *increase of ketogenesis → ketoacidosis*

c) Ketoacidosis from FASTING → occurs in conditions of **low** blood sugar
- **Pathogenesis**:
  - cessation of exogenous carbohydrate intake causes depletion of glycogen stores → *hypoglycemia*
  - acidosis is exacerbated by *physical exertion* (increased anaerobic glycolysis)

3. TOXIC METABOLIC ACIDOSES

a) METHANOL and ETHYLENE GLYCOL Poisoning
- **Pathogenesis**: the metabolism of methanol and ethylene glycol generates non-volatile acidic metabolites that accumulate in the plasma (Tab. 17).

Table 17. Characteristics of methanol and ethylene glycol poisoning.

Characteristics	METHANOL poisoning	ETHYLENE GLYCOL poisoning
Acid production	▪ Metabolism of formaldehyde into **formic acid** → source of NADH + H^+ for the ↑ production of **lactic acid**	▪ Metabolism of ethylene glycol into **oxalic acid** → source of NADH + H^+ for the ↑ production of **lactic acid**
Symptoms	▪ They appear after 24 hours and consist of: – **optic nerve** damage – **CNS** damage	▪ **Neurological:** state of "drunkenness" → coma ▪ **Cardiorespiratory:** tachycardia, pulmonary edema ▪ **Renal failure:** oxalate crystals
Lethal dose	30 ml	100 ml

- **Treatment**:
  - administration of **FOMEPIZOLE** - alcohol dehydrogenase inhibitor

- *administration of ethanol* → prevents the conversion of methanol and ethylene glycol into their toxic metabolites because the *affinity of alcohol dehydrogenase is 10 – 20 x higher for ethanol*
- *correction of acidosis* → with $NaHCO_3$ in severe forms
- *hemodialysis* → removal of methanol and ethylene glycol from the body

b) SALICYLATE intoxication

- **Pathogenesis**:
  - acetylsalicylic acid (aspirin) is metabolized in plasma into **salicylic acid** (diffusible), a weak acid that dissociates into: salicylate (less diffusible) and H^+
  - salicylic acid crosses the blood-brain barrier, stimulates the respiratory centers and causes **RESPIRATORY ALKALOSIS in the initial phases of intoxication** → the kidney compensates for respiratory alkalosis by ↓ plasma [HCO_3^-]
  - salicylic acid uncouples oxidative phosphorylation with **3 consequences**:
    - ✓ energy loss under the form of heat → *hyperthermia* (41°C)
    - ✓ accumulation of pyruvic acid + NADH → *lactic acid* and **METABOLIC ACIDOSIS in advanced stages of intoxication**
    - ✓ accumulation of acetyl-CoA → generation of ketone bodies with *ketoacidosis*
- **Lethal dose**: 10 – 30 g in adults and 3 g in children
- **Treatment**: increase in pH in order to favor the dissociation of salicylic acid (with high toxicity) into salicylate (with low toxicity)
  - alkalinization of plasma → favors the exit of salicylic acid from cells
  - alkalinization of urine → favors urinary excretion in the form of salicylate

4. Acidosis in CHRONIC KIDNEY DISEASE (CKD)

- **Characteristics**:
  - the most common cause of **CHRONIC metabolic acidosis**
  - acidosis is **moderated** by the intervention of *bone buffer systems* BUT leads to *bone demineralization* and hypercalciuria, being a **major risk factor for renal osteodystrophy**
- **Cause**: CKD especially in the terminal stage of RENAL FAILURE with UREMIA
  - **Glomerular dysfunction** → Decrease in glomerular filtration rate (GFR) with:
    - *Retention of inorganic (phosphate, sulfate) and organic acids*:
      - ✓ $H_3PO_4 \rightarrow HPO_4^{2-}$ + **$2H^+$**
      - ✓ $H_2SO_4 \rightarrow SO_4^{2-}$ + **$2H^+$**
    - *Consumption of HCO_3^- for buffering*: HCO_3^- + **H^+** → decrease in plasma [HCO_3^-]
  - **Tubular dysfunction** → Decrease in the capacity for: i) tubular secretion of H^+ and restoration of HCO_3^- and ii) reabsorption of HCO_3^- with loss of bicarbonate

<u>B. Metabolic acidoses with NORMAL AG</u>

- **Definition**: **decreased [HCO_3^-]** is the consequence of ***digestive/renal loss of HCO_3^-***, of decreased renal excretion of H^+ or of ***Cl^- overload*** *in HYPERCHLOREMIC acidoses*
- **Etiopathogenic forms:**

1. Acidosis due to increased DIGESTIVE losses of bicarbonate

- **Causes**: loss of digestive fluids (intestinal, pancreatic juice, bile) rich in HCO_3^-
  - **Severe/chronic diarrhea** – the main cause in children
  - **Ileostomy** – surgical opening of an artificial anus at the level of the ileum in: severe inflammatory bowel disease (most common), colorectal cancer
  - **Ureterosigmoidostomy** – drainage of the ureters into the sigmoid colon in resection for bladder cancer) → colonic mucosa absorbs Cl^- and secretes HCO_3^-

2. Acidosis due to increased RENAL losses of bicarbonate

- **Causes**: decreased tubular reabsorption of HCO_3^- with bicarbonateuria
  - **Acetazolamide treatment** → carbonic anhydrase inhibition at the PCT level
  - **Hyperparathyroidism** → decreased urine acidification capacity
  - **Tubular lesions** induced by drugs, heavy metals, paraproteins
  - **Renal tubular acidosis** (RTA): systemic acidosis caused by an alteration in the ability of the renal tubules to maintain acid-base balance:
    - ✓ **Proximal RTA (type 2):** *decreased reabsorption of HCO_3^- at the PCT level*, most frequently in *Fanconi syndrome* - complex tubular defect that associates glycosuria, aminoaciduria, phosphaturia

3. Decreased renal excretion of H^+

- **Causes**:
  - **Hypoaldosteronism**: decreased distal secretion of H^+ with decreased generation of HCO_3^- at the DCT level
  - **Renal tubular acidosis (RTA)**:
    - ✓ **Type 1 (distal) RTA**: *decreased secretion of H^+ at the DCT level* with the impossibility of lowering the urinary pH below 5.3 despite systemic acidosis
    - ✓ **Type 4 ATR**: deficient or absent response to *aldosterone* with *hyperkalemia*

4. Acidosis by CHLORIDE OVERLOAD (hyperchloremic acidosis)

- **Causes**:
  - **Increased administration of ammonium chloride** (NH_4Cl) used in the treatment of alkalosis
  - **Parenteral hyperalimentation** with AA solutions containing Cl^- – increased catabolism of arginine, lysine
    → Excess Cl^- stimulates the secretion of HCO_3^- at the level of type B intercalated cells
    → decreased plasma [HCO_3^-]

MANIFESTATIONS of metabolic acidosis:

1. Manifestations determined by the CAUSES of metabolic acidosis

- *diabetic ketoacidosis*: hyperglycemia, glycosuria, acetone breath
- *chronic kidney disease / end-stage renal failure*: nitrogen retention, uremic breath

2. Manifestations determined by the DECREASE in pH and [HCO_3^-]

Mechanisms:

- *systemic vasodilation* (indirect effect) → acidosis decreases the response of vessels to sympathetic stimulation
- *DECREASE in neuromuscular excitability* → acidosis mobilizes Ca^{2+} bound to plasma proteins and causes the increase in [Ca^{2+}] in the ECF

Manifestations:

- *neurological*: increased intracerebral pressure, cerebral edema, headache, lethargy, coma
- *cardiovascular*: decreased heart rate and contractility, decreased CO, cardiac arrhythmias, hypotension
- *gastrointestinal*: anorexia, nausea, vomiting, abdominal pain
- *muscular*: muscle hypotonia, muscle weakness
- *skin changes*: warm, hyperemic (cutaneous vasodilation)

3. Manifestations determined by the acid-base COMPENSATION / CORRECTION

- *respiratory*: Kussmaul breathing (increased frequency and amplitude of respirations)
- *renal*: elimination of acidic urine
- *transcellular H^+/K^+ exchange*: hyperkalemia
- *bone*: bone demineralization (in chronic acidosis)

METABOLIC ALKALOSIS

DEFINITION:

- primary increase in [HCO_3^-] > 26 mmol/L
- arterial pH > 7.45

MECHANISMS OF pH RESTORATION:

1. ***Respiratory compensation through HYPOVENTILATION*** is *PARTIAL*
2. ***Renal correction*** $\rightarrow$ elimination of **alkaline urine**
3. ***Activation of transcellular H^+/K^+ exchange*** $\rightarrow$ H^+ exit into the ECF + K^+ entry into cells with consequent **hypokalemia**

ETIOPATHOGENIC CLASSIFICATION:

A. CHLORIDE-RESPONSIVE metabolic alkalosis
B. CHLORIDE-RESISTANT metabolic alkalosis

<u>A. CHLORIDE-RESPONSIVE metabolic alkalosis:</u>

1. DIGESTIVE loss of H^+

- **Causes:**
  - **prolonged vomiting**, bulimia
  - gastrointestinal fistulas
- **Pathogenesis**: gastric juice losses associated with increased transfer of HCO_3^- into the blood (at the level of gastric parietal cells for every 1 mEq/L of HCl secreted into the gastric lumen, 1 mEq/L of HCO_3^- passes into the blood)
- **Consequences**: increased plasma [HCO_3^-], volume depletion (water + Na^+), decreased chloremia and potassemia

2. RENAL loss of H^+

- **Causes: excessive administration of *loop diuretics* or *thiazide diuretics*** causes increased tubular secretion of H^+ with reabsorption of HCO_3^-

- **Consequences**: increased plasma [HCO_3^-], volume depletion (Na^+ + water), decreased chloremia and potassemia

Remember!

In volume depletion (Na^+, water) from *prolonged vomiting* and *excessive diuretic administration*, the decrease in GFR activates the RAA system that will ***maintain metabolic alkalosis*** by:

- ✓ Angiotensin II: increases H^+ secretion and HCO_3^- reabsorption in PCT
- ✓ Aldosterone: increases H^+ secretion and HCO_3^- generation in DCT + CD

The kidney **cannot correct alkalosis** until the hydro-electrolyte imbalance is corrected. Correction of volume depletion by administering 0.9% NaCl (± KCl) allows the kidney to correct metabolic alkalosis (by eliminating alkaline urine). In addition, administration of Cl^- allows the resumption of the activity of the Cl^-/HCO_3^- exchange transporter at the level of intercalated cells with rapid bicarbonate excretion → therefore, **metabolic alkalosis is chloride sensitive**.

B. CHLORIDE-RESISTANT metabolic alkalosis:

1. Alkalosis from HYPERALDOSTERONISM

- **Particularity**: the most common cause of CHRONIC metabolic alkalosis
- **Pathogenesis**: increased H^+ secretion and HCO_3^- generation in DCT + CD

2. Alkalosis due to INCREASED BASE INTAKE

- **Particularity**: the most common cause of ACUTE metabolic alkalosis
- **Causes**:
  - Excessive $NaHCO_3$ intake (infusion solution)
  - Milk-alkali syndrome (in the past)

3. POSTHYPERCAPNIC alkalosis

- **Cause**: sudden correction of chronic respiratory acidosis by mechanical ventilation
- **Pathogenesis**:
  - Chronic respiratory acidosis (increased $PaCO_2$) is compensated by the kidneys by increasing plasma [HCO_3^-]
  - Mechanical ventilation causes a rapid decrease in $PaCO_2$
  - [HCO_3^-] remains elevated because renal correction of alkalosis is slower

MANIFESTATIONS OF METABOLIC ALKALOSIS

1. Manifestations determined by the CAUSES of metabolic alkalosis

2. Manifestations caused by the increase in pH and [HCO_3^-]

Mechanisms:

- *INCREASE in neuromuscular excitability* → alkalosis binds Ca^{2+} to plasma proteins with a decrease in [Ca^{2+}] in the ECF

Manifestations:

- *neurological*: accentuated osteotendinous reflexes, tetany, convulsions
- *cardiovascular*: hypotension, cardiac arrhythmias
- *gastrointestinal*: nausea, vomiting, diarrhea

3. Manifestations determined by ACID-BASE COMPENSATION / CORRECTION
- *respiratory*: hypoventilation
- *renal*: elimination of alkaline urine
- *transcellular H^+/K^+ exchange*: hypokalemia

RESPIRATORY ACIDOSIS

DEFINITION:
- primary increase in $PaCO_2$ > 45 mmHg
- arterial pH < 7.35

MECHANISMS OF pH RESTORATION:
1. ***RENAL compensation*** *by elimination of acidic urine* is *PARTIAL*
2. ***Activation of transcellular H^+/K^+ exchange*** → intracellular accumulation of H^+ + K^+ exit from the cell with **HYPERkalemia** (more important in metabolic acidosis)

ETIOPATHOGENIC CLASSIFICATION:
A. ACUTE respiratory acidosis
B. CHRONIC respiratory acidosis

A. ACUTE RESPIRATORY ACIDOSIS
- **Causes – ACUTE pulmonary hypoventilation** from:
  - depression of bulbar respiratory centers → barbiturate intoxication, overdose in general anesthesia
  - airway obstruction → foreign body aspiration, laryngeal spasm, *severe* asthma attack
  - acute lung diseases → severe pneumonia, atelectasis, pneumothorax
  - acute chest trauma
  - paralysis of the respiratory muscles → myasthenia gravis
  - defective mechanical ventilation → increased concentration of CO_2 in the inhaled air
- **Particularity**: rapid increase in $PaCO_2$ (hypercapnia), accompanied by decrease in PaO_2 (hypoxemia), determines a ***sharp decrease in pH*** due to the minimal increase in $[HCO_3^-]$ → the kidney fails to compensate for the rapid increase in $PaCO_2$

B. CHRONIC RESPIRATORY ACIDOSIS
- **Causes – CHRONIC pulmonary hypoventilation** from:
  - chronic obstructive pulmonary disease (COPD)
  - extreme obesity (Pickwick syndrome)
- **Particularity**: persistent increase in $PaCO_2$ causes:
  - *compensatory increase in $[HCO_3^-]$* → ***decrease in pH is less important***
  - *adaptation of respiratory centers to hypercapnia*
  - activity of respiratory centers is maintained because hypoxemia stimulates peripheral chemoreceptors
  - administration of pure O_2 eliminates the hypoxic stimulus and can trigger an episode of apnea

MANIFESTATIONS OF RESPIRATORY ACIDOSIS

1. Manifestations determined by HYPERCAPNIA

Mechanisms:

- ***cerebral vasodilation*** *by direct effect* (CO_2 crosses the blood-brain barrier)
- *DECREASE in neuromuscular excitability* → acidosis mobilizes Ca^{2+} bound to plasma proteins and causes the increase in [Ca^{2+}] in the ECF

Manifestations:

- *neurological*: headache, drowsiness, confusion, muscular asthenia, paralysis, coma
- *cardiovascular*: hypotension

2. Manifestations determined by hypoxemia (see Respiratory Failure)

3. Manifestations determined by ACID-BASE COMPENSATION / CORRECTION

- *acidic urine*
- *hyperkalemia*

RESPIRATORY ALKALOSIS

DEFINITION:

- primary decrease in [$PaCO_2$] < 35 mmHg
- arterial pH > 7.45

MECHANISMS OF pH RESTORATION

1. ***RENAL compensation by elimination of alkaline urine*** is *PARTIAL*
2. ***Activation of transcellular H^+/K^+ exchange*** → H^+ exit into the ECF + K^+ entry into the cell with **hypokalemia**

ETIOPATHOGENIC CLASSIFICATION:

A. ACUTE respiratory alkalosis

B. CHRONIC respiratory alkalosis

A. ACUTE respiratory alkalosis

- **Causes – ACUTE pulmonary hyperventilation** in:
  - DIRECT stimulation of respiratory centers → salicylate intoxication in the early stages, hyperpyrexia
  - REFLEX stimulation of respiratory centers determined by:
    - ✓ *psychological factors* → anxiety ("panic attack"), severe pain
    - ✓ *hypoxia* → altitude, lung diseases, e.g. acute pulmonary edema at onset (in the interstitial edema phase), pulmonary embolism at onset, *moderate* asthma attack
  - excessive mechanical ventilation

B. CHRONIC respiratory alkalosis

- **Causes – CHRONIC pulmonary hyperventilation** in:
  - chronic stimulation of respiratory centers encountered during *pregnancy* (last trimester) by the direct effect of progesterone (and estrogens)

MANIFESTATIONS OF RESPIRATORY ALKALOSIS

1. Manifestations determined by ACUTE hypocapnia

Mechanisms:

- ***cerebral vasoconstriction*** *by direct effect*
- *INCREASE in neuromuscular excitability* → alkalosis binds Ca^{2+} to plasma proteins and determines the decrease in [Ca^{2+}] in the ECF

Manifestations:

- *neurological*: paresthesias, tetany, convulsions
- *cardiovascular*: tachyarrhythmias

2. Manifestations determined by CHRONIC hypocapnia

- *reduction in the seizure threshold*
- *hypophosphatemia* (hypocalcemia induces secondary hyperPTH)

3. Manifestations determined by ACID-BASE COMPENSATION / CORRECTION

- *alkaline urine*
- *hypokalemia*

7. PATHOPHYSIOLOGY OF FLUID AND ELECTROLYTE DISORDERS

WATER AND SODIUM BALANCE - Brief physiology overview

THE HYDRIC SPACES OF THE BODY

The total water in the body varies depending on age, gender and constitutional type as follows:

- ✓ in newborns – 75% of body weight (in kg)
- ✓ in adults – 60% in M and 50% in F
- ✓ in obese people – 55%
- ✓ in the elderly (> 65 years) – 50% in M and 45% in F

Total water (TW) is distributed into specific compartments:

- the **intracellular compartment (IC)** which represents 2/3 of TW
- the **extracellular compartment (EC)** which represents 1/3 of TW and includes:
  - **intravascular fluid (plasma)**
  - **interstitial fluid**
  - **lymph**
  - **transcellular fluid** (becomes the **"third space"** in pathological conditions: ascites, pleural and pericardial collections, burns)

Observation!

Although the composition of the extra- and intracellular compartments is different, they are in osmotic balance because water diffuses freely through the cell membranes from the **hypotonic** compartment (with low osmolarity) to the **hypertonic** compartment (with high osmolarity).

a. Water balance

The body's water content is a balance value between:

- ***Water intake:***
  - Exogenous: **ingested liquids** (approx. 1500 ml) and from **solid foods** (approx. 750 ml)
  - Endogenous: **metabolic water** (*result of oxidation of energy substrates*, approx. 250 ml/day)
    ⇒ total = 2500 ml/day
- ***Water elimination:***
  - **Renal**:
    - **main route** - normally, *diuresis* (on average 1500 ml/day) is *regulated by ADH*:
      - ✓ if ADH secretion is maximal ⇒ 500 ml/day with urinary osmolarity = 800 -1400 mosm/kg
      - ✓ if ADH is absent (diabetes insipidus) ⇒ 15 -20 l/day with urinary osmolarity = 40 - 80 mosm/kg
  - **Extrarenal**:
    - **Cutaneous and respiratory (insensible losses):**
      - ✓ skin (350 ml) and lungs (350 ml) = 700 ml/day (increases in case of fever by 50 ml/day for every 1°C > 37°C)
      - ✓ sweat = 200 ml/day (increases up to 1.5 l/hour during the intense thermoregulation effort)
    - **Gastrointestinal**:
      - ✓ normal, reduced - through feces = 100 ml/day
      - ✓ becomes important in pathological conditions: vomiting, diarrhea, digestive fistulas

    ⇒ total = 2500 ml/day

The regulation of water balance depends on: i) EC osmolarity and ii) circulating volume (Table 18).

Table 18. Regulation of water balance: effector mechanisms and consequences.

	Osmolarity	Circulating volume
Stimuli	Increase by 2%	Decrease by 10%

Receptors	Hypothalamic and peripheral osmoreceptors	① Baroreceptors in high-pressure areas (carotid sinus and aortic arch) ② Volume receptors in low-pressure areas (left atrium and large veins)
Effectors	Release of antidiuretic hormone (ADH, vasopressin) from the posterior pituitary	① Sympathetic stimulation (circulating catecholamines increase) ② Activation of the renin-angiotensin-ALDO system ③ Inhibition of atrial natriuretic peptide (ANP) release
Response	Antidiuretic	① Increased CO and PVR ② Renal/systemic vasoconstriction & Na^+ reabsorption Stimulation of the thirst sensation
Result	**Water retention**	**Hydro-saline retention** **Increased water intake**

b. Sodium balance

The total sodium content in the body depends on the balance between:

- **Na^+ intake** - *dietary* (normally, 5-10 g NaCl/day).
- **Na^+ excretion** - *renal* (extrarenal excretion being negligible under normal conditions).

Renal Na^+ excretion is regulated so as to quickly compensate for variations in intake through the following **mechanisms**:

1. **Modification of glomerular filtration (GF)** and ***proximal*** **tubular reabsorption of Na^+** depending on **hemodynamic factors**:
   - Na^+ loading (Na^+ retention) $\rightarrow$ increases volemia and causes:
     - ✓ the increase of GF
     - ✓ the decrease of proximal tubular reabsorption $\rightarrow$ increases renal excretion of Na^+
   - Na^+ loss (Na^+ depletion) $\rightarrow$ decreases volemia and causes:
     - ✓ the decrease of GF
     - ✓ the increase of proximal tubular reabsorption $\rightarrow$ decreases renal excretion of Na^+
2. **Modification of *distal* tubular reabsorption of Na^+**

   $\Rightarrow$ depending on **mineralocorticoid - aldosterone (ALDO) secretion**:
   - hyponatremia and/or hypovolemia $\rightarrow$ decreases Na^+ load at the macula densa level in the distal tubule and promotes the *activation of the renin-angiotensin-aldosterone system (RAA)* $\rightarrow$
     - ✓ AII increases Na^+ and water reabsorption at the PCT level
     - ✓ ALDO increases Na^+ reabsorption at the DCT and collecting duct level

   $\Rightarrow$ depending on the **secretion of natriuretic factors**:
   - hypervolemia & increased venous return $\rightarrow$ atrial distension $\rightarrow$ increased release of atrial natriuretic peptide (ANP) by atrial myocytes which at the renal level causes:
     - ✓ the increase of GFR through vasodilation of the afferent arteriole and constriction of the efferent one
     - ✓ decreased Na^+ reabsorption in the PCT, DCT and collecting duct
     - ✓ stimulation of diuresis

   $\Rightarrow$ depending on **prostaglandins (PG) released by the renal medulla** which cause:
   - vasodilation and increased GFR
   - stimulation of diuresis and natriuresis

Observation!

The major determinants of EC osmolarity are: **sodium, glucose and urea**. With the exception of hyperglycemia and azotemia, **over 90% of EC osmolarity is given by Na^+ salts**.

PLASMA VOLUME CHANGES

HYPOVOLEMIA

DEFINITION: decrease in extracellular compartment volume caused by the **combined loss of sodium and water** in various proportions (synonyms: volume depletion, volume contraction)

CLASSIFICATION: Tab. 19 - 21

1. Iso-osmolar hypovolemia
2. HYPER-osmolar hypovolemia
3. Hypo-osmolar hypovolemia

Table 19. Iso-osmolar hypovolemia / isotonic volume depletion

Definition	▪ **Decrease in extracellular compartment (EC) volume caused by loss of SODIUM equal to that of WATER**
Etiology	▪ **Loss of isotonic fluids** (with osmolarity equal to that of plasma): ✓ **Hemorrhages** ✓ **Plasmorrhagia - in third degree burns** ✓ Loss of digestive fluids: diarrhea, intestinal fistulas, repeated evacuation of ascites fluid ✓ Phase of diuresis resumption in acute kidney injury
Pathogenesis	▪ Decrease in EC, **WITHOUT modification of osmolarity** and intracellular compartment (IC) ▪ NO movement of water from/towards the intracellular compartment occurs - EC decreases, IC N ▪ Activation of compensatory mechanisms to *restore volemia* (RAA system, ADH) ▪ Sympathetic stimulation
Clinically	▪ Signs of **EXTRACELLULAR dehydration** predominate: ✓ **Tachycardia, jugular vein collapse, normal or low BP (in orthostatic position)** ✓ Vascular collapse in severe forms
Laboratory	▪ **Natremia and plasma osmolarity are normal** ▪ Signs of **hemoconcentration:** increased Ht, urea & creatinine (azotemia), proteins ▪ Oliguria and excretion of concentrated urine

Table 20. HYPER-osmolar hypovolemia / HYPERtonic volume depletion

Definition	▪ **Decrease in extracellular volume caused by loss of WATER greater than the loss of sodium (total water deficit)**
Etiology	▪ **Loss of hypotonic fluids** (with osmolarity lower than that of plasma): ✓ **Loss of digestive fluids: diarrhea (most common cause in children)** ✓ **Loss of sweat fluid:** profuse sweating/excessive sweating in febrile sdr. ✓ Osmotic diuresis (glucosuria, mannitol) ✓ Prolonged mechanical ventilation ✓ Pituitary or nephrogenic diabetes insipidus (rare)

	▪ **Reduced water intake:** lack of access to water in elderly immobilized patients, comatose patients ▪ **Parenteral nutrition with excess salt**
Pathogenesis	▪ Decrease in the volume of the extracellular compartment **WITH an increase in osmolarity** ▪ Water will diffuse from the intracellular compartment (relatively hypotonic) into the hypertonic extracellular compartment = **volume depletion will be achieved 2/3 due to the IC and 1/3 due to the EC** – IC and EC decrease ▪ **Increased osmolarity stimulates the sensation of thirst** and, respectively, water intake ▪ Activation of compensatory mechanisms to *restore volemia* (RAA system, ADH)
Clinically	▪ Signs of **INTRACELLULAR dehydration** predominate (with losses up to 2000 ml): ✓ **Thirst!** ✓ **Dry skin and mucous membranes** ✓ Decreased skin turgor ✓ Hypotonic eyeballs ▪ Later, signs of **EXTRACELLULAR dehydration**: **decreased BP, thready pulse,** cyanotic and cold extremities (with losses of approx. 3000 ml) ▪ Alteration of mental status (hallucinations, confusion) and coma in *acute* forms (when serum Na^+ quickly reaches 160 mEq/L)
Laboratory	▪ **HYPERnatremia (Na^+ > 145 mEq/L) and plasma HYPER-osmolarity** ▪ Signs of **hemoconcentration:** increased Ht, urea & creatinine (azotemia), proteins ▪ Oliguria and excretion of concentrated urine

Table 21. Hypo-osmolar hypovolemia / hypotonic volume depletion

Definition	▪ **Decrease in extracellular volume caused by loss of SODIUM greater than the loss of water - always associated with hyponatremia**
Etiology	▪ **Fluid loss:** ✓ **Renal:** – Addison's disease (adrenal cortex failure - ALDO deficiency decreases Na^+ reabsorption with decreased osmolarity which will inhibit ADH release and lead to volume depletion; hypovolemia will subsequently stimulate ADH release with hemodilution and hyponatremia) – excessive administration of loop diuretics – advanced chronic kidney disease ✓ **Digestive:** diarrhea (possibly) ▪ **Increased intake of fluids with low sodium content -** e.g., endovenous perfusions with glucose: decreased osmolarity which will inhibit ADH release and lead to volume depletion (since only water is lost, natremia will return to normal, but due to the volume depletion; therefore, postoperative hydroelectrolyte rebalancing consists of administering saline) ▪ **Low-sodium regimens**

Pathogenesis	▪ Decrease in the volume of the extracellular compartment **WITH a decrease in osmolarity** ▪ Water will diffuse from the extracellular compartment (hypotonic) to the intracellular compartment (relatively hypertonic) - EC decreases and IC increases ▪ **Decrease in osmolarity inhibits the sensation of thirst ⇒ thirst is ABSENT** ▪ Activation of compensatory mechanisms to restore volemia (activation of the RAA system) ▪ Sympathetic stimulation
Clinically	▪ Signs of: ✓ **EXTRACELLULAR dehydration:** hypotension, tachycardia, cyanotic and cold extremities ✓ **CELLULAR hyperhydration**: – cramps and muscle weakness – CNS: headache, disorientation, convulsions and coma - cerebral edema due to water intoxication
Laboratory	▪ **Hyponatremia ($Na^+ \leq 135$ mEq/L) and plasma hypoosmolarity** ▪ Signs of **hemoconcentration**: increased Ht, urea and creatinine (azotemia)

HYPERVOLEMIA

DEFINITION: increase in the volume of the extracellular compartment determined almost exclusively by the **increase in the SODIUM content of the body**

CLASSIFICATION: Tab. 22 - 24

1. Iso-osmolar HYPERVOLEMIA
2. Hypo-osmolar HYPERVOLEMIA
3. HYPER-osmolar HYPERVOLEMIA

Table 22. Iso-osmolar HYPERVOLEMIA / isotonic volume excess

Definition	▪ **Increase in extracellular volume caused by an excess of SODIUM equal to that of WATER**
Etiology	▪ **Increased intake of isotonic solutions** (with osmolarity equal to that of plasma) in patients with: ✓ Heart failure ✓ Liver cirrhosis ✓ Kidney diseases: nephrotic syndrome, chronic uremia
Pathogenesis	▪ Increase in hydrostatic pressure with the passage of water into the interstitial space ▪ NO movement of water from/to the intracellular compartment - IC N ▪ Activation of compensatory mechanisms to **restore volemia** (RAA system, ADH)
Clinically	▪ Signs of **EXTRACELLULAR hyperhydration** predominate: ✓ Weight gain ✓ High blood pressure with a strong pulse

	✓ Jugular turgescence ✓ Peripheral edema ✓ Pulmonary edema → dyspnea
Laboratory	▪ **Plasma natremia and osmolarity are normal** ▪ Signs of **hemodilution:** decreased Ht, urea & creatinine (azotemia), proteins Forcing diuresis is necessary.

Table 23. HYPER-osmolar HYPERvolemia / HYPERtonic volume excess

Definition	▪ **Extracellular volume increase associated with actual SODIUM excess**
Etiology	▪ **Excess mineralocorticoids**: ✓ Primary HyperALDO (Conn sdr.) ✓ Cushing sdr. (adrenal cortex hyperfunction): – excess ALDO increases Na^+ reabsorption with increased osmolarity which will stimulate ADH release and lead to increased volemia; hypokalemia and metabolic alkalosis may associate ▪ **Increased intake of fluids with high sodium content:** excessive infusions of hypertonic NaCl solutions, $NaHCO_3$, antibiotics containing Na^+ ▪ Administration of **high doses of corticosteroids**
Pathogenesis	▪ Increase in extracellular compartment volume **WITH increase in osmolarity** ▪ Water will diffuse from the intracellular compartment (relatively hypotonic) into the extracellular compartment (hypertonic) ⇒ IC decreases ▪ **Increase in osmolarity stimulates the sensation of thirst**
Clinically	▪ Combination of signs of: ✓ **EXTRACELLULAR hyperhydration /** VOLEMIC expansion**:** peripheral edema, pulmonary edema, increased venous pressure, risk of heart failure ✓ **CELLULAR dehydration /** NEUROLOGICAL impairment: – at the CNS level: headache, agitation, delirium, convulsions and coma on the background of neuronal dehydration
Laboratory	▪ **HYPERnatremia (Na^+ > 145 mEq/L) and plasma HYPERosmolarity** ▪ Signs of **hemodilution**: decreased Ht ▪ Oliguria and excretion of concentrated urine

Table 24. Hypo-osmolar HYPERvolemia / Hypotonic volume overload

Definition	▪ **Increase in extracellular volume caused by an excess of WATER with decreased serum osmolarity**
Etiology	▪ **Oligo-anuric phase of acute kidney injury** ▪ **Glomerulonephritis** ▪ **Excess ADH** from**:** ✓ Syndrome of inappropriate ADH secretion (SIADH, Schwartz-Bartter syndrome) - **hypersecretion of ADH** in: o lung tumors: small cell lung cancer - **most common** o head trauma, brain tumors

	✓ Severe liver disease with/without ascites (ADH inactivation deficiency) ✓ Congestive heart failure treated with diuretics ▪ **Increased intake of fluids that dilute sodium:** excessive infusions of 5% glucose solution
Pathogenesis	▪ Increase in the volume of the extracellular compartment **WITH a decrease in osmolarity** ▪ Water will diffuse from the extracellular compartment (hypotonic) into the intracellular compartment (relatively hypertonic) ⇒ increase in IC ▪ **Decrease in osmolarity inhibits the sensation of thirst ⇒ thirst is ABSENT**
Clinically	▪ Signs of: ✓ **EXTRACELLULAR hyperhydration** / VOLEMIC expansion: peripheral edema, pulmonary edema, increased venous pressure, fluid retention ✓ **CELLULAR hyperhydration**: – cramps and muscle weakness – CNS: headache, disorientation, convulsions and coma (cerebral edema due to water intoxication)
Laboratory	▪ **Hyponatremia (Na^+ < 135 mEq/L) and plasma hypoosmolarity** ▪ Signs of **hemodilution**: Ht decreases ▪ Urinary Na^+ is reduced

PLASMA ELECTROLYTE CHANGES

ALTERATION OF SODIUM BALANCE

I. HYPONATREMIA

▪ **Definition**: **decrease in plasma Na^+ concentration < 135 mEq/L** accompanied or not by hypoosmolarity

▪ **Classification**:

1. Hypovolemic hyponatremia - loss of SODIUM is greater than that of water due to:

- **Renal losses:**
  - Diuretics - especially thiazides
  - Osmotic diuresis - hyperglycemia (decompensated/complicated DM), severe uremia
  - Addison's disease (adrenal cortex insufficiency) or ALDO deficiency
  - Renal tubular acidosis (associated with decreased bicarbonate and hyperchloremic metabolic acidosis)
- **Extrarenal losses:**
  - Severe diarrhea
  - Prolonged vomiting
  - Water retention in the 3rd space (burns, intestinal occlusions, pancreatitis)
  - Hemorrhages

Observation!

Evaluation of urinary Na^+ is useful in the differential diagnosis of hyponatremia of ***renal*** or ***extra-renal*** origin:

- Urinary Na^+ > 20 mEq/L in renal losses

- Urinary Na^+ < 20 mEq/L in extrarenal losses (increases tubular sodium reabsorption)

2. Hypervolemic hyponatremia - "dilutional hyponatremia"
- Congestive heart failure
- Nephrotic syndrome, hypoalbuminemia
- Chronic renal failure
- Liver cirrhosis

3. Isovolemic hyponatremia - normal EC, absence of edema
- **Inadequate secretion of ADH** at the pituitary or non-pituitary level (associated with urinary Na^+ > 20 mEq/L):
  - ✓ **CNS disorders**: stroke, trauma, tumors, psychoses - increased release of pituitary ADH
  - ✓ **Pulmonary diseases**: pneumonia, atelectasis, acute resp. failure (associated with **decreased** PaO_2)
  - ✓ **Drugs**: carbamazepine, chlorpropamide, opiates - potentiation of the pituitary ADH effect
  - ✓ **Tumors**: small cell lung cancer, occasionally head and neck tumors - ectopic (non-pituitary) release of ADH-like peptides
- **Hormonal deficiencies: hypothyroidism, glucocorticoid deficiency**
- **Psychogenic polydipsia**: history of **schizophrenia** with massive water intake (normal renal dilution function allows increased water elimination)

Pseudohyponatremia:
It is a condition associated with decreased Na^+ < 135 mEq/L with *normal plasma osmolarity*, in the following conditions:
- hyperlipidemia - familial hypercholesterolemia, hypertriglyceridemia
- hyperproteinemia - e.g., multiple myeloma, chronic lymphoproliferative disorders
- laboratory errors - requires repetition

- **Clinical manifestations:**

The major disturbance in hyponatremia is WATER INTOXICATION which occurs when plasma osmolarity drops < 240 mOsm/L:
– water will diffuse from the hypotonic extracellular compartment to the intracellular one (relatively hypertonic)
– cellular hyperhydration occurs ⇒ cellular swelling
– at the **CNS level hyponatremic encephalopathy** occurs characterized by **cerebral edema** with altered mental status (lethargy, confusion) and then coma.

II. HYPERNATREMIA
- **Definition: increased plasma Na^+ concentration > 145 mEq/L always accompanied by extracellular HYPERosmolarity and intracellular dehydration, respectively**.
  In most cases, it signifies a water deficit and is associated with increased mortality.

- **Classification**:

According to the changes in volemia (important in clinical practice):
1. **Hypovolemic hypernatremia** - loss of WATER greater than that of sodium, with elimination of **hypotonic fluids**:

- **sweating and perspiration (with hyperventilation) in febrile patients** = *the most common cause of hypernatremia*
- severe and incorrectly compensated gastrointestinal losses: diarrhea, vomiting, fistulas
- osmotic diuresis (presence of osmoactive compounds: glucose, urea) in:
  - ✓ DM with glycosuria
  - ✓ acute kidney injury (AKI) in the phase of diuresis resumption (polyuric)

2. **Isovolemic hypernatremia** - loss of WATER without loss of sodium
   - diabetes insipidus (pituitary or nephrogenic):
     - ✓ severe hypernatremia occurs only in the case of rapid onset of diabetes - e.g., after brain trauma or neurosurgical interventions (when water intake is inadequate)

3. **Hypervolemic hypernatremia** is usually **iatrogenic** and occurs after:
   - excessive infusions of hypertonic solutions (e.g., sodium bicarbonate for correction of metabolic acidosis, antibiotics)
   - total parenteral nutrition

- **Clinical manifestations:**
  - are determined by the increase in plasma osmolarity
  - water will diffuse from the intracellular compartment (relatively hypotonic) into the hypertonic extracellular compartment with the installation of a **global, extra- and intracellular dehydration**

The major disorder in hypernatremia is represented by **neuronal DEHYDRATION**.

- **at the CNS level**, an alteration of mental status (lethargy, confusion) and then coma occurs. Against the background of neuromuscular hypoexcitability, respiratory paralysis may occur.
  - in **acute** forms, disorders occur when plasma osmolarity > 320 mOsm/L
  - in **chronic** forms, nervous disorders are minimal (adaptation to chronic hyperosmolarity through i.c. accumulation of osmoactive substances)
- severe hypernatremia is associated with convulsions.

ALTERATION OF POTASSIUM BALANCE

POTASSIUM METABOLISM – Brief physiology overview

a. K^+ distribution in the body

- potassium (K^+) is distributed asymmetrically between the extra- and intracellular environment due to the activity of the *Na^+/K^+-dependent membrane ATPase* which makes it the **main intracellular cation** (~ 155 mEq/L), while at the extracellular level **potassemia registers values between 3.5 - 5 mEq/L**.
- K^+ plays an important regulatory role on:
  - ✓ neuromuscular excitability and muscle contraction
  - ✓ insulin secretion: hypokalemia inhibits and hyperkalemia stimulates insulin secretion

Potassemia (kalemia) depends on the following factors:

- total K^+ content in the body
- humoral factors:
  - ✓ acidosis ***removes*** K^+ from the cell → risk of HYPERkalemia
  - ✓ alkalosis ***introduces*** K^+ into the cell → risk of hypokalemia
- hormonal factors

- ✓ insulin
- ✓ catecholamines

} → determines the entry of K^+ into the cells

b. K^+ balance

The K^+ content in the body depends on the balance between:

- **K^+ intake** – *food*
- **K^+ excretion**:
  - *renal* (most important)
  - *gastrointestinal* (through feces)

Renal K^+ excretion is regulated by:

1. **Aldosterone** (whose release is stimulated by hyperkalemia):
   - ✓ HYPERALDO → increases tubular K^+ secretion at the distal nephron level → increases urinary K^+ excretion
   - ✓ hypoALDO has the opposite effect
2. **Urine flow to the distal (dilutional) segment** of the nephron:
   - ✓ increased urine flow → increased K^+ excretion
3. **Electrochemical gradient of K^+ between the tubular cell and the tubular lumen:**
   - ✓ negative charge of the tubular lumen (by increased anion concentration) → increases K^+ excretion
   - ✓ change in K^+ concentration in the tubular cell:
     - increase in concentration (in alkalosis) → increases K^+ excretion
     - decrease in concentration (in acidosis) → decreases K^+ excretion

I. HYPOPOTASSEMIA

- **Definition: decreased potassemia < 3,5 mEq/L**
- **Etiopathogenesis:** Tab. 25.

Table 25. Causes of hypokalemia.

Mechanism	Cause
1. Increased RENAL elimination (urinary K^+ > 20 mEq/day)	▪ **Excessive diuretics administration:** loop diuretics (furosemide), thiazide diuretics (indapamide) **- MOST FREQUENT CAUSE** ▪ **Osmotic diuresis:** glycosuria (decompensated DM), mannitol ▪ **Excess mineralocorticoids:** – Primary HYPERaldosteronism (Conn's syndrome) – Secondary HYPERaldosteronism from: congestive heart failure, liver failure from advanced cirrhosis, renal artery stenosis, nephrotic syndrome – Cushing's syndrome ▪ **Medications:** – aminoglycosides and amphotericin (tubular toxicity) – prolonged corticosteroid therapy ▪ **AKI in the polyuric phase** ▪ **Renal tubular acidosis** (distal and proximal)
2. Increased DIGESTIVE losses (urinary K^+ < 20 mEq/day)	▪ **Diarrhea** (approx. 30 mEq/L) and **laxative abuse** ▪ **Vomiting** (approx. 5 mEq/L) ▪ **Intestinal fistulas, ileostomy**

	▪ **Zollinger-Ellison syndrome** ▪ **Villous adenoma of the colon** (K^+-rich mucus)
3. REDISTRIBUTION in the intracellular space	**The entry of potassium into cells is favored by:** ▪ **Drugs that increase the activity of Na^+-K^+-dependent ATPase:** – beta2-adrenergic agonists: bronchodilators, nasal decongestants – theophylline, caffeine ▪ **Insulin therapy and administration of glucose solutions** ▪ **Alkalosis** (metabolic and respiratory) ▪ **Cyanocobalamin therapy of pernicious anemia**
4. Decreased INTAKE (rare)	▪ **Inability to swallow** (tumors, pharyngeal/esophageal stenosis, inflammation of the oral cavity) ▪ **Starvation** ▪ **Alcoholism**

- **Clinical manifestations and ECG changes:** Tab. 26.

Table 26. Clinical manifestations and ECG changes in hypokalemia.

Clinical manifestations	ECG changes
1. Muscular: – **muscle asthenia** – **hypotonia of the muscles of the extremities or flaccid paralysis** – **exercise rhabdomyolysis** (hypokalemia inhibits insulin release with a consequent decrease in muscle glycogen reserves) – **ventilatory failure** **2. Digestive:** – **anorexia, nausea, vomiting** – **constipation** – **paralytic ileus** **3. Renal:** – **kaliopenic nephropathy** (damage to the collecting tubules - lack of response to ADH with decreased urine concentration capacity and polyuria)	▪ **Increased amplitude of the U wave** ▪ Flat and negative T wave ▪ ST segment depression ▪ QT interval prolongation – risk of ventricular tachyarrhythmias (against the background of a pathological heart) ▪ Increased sensitivity to catecholamines ***Observation!*** Hypokalemia potentiates the arrhythmogenic properties of digitalis.

II. HYPERPOTASSEMIA

- **Definition: increased potassemia > 5 mEq/L.**
- **Etiopathogenesis:** Tab. 27.

Table 27. Causes of HYPERkalemia.

Mechanism	Cause
1. Decreased RENAL elimination	▪ **Chronic kidney disease (CKD) and renal failure** of any

	etiology (if GFR < 5 ml/min) - **MOST FREQUENT CAUSE** ▪ **K^+-sparing diuretics**: spironolactone, eplerenone, amiloride ▪ **Medications**: NSAIDs, cyclosporine ▪ **Mineralocorticoid deficiency**: – **Addison's disease** – **hyporeninemic hypoaldosteronism** (in the destruction of the juxtaglomerular apparatus, distal tubular acidosis type 4) – heparin and low MW heparins (inhibit aldosterone release) – association of ACE inhibitors with AII receptor blockers
2. Release through TISSUE DESTRUCTION	▪ **Pathological hemolysis** ▪ **Tumor lysis syndrome** ▪ **Rhabdomyolysis - e.g., crush syndrome** ▪ **DIC** ▪ **Burns** ▪ **Reperfusion of ischemic tissue** ▪ **Spinal cord injuries, denervation syndrome** ▪ **Intense physical effort**
3. REDISTRIBUTION in the extracellular space	**The exit of potassium from cells is favored by*:*** ▪ **Drugs that decrease the activity of the Na^+-K^+-dependent ATPase:** – beta-adrenergic antagonists (beta-blockers): propranolol – digitalis toxicity! ▪ **Infusions with solutions that increase plasma osmolarity (hypertonic glucose, mannitol)** - induce K^+ outflow by osmotic mechanism ▪ **Acidosis** (lactic) ▪ **Insulin deficiency** (in diabetic ketoacidosis)
4. Increased INTAKE	▪ Penicillin administration ▪ Transfusions with preserved blood ▪ **Consumption of foods rich in K^+ (e.g., bananas) in patients with CKD - risk in the elderly with diuresis < 500 ml/day**
5. Pseudo-HYPERkalemia	▪ **Traumatic vein puncture/prolonged tourniquet application** ▪ **Exposure to cold**

- **Clinical manifestations and ECG changes:** Tab. 28.

Table 28. Clinical manifestations and ECG changes in HYPERkalemia.

Clinical manifestations	ECG changes
1. Muscular: – **muscular asthenia** – **muscular hypotonia** – **muscular cramps** – **paresthesias** – **flaccid paralysis** **2. Digestive:** – **nausea, vomiting** – **intestinal cramps** – **diarrhea** **3. Cardiac:** – **risk of cardiac arrest**	**K^+ = 5,5 mEq/l** → tall, symmetrical T wave ("tent-shaped") **K^+ = 6 - 7 mEq/l** → PR interval prolongation decreased P wave amplitude **K^+ = 8 - 10 mEq/l** → absence of P wave Prolonged QRS (S deep and wide, low R) short QT interval **K^+ > 10 mEq/l** → asystole or ventricular fibrillation (rare)

8. PATHOPHYSIOLOGY OF DIABETES MELLITUS AND HYPOGLYCEMIAS

ENDOCRINE REGULATION OF THE INTERMEDIARY METABOLISM – Brief physiology and biochemistry overview

The storage and utilization of energy substrates as well as blood glucose levels are controlled by **insulin** and **counterregulatory hormones**.

a) Regulation of STORAGE and USE of energy substrates (Tab. 29)

1. ANABOLIC Phase

- *Activation conditions* - exogenous energy intake is *higher* than the energy requirements of the body
- *Hormonal regulation* - is carried out by INSULIN (the main ANABOLIC hormone), released in response to an increase in blood glucose, amino acid concentration or free fatty acids in plasma
- *Main metabolic substrate* – GLUCOSE
- *Activated metabolic pathways* - ensure the storage of energy reserves

2. CATABOLIC Phase

- *Activation conditions* - exogenous energy intake is *lower* than the energy requirements of the body
- *Hormonal regulation* - is carried out by counter-regulatory hormones (insulin antagonists) - GLUCAGON (mainly), CORTIZOL, EPINEPHRINE, STH, THYROID HORMONES
- *Main metabolic substrate* - FATTY ACIDS
- *Activated metabolic pathways* - determine depletion of energy reserves

Table 29. Metabolic pathways activated in the ANABOLIC and CATABOLIC phases of the intermediary metabolism.

Phase	Hormones	CARBOHYDRATE Metabolism	LIPID Metabolism	PROTEIN Metabolism
ANABOLIC	Insulin	↑ glucose uptake ↑ glycolysis ↑ glycogenogenesis	↑ FA uptake ↑ FA synthesis ↑ lipogenesis	↑ AA uptake ↑ protein synthesis
CATABOLIC	Counter-regulatory hormones	↑ glycogenolysis ↑ gluconeogenesis	↑ lipolysis ↑ FA β-oxidation ↑ ketogenesis	↑ protein catabolism

FA = fatty acids, AA = amino acids

b) Blood sugar regulation - postprandial and in "*fasting*" or starvation conditions

1. Postprandial

- *Hormonal regulation*: **increased blood glucose levels** cause **increased insulin** (activation of pancreatic β cell secretion) and **decreased glucagon** (inhibition of pancreatic α cell secretion)
- *Effects of increased insulin secretion*:
  - increased glucose uptake into insulin-dependent tissues: adipose tissue, skeletal muscle
  - increased glycolysis, glycogenogenesis and lipogenesis in the liver
- *Consequence*: decreased blood glucose levels (negative humoral feed-back mechanism)

2. "Fasting" or starvation state

- *Hormonal regulation*: **decreased blood glucose levels** cause **decreased insulin** (inhibition of pancreatic β cell secretion) and **increased glucagon** (activation of pancreatic α cell secretion)
- *Effects of decreased insulin secretion:*
  - increased lipolysis in the adipose tissue → source of FA
  - increased catabolism of skeletal muscle proteins → source of AA

- *Effects of increased glucagon secretion:*
  - increased glycogenolysis, hepatic gluconeogenesis (AA utilization) → source of *glucose*
  - increased hepatic ketogenesis (FA utilization) → source of *ketone bodies* used for energy by peripheral tissues (myocardium, muscle, brain)
- *Consequence*: increased blood glucose (negative humoral feedback mechanism)

Insulin receptors

- Insulin exerts its actions by binding to a receptor, located at the level of the cell membrane of numerous cells. It comprises a dimer with two alpha subunits (which include the binding sites for insulin), and two beta subunits (which cross the cell membrane). When insulin binds to the alpha subunits, it induces a change in the conformation of the beta subunits, resulting in the activation of tyrosine kinase and the initiation of a cascade response involving a series of other intracellular substrates. The insulin-receptor complex is then internalized by the cell, insulin is degraded and the receptor is recycled to the cell surface.

Glucose transport

- Cell membranes are not permeable to glucose. A family of specialized glucose transport proteins (GLUTs) transport glucose across the membrane into cells. The function of GLUT-1-3 is independent of insulin, but insulin stimulates glucose uptake into muscle and adipose tissue via GLUT-4. GLUT-4 is normally present in the cytoplasm, but after insulin binds to its receptor, GLUT-4 moves to the cell surface where it creates a pore for glucose entry.

DIABETES MELLITUS (DM)

DEFINITION: the most complex metabolic condition, *heterogeneous* in terms of *etiopathogenesis, clinical and therapeutic aspects*, with **pandemic** evolution and presenting **3 main characteristics**:

- *Chronic (persistent)* **hyperglycemia**
- **Deficiency** of ***insulin secretion and/or action***
- **Progressive** evolution towards ***acute and chronic complications***

DIAGNOSTIC CRITERIA (American Diabetes Association/ADA, 2022):

a) **DIABETES MELLITUS** is defined as the presence of **<u>one</u>** of the following criteria:

- Fasting blood glucose **≥ 126 mg/dL** in **two repeated determinations**
- 2-hour blood glucose **≥ 200 mg/dL** in the **OGTT test**
- Blood glucose **≥ 200 mg/dL** at any time of the day (unrelated to the last meal) **in the presence of classic symptoms of DM**
- HbA1c **≥ 6.5%**

b) **PREDIABETES** is defined as the presence of **<u>one</u>** of the following criteria:

- Fasting blood glucose = **110 - 125 mg/dL** - condition called **impaired fasting plasma glucose (IFG)**
- 2-hour blood glucose = **140 - 199 mg/dL** in the **OGTT test** - **impaired glucose tolerance (IGT)**
- HbA1c = **5.7-6.4%**

CLASSIFICATION:

1. Type 1 DM (5-10% of cases): with **absolute insulin deficiency**, **immunologically** mediated
2. Type 2 DM (90% of cases): with **relative insulin deficiency** due to **insulin resistance** and **pancreatic β-cell secretory dysfunction**

3. **Other specific types of diabetes:**
   - *genetic defects of pancreatic β-cells with insulin secretory dysfunction*: Maturity Onset Diabetes of the Young (MODY)
   - *exocrine pancreatic diseases*: chronic pancreatitis, neoplasm, cystic fibrosis
   - *endocrinopathies*: Cushing's syndrome, pheochromocytoma, acromegaly, hyperthyroidism
   - *viral infections*: cytomegalovirus, congenital rubella, coxsackie B
   - *drug-induced*: corticosteroid therapy, thiazide diuretics, beta-adrenergics, thyroid hormones, interferon-alpha, etc.
   - *genetic syndromes associated with DM*: Down, Klinefelter, Turner, Prader-Willi
4. **Gestational DM**: occurs in the second half of pregnancy, is characterized by *insulin resistance* induced by chorionic gonadotropin, progesterone and cortisol and is associated with the risk of subsequent development of type 2 DM.

CLASSICAL SYMPTOMS

The classic symptoms of DM in association with pathogenic mechanisms are presented in Tab. 30.

Table 30. PATHOGENIC mechanisms of the CLASSIC symptoms of DM.

Symptom	PATHOGENIC mechanisms
Polyuria	▪ hyperglycemia > 160-180 mg/dL, which exceeds the renal threshold for glucose reabs. → *glycosuria* ▪ glycosuria causes *osmotic diuresis* → *elimination of large amounts of water, Na^+ and K^+* ▪ polyuria causes water and Na^+ depletion → *hypovolemia*
Polydipsia	▪ hyperglycemia causes increased plasma osmolarity → *plasma hypertonicity* ▪ plasma hypertonicity causes water migration from ICF into the ECF → *intracellular dehydration*
Polyphagia	▪ mainly present in uncontrolled type 1 diabetes → absolute insulin deficiency causes: – a catabolic state → *depletion of cellular energy stores* – decreased activity of the satiety center in the hypothalamus → *feeling of hunger*
Rapid weight loss	▪ mainly present in uncontrolled type 1 diabetes: – absolute insulin deficiency → loss of *muscle mass* (protein catabolism) and *adipose tissue* (lipid catabolism) – hyperglycemia → *intracellular dehydration and hypovolemia through osmotic diuresis*

The MAJOR CHARACTERISTICS of type 1 and 2 diabetes are presented in Tab. 31.

Table 31. MAJOR **CHARACTERISTICS** of type 1 and 2 diabetes.

CHARACTERISTICS	DM type 1	DM type 2
Prevalence	**5-10% of cases** (*the most common chronic condition encountered in pediatrics!)*	**90% of cases**
Age of onset	**< 30 years** (peak dg. at puberty - 11-13 years)	**> 40 years** (peak dg. > 65 years, but **increasing incidence in children** due to childhood obesity)
Body weight	Normal or **Recent weight loss**	**Obesity in 85% of cases**
Type of onset	**Acute** Classic symptomatology (polyuria, polydipsia), frequently with ketoacidosis	**Progressive** Nonspecific signs (asthenia, pruritus, paresthesias)
Major acute complication	**Ketoacidotic** coma	**Hyperosmolar** coma
Insulin secretion (insulinemia)	**Absolute deficiency** *Initially*: mild deficiency ("honeymoon period") responding to low doses of insulin *Later*: severe deficiency	**Relative deficiency** *Initially:* normal insulinemia or hyperinsulinism due to insulin resistance *Later:* low insulinemia due to secretory dysfunction of β cells
Pathogenic mechanism	**Destruction of β cells by autoimmune mechanism** The mass of destroyed β cells is replaced by *fibrous tissue*	**Insulin resistance and secretory dysfunction of β cells** The mass of destroyed β cells is replaced by *amyloid* deposits
Auto-antibodies	**Present**	**Absent**
Genetic predisposition	**Reduced** (family history positive in 5-10% of cases)	**Important** (family history positive in 40-50% of cases)
Environmental factors involved	***Viral infections*** Dietary factors	***Central obesity (android type)*** Sedentary lifestyle, chronic stress
Treatment	Diet **Insulin required for life** (insulin-dependent)	Diet **Non-insulin medication** ± insulin (insulin-independent)

ETIOPATHOGENESIS:

A. Etiopathogenesis of type 1 diabetes

Patients with type 1 diabetes have a **normal number of β cells at birth**, but these will be destroyed by triggering a **cellular and humoral autoimmune process**. Exposure to **environmental factors** (infectious, nutritional, deficiencies) constitutes the **"trigger" event**

that initially causes the destruction of a reduced number of β cells and the consequent release of **auto-antigens (auto-Ag)**.

The subsequent **autoimmune response** determines the **progressive reduction of the β cell mass** over several years (5-7 years) corresponding to the 3 evolutionary stages of diabetes:

I. **Stage I** with **normoglycemia** and the **presence of antipancreatic autoantibodies** in the serum (important for early diagnosis)
II. **Stage II** with **dysglycemia** manifested by impaired fasting plasma glucose (IFG), impaired glucose tolerance (IGT) and HbA1c = 5.7-6.4%
III. **Stage III** with **HYPERGLYCEMIA** and the **onset of characteristic symptoms**.

Observation!

Hyperglycemia becomes manifest when **80-90% of β cells** are destroyed, with the onset of **ABSOLUTE insulin deficiency.**

1. ENVIRONMENTAL factors

They have an **important role** in the pathogenesis of type 1 diabetes because they represent the **"trigger" event** responsible for the onset of the autoimmune reaction. They include:

- **Dietary factors**: early introduction of:
  - *cow's milk* (before 4 months) in the diet
  - *gluten*-containing foods in the first months of life (frequent association of type 1 diabetes with celiac disease)

  ⇒ triggering of autoimmune processes through **molecular mimicry**
- **Viral infections**: e.g., ***enteroviruses (Coxackie, Echo), rubella virus, cytomegalovirus, Epstein-Barr virus, mumps virus***, which cause:
  - direct **cytolytic** effect on β cells
  - initiation of the chronic inflammatory process of the islets of Langerhans called **INSULITIS** through the release of proinflammatory cytokines: TNF-alpha, IL-1
  - triggering of autoimmune processes through **molecular mimicry**
- **Deficiency factors**: e.g., ***vitamin D deficiency*** - responsible for a large number of cases of type 1 diabetes in the Nordic countries

Observation!

Molecular mimicry represents the immune response to foreign antigens (e.g. viral proteins, cow's milk albumin, gluten gliadin) **structurally similar** to auto-Ag found in β cells (e.g. *glutamic acid decarboxylase*) with the appearance of antipancreatic auto-Abs.

2. GENETIC predisposition

It has a **lower** significance in the etiopathogenesis of type 1 DM (30% concordance in monozygotic twins) compared to type 2 DM (90% concordance in monozygotic twins) which is based on **polygenic defects** and the involvement of the major histocompatibility complex that encodes the synthesis of **HLA antigens** ("Human Leukocyte Antigens"). Thus, the association of **type 1 DM** with **HLA haplotypes DR3, DR4, DQ2, DQ8, B8, B15** (present in approx. 90% of patients) has been observed.

3. Cellular and humoral AUTOIMMUNE mechanism

It plays the **MAJOR role** in the pathogenesis of type 1 diabetes, being responsible for the **progressive** destruction of **β pancreatic cells**.

- **The role of CELLULAR immunity**: β cells damaged by environmental factors release auto-Ag that will be captured, processed and presented by pancreatic dendritic cells to the following subclasses of **T lymphocytes**:
  - **cytotoxic T lymphocytes** → exert a **direct cytotoxic effect** on pancreatic β cells - being the main ones responsible for their **destruction** through **perforins** (pore-forming proteins - similar to the membrane attack complex of C) and **granzymes** (proteases that induce the apoptosis of β cells)
  - **Th1 lymphocytes** → stimulate (via IFN-gamma secretion) the synthesis of **pro-inflammatory cytokines, IL-1 and TNF-α** by **activated macrophages** with the onset of **insulitis** (local chronic inflammation)
  - **Th2 lymphocytes** → activate (via IL-4 and Il-13 secretion) **B lymphocytes that differentiate into plasma cells** secreting **anti-pancreatic autoantibodies**.
- **The role of HUMORAL immunity**: consists in the presence in circulation of **antipancreatic autoantibodies** which are considered **MARKERS** and **NOT necessarily effectors of β-cell destruction** - being of two types:
  a) **Autoantibodies against Langerhans islet cells**: e.g. autoAb anti-islet antigen-2 (IA2), autoAb anti-glutamic acid decarboxylase (GAD)
  b) **Anti-insulin autoantibodies** with 2 characteristics:
     - are formed by the release of insulin during the destruction of β-cells = primary autoimmune mechanism
     - may appear following insulin treatment and will determine a suboptimal response to treatment = secondary autoimmune mechanism

Remember!
The assessement of anti-islet cell and anti-insulin autoAbs is useful for the **differential diagnosis** of type 1 and type 2 diabetes. These autoAbs can be detected in the serum of type 1 diabetes patients 5-7 years before the onset of **hyperglycemia**, allowing **early diagnosis** of the disease, as well as in *first-degree relatives* of patients, with a role in **predicting the onset** of the disease.

B. Etiopathogenesis of type 2 diabetes
Type 2 diabetes patients are individuals with **central obesity** responsible for the onset of **insulin resistance**. This induces a **compensatory hyperinsulinism** that **precedes** (by 5-10 years) the **onset of hyperglycemia**. The **main pathogenic mechanisms involved in the etiopathogenesis of type 2 diabetes** are: insulin resistance, secretory dysfunction of β cells (favored by **genetic predisposition**), exaggerated hepatic glucose production, decreased secretion and action of incretins, increased glucose renal tubular reabsorption.

1. ENVIRONMENTAL factors
They play an **important** role in the pathogenesis of type 2 diabetes because they represent **MODIFIABLE risk factors**.
The **main environmental factor** involved is **central obesity** (**abdominal**, **android** type), to which a **sedentary** lifestyle and **chronic psychological stress** are added. Abdominal obesity is present in **85%** of patients with type 2 diabetes and intervenes in the pathogenesis of type 2 diabetes through **3 mechanisms**:

- **Excessive intake of carbohydrates and lipids through overeating** → "**toxic**" effect on ***pancreatic β cells*** with their ***dysfunction/apoptosis*** along with the dysfunction of ***peripheral tissues*** responsible for insulin resistance through:
  - **glucotoxicity**: activation of **secondary pathways** for metabolizing excess glucose, with the generation of toxic metabolites in ***insulin-independent tissues***
  - **lipotoxicity**: increased **triglyceride (TG)** deposits in ***non-adipose tissues***
- **Increased adipose tissue mass** → modification of **adipokine** secretion responsible for insulin resistance through: *increased resistin, decreased adiponectin and leptin*
- **Production of inflammatory cytokines in the adipose tissue** → hypertrophied adipocytes and macrophages activated by lipotoxicity produce **TNF-alpha** and **IL-6** which reduce the uptake of glucose by decreasing GLUT-4 activity and lipogenesis in the adipose tissue.

2. GENETIC predisposition

It plays an **important** role in the pathogenesis of type 2 diabetes (90% concordance in monozygotic twins, familial aggregation of the disease - first-degree relatives have a 20-30% risk of developing diabetes) and is based on **polygenic defects** that affect:

- the mass or functionality of β pancreatic cells with the onset of **β cell secretory dysfunction**
- the response of peripheral tissues to insulin with the onset of **insulin resistance**

3. INSULIN RESISTANCE

It plays the **MAJOR role** in the pathogenesis of type 2 diabetes and is defined as the **suboptimal response of peripheral tissues to insulin** = skeletal muscle and adipose tissue, the CAUSES being:

i) **receptor** defects - **decrease in no. of insulin receptors** and
ii) **postreceptor** defects - **altered intracellular signaling**

CONSEQUENCES of insulin resistance: **decreased glucose utilization in peripheral tissues** and, respectively, **increased *hepatic* glucose production**, through:

- decreased stimulatory effect of insulin on:
  = *glucose uptake* and *glycogenogenesis* in **muscle tissue**
  = *lipoprotein lipase* and *lipogenesis* in **adipose tissue**
- decreased inhibitory effect of insulin on ***glycogenolysis*** and ***hepatic gluconeogenesis***

4. SECRETORY DYSFUNCTION of β cells

Secretory dysfunction of β cells is defined as **inadequate insulin secretion** (relative deficit) necessary to correct **hyperglycemia** induced by **insulin resistance** and **excess counterregulatory hormones.**

The factors responsible for the secretory deficiency of β cells are:

a) **Genetic predisposition** (polygenic defects)
b) **Progressive reduction of the mass of β pancreatic cells** → apoptosis of β cells exposed to gluco- and lipotoxicity, with the formation of insoluble amyloid deposits
c) **Decrease in the effect of incretins**:

- Incretins are peptides secreted postprandially by the **mucosa of the small intestine**, the main ones being:
  - GIP (Gastric Inhibitory Peptide or Glucose-dependent Insulinotropic Polypeptide)
  - GLP-1 (Glucagon Like Peptide-1)
- The **effect of incretins** consists of ***hypoglycemia*** caused through:
  - potentiation of insulin secretion at the level of β cells
  - inhibition of glucagon secretion at the level of α cells

  the effects being ***dependent on blood glucose*** (their effect decreases as blood glucose normalizes)
  - delay in gastric emptying with prolongation of the feeling of satiety

Both incretins are rapidly inactivated by the enzyme called **dipeptidyl peptidase 4** (DPP-4).

Remember!
The discovery of the *"incretin effect"* led to the introduction of **2 new classes** of **antidiabetic drugs** designed to lower blood sugar in type 2 diabetes, namely:

- **GLP-1 receptor agonists** $\rightarrow$ with stimulation of the *"incretin effect"* - e.g., *Exenatide, Liraglutide, Dulaglutide, Semaglutide*
- **DPP-4 enzyme inhibitors** $\rightarrow$ with *delay in the degradation of incretins in plasma* - e.g., *Sitagliptin, Saxagliptin, Vildagliptin*

5. Increased RENAL REABSORPTION of glucose
Reabsorption of glomerularly filtered glucose (approx. 160-180 g/day) occurs entirely at the level of the **proximal convoluted tubule** with the help of **Sodium-GLucose co-Transporters**: **SGLT-2** (reabsorbs 90% of the filtered glucose) and **SGLT-1** (the remaining 10%). In DM, **increased expression and activity of SGLT-2** at the **PCT** level and, respectively, of **glucose reabsorption**, with **worsening hyperglycemia**, has been described.

Remember!
The discovery of the SGLT-2 effect led to the introduction of the latest class of oral antidiabetic drugs, the **sodium-glucose co-transporter inhibitors, SGLT-2 inhibitors** - the "*gliflozin*" type drugs, e.g. *Canagliflozin, Dapagliflozin, Empagliflozin, Sotagliflozin*.

ACUTE COMPLICATIONS of DM - METABOLIC complications

A. DIABETIC KETOACIDOSIS (DKA)

DEFINITION: the **MAJOR acute** complication of **type 1 DM** caused by **SEVERE insulin deficiency**, but can also be occasionally encountered in patients with type 2 DM as well

PRECIPITATING FACTORS:

- activation of the sympatho-adrenergic system in:
  - intercurrent disorders: infections, trauma, acute myocardial infarction, surgical interventions
  - psychological stress
  - pregnancy
  - sympathomimetics
- interruption of insulin administration

PATHOGENESIS - the onset of the condition involves the association of **2 factors**:

1. **ABSOLUTE insulin deficiency** - with the following consequences:
   - increased lipolysis in the adipose tissue and increased plasma levels of FFA
   - increased uptake of FFA by the liver and increased substrate availability for ketogenesis

2. **GLUCAGON excess** - with the following consequences:
   - increased transport of FA from the cytosol into mitochondria at the hepatic level by activating the carnitine system
   - increased mitochondrial β-oxidation of FA with the formation of excess acetyl-CoA that will be used for ketogenesis

Hyperglycemia is the consequence of both hormonal imbalances, being caused both by the *decrease in glucose utilization* and by the *increase in its production through hepatic gluconeogenesis* (excess of FFA and AA through proteolysis).

DKA MANIFESTATIONS - Tab. 32.

Table 32. **Clinical** and **paraclinical** manifestations of DKA.

Consequence	Characteristics
Metabolic acidosis with INCREASED ANIONIC GAP (ketoacidosis)	▪ **Clinically:** – acetone breath smell (green apples) – flushed and warm skin – nausea, vomiting (gastric stasis) – Kussmaul breathing (compensatory hyperventilation) in advanced stages ▪ **Paraclinically:** – **pH < 7,3** – **HCO_3^- < 15 mmol/L** – **Ketonemia ≥ 3 mmol/l or significant Ketonuria**
Hyperglycemia	**Glycemia: >200 mg/dL** **Glycosuria +++**
Plasma hypertonicity	**Osmolarity < 330 mOsm/L**
Hyponatremia (frequently)	Na^+ loss through *osmotic diuresis* and *vomiting* + dilutional mechanism – an increase in blood glucose by 100 mg/dl above the normal value of 100 mg/dl causes a reduction in natremia by approximately 1.6 mmol/l, the value returning to normal with the decrease in blood glucose; it is necessary to correct sodium according to the formula: corrected Na^+ = current Na^+ + 1.6 x [blood glucose – 100]/100
Hypokalemia	K^+ loss through *osmotic diuresis* and *vomiting/diarrhea* – can be *masked* by the exit of K^+ from the cells in exchange for

	H^+ determined by metabolic acidosis – becomes *manifest* after the administration of insulin which corrects the metabolic acidosis and stimulates the Na^+/K^+ pump

DKA staging - Tab. 33.

Table 33. DKA staging.

Stage	Serum pH	Serum bicarbonate (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

Observation!
The treatment of DKA is based on **fluid and electrolyte rebalancing AND insulin administration**:
- fluid and electrolyte rebalancing **increases renal perfusion** and **glucose excretion in the urine**
- insulin administration **without** fluid and electrolyte rebalancing causes **hypokalemia** (correction of acidosis causes K^+ entry into the cells) and **vascular collapse** (correction of glycemia causes water to exit the vascular lumen and enter into the tissues).

B. HYPEROSMOLAR COMA

DEFINITION: it is the **major ACUTE complication of type 2 diabetes**

PRECIPITATING FACTORS:
It frequently occurs in **elderly diabetics**, against the background of **dehydration/insufficient fluid intake** (factors that increase blood glucose and accentuate the dehydration caused by osmotic diuresis):
- intercurrent disorders (e.g., infections with fever)
- acute cardiovascular events (AMI, stroke)
- excessive administration of diuretics

PATHOGENESIS: **RELATIVE** insulin deficiency causes hyperglycemia, but the ***insulinemia is sufficient to inhibit ketosis***

MANIFESTATIONS of hyperosmolar coma - Tab. 34.

Table 34. **Clinical** and **paraclinical** manifestations of HYPEROSMOLAR COMA.

Consequence	Characteristics
Hyperglycemia	**Glycemia: > 600 mg/dL** **Glycosuria ++++** ☞ *Osmotic diuresis* with severe hypovolemia responsible for: – prerenal azotemia – hypovolemic shock/vascular collapse
Plasma hypertonicity	**Osmolarity > 330 mOsm/L** ☞ at values > 360 mOsmol/L coma sets in (common in these patients)

ABSENCE OF KETOACIDOSIS	Insulin deficiency is **RELATIVE:** – activation of ***glycogenolysis/gluconeogenesis*** → *hyperglycemia* – **WITHOUT *significant*** activation of ketogenesis ☞ **pH > 7,3, HCO_3^- > 15 mmol/L, ketonuria +**
Possibly METABOLIC ACIDOSIS from other causes	Prerenal azotemia → *nonvolatile acid retention* Shock/vascular collapse → *lactic acidosis* Starvation → *starvation ketosis*

C. HYPOGLYCEMIA

DEFINITION: low **blood sugar < 70 mg/dL that is severe** due to **CNS lesions**
It is the MOST COMMON acute complication of DM.

CAUSE: overdose of insulin or, more rarely, of oral antidiabetics
- **frequent** complication of *parenteral* treatment with **INSULIN** → occurs in > 90% of patients with **type 1 diabetes**
- **rare** complication of *oral* treatment with **SULFONYLUREA derivatives** (e.g., Glibenclamide, Gliclazide, Glipizide, Glimepiride) → occurs in patients with **type 2 diabetes**

PRECIPITATING FACTORS:
- inadequate food intake for the doses of insulin/oral antidiabetics administered
- increased physical effort - increases glucose uptake by the striated muscle
- alcohol abuse - inhibits gluconeogenesis

CONSEQUENCES: functional disorders and neuronal damage proportional to the *degree, duration and speed of hypoglycemia onset*

CLINICAL Forms:
1. ***Acute* hypoglycemia** = **adrenergic (warning) symptoms** → tremors, tachycardia, sweating, dizziness, anxiety, imperative hunger
2. ***Chronic* hypoglycemia** = **neuroglycopenic symptoms** → headache, irritability, visual disturbances, seizures, coma
3. ***Nocturnal* hypoglycemia** → night sweats, morning headache, difficulty waking up

Remember!
- adrenergic (warning) symptoms **disappear** in patients with *autonomic neuropathy* or in those who are undergoing *treatment with beta-blockers*
- repeated episodes of hypoglycemia *reduce the sympathetic reaction over time* and cause a **delayed response** to significant hypoglycemia

CHRONIC COMPLICATIONS of DM

CLASSIFICATION:
I. Microvascular complications: retinopathy and nephropathy
II. MACROvascular complications: accelerated ATS and HT

III. Diabetic neuropathy
IV. Foot ulcer
V. Infectious complications

Observation!
They have the highest INCIDENCE and increase the **morbidity** and **mortality** of diabetic patients.

I. MICROVASCULAR complications of DM – diabetic MICROANGIOPATHY

- **PATHOGENESIS**: chronic hyperglycemia activates **4 SECONDARY METABOLIC PATHWAYS** that are responsible for the occurrence of **microvascular complications** through **GLUCOTOXICITY**, as follows:
  1. **Non-enzymatic PROTEINS glycation** → glomerular and retinal lesions with **diabetic nephropathy & retinopathy**
  2. **Activation of PROTEIN KINASE C** → glomerular and retinal lesions with **diabetic nephropathy & retinopathy**
  3. **Activation of the POLYOL PATHWAY** → nerve damage (demyelination) and crystallin lens lesions with **diabetic neuropathy & cataracts**
  4. **Activation of the HEXOSAMINE PATHWAY** → worsening of **insulin resistance** through glucotoxicity

1. Non-enzymatic glycation of PROTEINS

- **Characteristics:**
  - represents the **irreversible binding** of glucose molecules onto **proteins** under *hyperglycemic* conditions
- **Glycation products** are:
  - **HbA1c**: is the result of Hb glycation and reflects the average level of glycemia to which erythrocytes have been exposed in 120 days of life → *diagnostic and monitoring indicator and "therapeutic target" in DM*
  - **Advanced Glycation End-products** (AGE) → are the result of **glycation of tissue and plasma proteins** and are classified into:
    - *Intracellular AGEs* → accumulate in cells and modify the function of intracellular proteins
    - *Extracellular AGEs* → modify extracellular matrix proteins
    - *Plasma AGEs* (soluble) → act on specific receptors called **RAGE (Receptor of Advanced Glycation Endproducts)** at the level of endothelial cells, macrophages and vascular smooth muscle cells (VSMC)
- **Consequences of increased AGEs:**
  a) Increased release of *proinflammatory cytokines* (IL-1, TNF-a) and *growth factors* (TGF-β, VEGF) at the *vascular wall level*
  b) Increased endothelial permeability
     - for *proteins* → at the glomerular basement membrane level with proteinuria
     - for *LDL-C particles* → at the arterial endothelium level with accelerated ATS
  c) Procoagulant status - through ↑ release of tissue factor
  d) Increased oxidative stress

- **CLINICAL FORMS:**

1. DIABETIC RETINOPATHY

- **Characteristics**: major cause of **BLINDNESS**, correlates with the **duration of hyperglycemia** and the **presence of HT**
- **CLINICAL Forms:**
  - **a) NON-PROLIFERATIVE Retinopathy (R1)**
    - punctate hemorrhages (capillary microaneurysms)
    - spot hemorrhages (in the deeper retinal layers)
    - hard (precipitation of effused lipids) and soft exudates (retinal microinfarctions)
  - **b) PREPROLIFERATIVE Retinopathy (R2)**
    - venous dilatations/loops
    - intraretinal microvascular anomalies
    - multiple, deep round hemorrhages
  - **c) PROLIFERATIVE Retinopathy (R3)**
    - neoformation vessels
    - preretinal or subhyaloid hemorrhages
  - **d) ADVANCED Retinopathy**
    - retinal fibrosis
    - partial/total retinal detachment by traction

2. DIABETIC NEPHROPATHY

- **Characteristics**: major cause of **RENAL FAILURE, dialysis, renal transplantation**
- **CLINICAL Forms:**

- Glomerular nephropathy: **diabetic glomerulopathy** → the **most common cause of chronic GN!**
- Tubulo-interstitial nephropathy: acute/chronic pyelonephritis (PN)
- Vascular nephropathy: ATS and renal arteriolosclerosis

Diabetic GLOMERULOPATHY

PATHOGENESIS:

- **Vasodilation of the afferent arteriole** induced by hyperglycemia and AGE and **vasoconstriction of the efferent arteriole** induced by the RAA system causes:
  - hyperperfusion and glomerular hyperfiltration → *glomerular and systemic hypertension (HT)*
  - increased protein filtration → *mesangial cell injury* with chronic inflammation + glomerular fibrosis
  - increased tubular protein reabsorption → *tubular cell injury* with chronic inflammation + tubulo-interstitial fibrosis
- **Glomerulosclerosis ± nodular sclerosis** → progressive reduction of the glomerular exchange surface
- **Expansion of the mesangial matrix** → increased permeability of the filtering membrane with worsening *proteinuria* responsible for the progressive damage of the remaining functional nephrons

II. MACROVASCULAR complications of diabetes – diabetic MACROANGIOPATHY

1. Accelerated ATHEROSCLEROSIS

- **Characteristics**:
  - **early** onset in both forms of DM
  - affects both genders **equally**
  - responsible for the **increased incidence of acute complications** with **unfavorable** evolution
    - *coronary artery disease* → the risk of AMI increases 2-3 times
    - *cerebral vascular disease* → the risk of ischemic stroke increases 2-3 times
    - *peripheral artery disease* → 50% of non-traumatic amputations are due to diabetic arteriopathy
- **Causes**:
  - frequent association with HT and dyslipidemia → metabolic syndrome with increased ATS risk
  - potentiation of atherogenic mechanisms triggered by other risk factors through:
    - increased atherogenicity of LDL particles (small and dense LDL, oxidized LDL, glycosylated LDL)
    - existence of a procoagulant status
    - existence of a proinflammatory status
  - independent risk factor of ATS through:
    - *secondary hypertriglyceridemia* from poorly controlled DM (VLDL ↑)
    - direct vascular effects of *chronic hyperglycemia* (similar to microvascular complications), *hyperinsulinism and insulin resistance*

2. ARTERIAL HYPERTENSION

- **In type 1 DM:**
  - HT occurs **after** the onset of nephropathy (secondary HT)
  - is caused by the ↓ GFR → water-salt retention (volume HT)
- **In type 2 DM:**
  - HT occurs **before** the onset of nephropathy (primary HT)
  - is caused by the association of *central obesity* + *insulin resistance* + *hyperinsulinism*

III. DIABETIC NEUROPATHY

- **Characteristic**: the **most frequent CHRONIC complication** of prolonged hyperglycemia
- **Causes**: activation of the polyol pathway and impairment of the microcirculation (vasa vassorum) that serves the nerve
- **CLINICAL forms:**

1. DISTAL SYMMETRICAL POLYNEUROPATHY

- **Characteristics**:
  - the most **FREQUENT** clinical form of **somatic polyneuropathy**
  - begins **distally** - in a "sock" (or "glove") pattern
  - the involvement is **symmetrical**
  - predominantly **sensory** (possibly motor as well)

- pathogenic mechanism: activation of the polyol pathway at the level of nerve cells with demyelination of peripheral nerves

▪ **CLINICAL manifestations:**

- *subjective*: paresthesias (numbness, tingling) and burning pain at the level of the extremities
- *objective*: decreased vibratory, tactile, thermal and **pain** sensitivity which favors the evolution of a skin lesion towards ulceration

2. VEGETATIVE NEUROPATHY

▪ **Characteristic**: diabetic vegetative neuropathy is the most **FREQUENT** clinical form of **vegetative neuropathy**

▪ **CLINICAL Manifestations**:

- *cardiovascular system*: silent ischemia (non-painful AMI), orthostatic hypotension, tachycardia
- *digestive system*: esophageal atony, diabetic gastroparesis (nausea, vomiting), diabetic enteropathy (postprandial watery diarrhea), diabetic colopathy (colon atony/constipation), fecal incontinence
- *urogenital system*: diabetic cystopathy (decreased bladder fullness sensation and/or emptying difficulty), sexual dysfunction/impotence
- *thermoregulation disorders*: distal anhidrosis, heat intolerance
- *osteo-articular system*: diabetic osteoarthropathy (neuropathic Charcot foot)
- ***delayed sympathetic response (late alarm reaction) in conditions of significant hypoglycemia***

IV. FOOT ULCER ("perforating ulcer")

▪ **Causes**: **DIABETIC FOOT triad**
  1. Local ischemia - due to *micro- and macroangiopathy*
  2. Local infections favored by *ischemia*
  3. Sensitivity disorders due to *distal symmetrical neuropathy*

▪ **Location**: at the level of increased pressure points → fingertips, metatarsal support area

▪ **Complications**:

- phlegmons of the foot → damage to ligaments and muscles
- bacterial osteitis → damage to the bones of the foot
- diabetic gangrene → requires amputation

V. INFECTIOUS complications

▪ **FAVORING factors:**

1) **Micro- and macroangiopathy**: reduced tissue perfusion and hypoxia affect the *chemotaxis* and *phagocytosis capacity* of neutrophils responsible for:
   - disruption of the inflammatory response to microbial invasion
   - absence of inflammatory signs
   - defective wound healing

2) Chronic hyperglycemia:
- decreased tissue O_2 release from hemoglobin (HbA1c) → favors infections with anaerobic germs
- glycosuria → favors urinary tract infections

3) Pain sensitivity disorders due to neuropathy

4) High number of medical interventions

- **Common types of INFECTIONS**: lower urinary tract infections (cystitis) and upper urinary tract infections (pyelonephritis), persistent candidal infections of the skin and mucous membranes, soft tissue infections, respiratory infections (including TB), osteomyelitis
- **Consequence**: increased release of counter-regulatory hormones that cause the increase of insulin requirements → *favoring factors of acute metabolic complications of diabetes*

HYPOGLYCEMIAS

- **Definition**: low **blood sugar < 70 mg/dL**
- **Consequences**: low ATP in the nerve cell causes *initially reversible* and then *irreversible damage* to the CNS
- **CLINICAL manifestations:**
  1. **DIRECT Disorders** – low blood sugar causes **nerve suffering** by depriving the neuron of its energy substrate which manifests itself through **neuroglycopenic symptoms**:
     - headache, visual disturbances, weakness/fatigue, convulsions, coma
  2. **INDIRECT Disorders** – low blood sugar causes **intense sympatho-adrenergic stimulation** which manifests itself through:
     - cold sweats, agitation, anxiety, tremors of the extremities, tachycardia and palpitations, overwhelming feeling of hunger

Remember!

Depending on the type of onset of hypoglycemia, a certain type of disorder predominates:
- in **CHRONIC hypoglycemias**, ***DIRECT disorders*** determined by **nervous suffering** predominate
- in **ACUTE hypoglycemias, *INDIRECT disorders*** produced by **sympathetic stimulation** predominate
- in the *hypoglycemic coma* in DM, the sympathetic response may be absent due to vegetative neuropathy

- **Classification:**

A. POSTPRANDIAL hypoglycemias

Etiopathogenesis: excessive release of insulin *after food ingestion* (Tab.35.)

Table 35. Etiopathogenesis of **postprandial** hypoglycemias.

Cause	PATHOGENIC mechanism
▪ **DIETARY hyperinsulinism** - the most common cause in *adults* – postgastrectomy	▪ food ingestion is followed by rapid evacuation of gastric contents ▪ massive glucose absorption causes excessive insulin release

▪ **HEREDITARY fructose intolerance -** the most common cause in *children*	▪ aldolase B deficiency prevents the metabolism of fructose into glucose and causes the accumulation of fructose-1-phosphate responsible for: *hypoglycemia, fructosuria, liver and kidney damage, lactic acidosis*

B. HUNGER hypoglycemias or *fasting* hypoglycemias
Etiopathogenesis: decreased hepatic glucose mobilization/production (Tab. 36) or excessive glucose utilization at the tissue level (Tab. 37).

Table 36. Hypoglycemias due to decreased hepatic glucose **mobilization/production**.

Cause	PATHOGENIC mechanism
▪ **COUNTERREGULATORY HORMONES deficiency** − pituitary insufficiency (low STH) − Addison's disease (low cortisol)	▪ decreased glycogenolysis ▪ decreased gluconeogenesis
▪ **SEVERE HEPATIC disorders** – chronic active hepatitis – cirrhosis	▪ decreased gluconeogenesis ▪ insulin inactivation deficiency
▪ **CHRONIC alcoholism**	▪ blocked gluconeogenesis by the increased $NADH/NAD^+$ ratio
▪ **SEVERE malnutrition**	▪ deficiency of gluconeogenesis substrates (e.g. alanine) due to decreased muscle mass

Table 37. Hypoglycemias due to **increased glucose utilization at the tissue level**.

Cause	PATHOGENIC mechanism
▪ **Hyperinsulinism** – insulinoma (tumor of pancreatic beta cells) – extrapancreatic tumors	▪ increased insulin secretion ▪ release of insulin-like substances
▪ **Intense and prolonged physical effort**	▪ decreased O_2 delivery at the muscle level causes: – decreased use of FA as an energy substrate – excessive use of glucose → lactic acidosis

9. PATHOPHYSIOLOGY OF DYS/HYPERLIPIDEMIAS

LIPOPROTEIN STRUCTURE AND FUNCTION – Brief physiology & biochemistry overview

- **Definition**: lipoproteins (LP) are structural complexes made up of *lipids* and *apoproteins* (Apo) that perform the function of transporting insoluble lipid molecules, *cholesterol* and *triglycerides*, in plasma.
- **Structure**: LP have **2 components** in their structure:
  - the **central HYDROPHOBIC core** containing: *esterified cholesterol and triglycerides*
  - the **outer HYDROPHILIC envelope** containing: *apoproteins, phospholipids* and *unesterified cholesterol*

Observation!
The assembly of lipoprotein components is done through **non-covalent bonds** that allow the transfer of *apoproteins* and *lipid* fractions between lipoprotein classes.

- **Lipoprotein classes**

By **ultracentrifugation**, **4 classes of LP** are obtained whose main characteristics are presented in Tab. 38:
- Chylomicrons (CHY)
- Very Low Density Lipoproteins (VLDL)
- Low Density Lipoproteins (LDL)
- High Density Lipoproteins (HDL)

Table 38. Characteristics of the main classes of plasma LIPOPROTEINS.

Characteristics	CHY	VLDL	LDL	HDL
ELFO mobility	Do not migrate	pre-β	β	α
Composition %				
– **Triglycerides**	**90**	**60**	10	10
– **Cholesterol**	5	20	**50**	**10-30**
Apo(lipo)proteins	Apo-B48 Apo-CII Apo-E	Apo-B100 Apo-CII Apo-E	Apo-B100	Apo-AI Apo-CII Apo-E
Transport function	*Exogenous triglycerides* - from the intestine to the adipose tissue and skeletal muscle	*Endogenous triglycerides* - from the liver to the adipose tissue and skeletal muscle	*Cholesterol* from the liver to the peripheral tissues	*Cholesterol* from peripheral tissues to the liver ("reverse transport")

Observations!
The remnants resulting from the hydrolysis of TG from the VLDL structure generate **IDL** (Intermediate Density Lipoproteins). These are intermediate density LPs, containing ApoB100 and E that migrate electrophoretically between the pre-β (VLDL) and β (LDL) bands and generate a **broad β band** when found in high concentration in plasma. The remnants resulting from the hydrolysis of chylomicrons are enriched in cholesterol and are pro-atherogenic.
A particular form of LP is lipoprotein (a) - Lp (a), which is an LDL that contains in addition to apo B100 also apo (a) and is a LP with a unanimously recognized pro-atherogenic role, its physiological function being currently unclear.

- **Apo(lipo)proteins**

Apoproteins (Apo-) are proteins synthesized in the **liver** (main source) and **enterocytes** (secondary source). **Four major classes of Apo-** are described, namely **A, B, C** and **E**, which fulfill **2 main roles**:

- **structural role**: ensures the *stability/solubility of the lipoprotein complex in plasma*
- **functional role**: *cofactors* (usually activators) *of enzymes involved in LP metabolism* and/or *ligands* for cellular receptors specific to a particular LP class (Tab. 39.).

Table 39. Functional role of the main classes of apo(lipo)proteins.

Apo- Class	LP Class	Functional role
Apo-AI	HDL	– activator of **LCAT** (the enzyme responsible for cholesterol esterification)
Apo-B100	LDL	– **LDLr** ligand in the liver and extrahepatic tissues
Apo-CII	CHY, VLDL	– activator of **LPL** (the enzyme responsible for TG metabolism) at the level of capillaries in adipose and muscle tissue
Apo-E	IDL, CHY remnants	– ligand for **remnants receptors** in the liver

LCAT – lecithin-cholesterol-acyl-transferase, **LDLr** – LDL receptors, **LPL** – lipoprotein lipase

LIPOPROTEIN PATHWAYS IN THE BODY

A. EXOGENOUS lipoprotein circuit - CHYLOMICRONS

CHY metabolism

CHY are synthesized in enterocytes and contain **mainly <u>exogenous TG</u>** and **small amounts of cholesterol** and on the exterior they present **Apo-B48**. CHY enter the general circulation via the lymphatic route (thoracic duct), receive **Apo-E** and **Apo-C-II** from HDL and reach the **capillaries** of the **adipose** and **muscle tissues** where TG will be hydrolyzed into **fatty acids (FA)** and glycerol under the action of **lipoprotein lipase (LPL)** - present at the capillary endothelium level and activated by **Apo-CII**. FA will be used for:

i) resynthesis and storage of TG in the *adipose tissue* and
ii) as an energy source in the *skeletal muscle* and *myocardium*.

CHY remnants - depleted in TG and **rich in cholesterol** are uptaken by the liver by binding of **Apo-E** to hepatic receptors for remnants and partially, to LDL receptors (LDLr). At the hepatocyte level, CHY remnants are degraded in lysosomes, and the released cholesterol will be used for the synthesis of bile acids or will be eliminated as such with bile in the intestine.

Observation!
Insulin increases LPL expression and enzyme activity is **reduced** in patients with **DM**, being responsible for the decrease in the metabolism of LP rich in TG, with **secondary hypertriglyceridemia**.

B. ENDOGENOUS lipoprotein circuit

a) VLDL metabolism

The liver synthesizes **<u>endogenous TG</u>** which it incorporates into **<u>VLDL</u>** together with a small amount of **cholesterol**. This is synthesized via the mevalonate pathway whose key enzyme is called *hydroxy-methylglutaryl CoA (HMG-CoA) reductase*, which is inhibited by *statins*, the main class of lipid-lowering drugs currently used in practice.

<u>VLDL</u> synthesized in the liver have **Apo-B100** on their exterior, they pass into the circulation where they receive **Apo-CII** and **Apo-E** from HDL and (similarly to CHY) they reach the **capillaries** in the **adipose** and **muscle tissues** where they are hydrolyzed into FA and glycerol under the action of **LPL** activated by **Apo-CII**.

VLDL remnants - depleted of TG and rich in cholesterol, are called **IDL** and are **catabolized via 2 pathways:**

- **50% of IDL particles** are uptaken by the **liver** by binding of **Apo-E** to the hepatic receptors (for remnants) and to LDLr, being endocytosed and processed by hepatocytes similarly to the metabolism of CHY remnants
- **50% of IDL particles** are additionally loaded with **cholesterol** transferred from **HDL** and generate a new class of LP, **LDL**.

Remember!

- Decreased **LPL** activity and/or **Apo-CII** deficiency causes the accumulation of **CHY** and **VLDL** with ***increased plasma triglycerides***.
- **Apo-E** deficiency causes the accumulation of **CHY remnants** and **IDL** with ***increased plasma cholesterol***.

b) LDL metabolism

1. <u>SPECIFIC</u> pathway - LDL receptor (LDLr)-DEPENDENT

Provides catabolism of **70% of plasma LDL** and involves **slow uptake of LDL** (by receptor-mediated endocytosis) via binding of **Apo-B100** to **specific high-affinity LDL receptors (LDLr)** found in:

- liver (mainly)
- extrahepatic tissues - e.g., adrenal cortex, gonads, etc.

After the Apo-B100/LDLr complex is broken down, LDLr is re-expressed on the cell surface, and the LDL particle is degraded at the lysosomal level into *cholesterol, phospholipids* and *amino acids*:

- cholesterol will be used mainly for the synthesis of *bile acids* (liver), the synthesis of *steroid hormones* (adrenal cortex), the *reconstruction of cell membranes* (the rest of the tissues)
- excess cholesterol will be stored with **2 consequences**:
  - *inhibition of HMG CoA-reductase* (the key enzyme in cholesterol synthesis)
  - *inhibition of the synthesis and membrane expression of LDLr* (through a negative feed-back mechanism).

Observation!

The action of removing LDL from plasma is prevented by the binding at the LDLr level of an enzyme (peptidase) called *proprotein convertase subtilisin-kexin type 9 (PCSK9)* which favors the degradation of receptors at the lysosomal level. **PCSK9 inhibitors** - *evolocumab* and *alirocumab* (monoclonal antibodies) represent the **newest class of cholesterol-lowering drugs** indicated in cases of severe hyperlipidemia resistant to classical therapy (their use in current practice being limited by the high costs of treatment).

2. <u>NON-SPECIFIC</u> pathway - INDEPENDENT of LDL receptors

Ensures the catabolism of **30% of plasma LDL** and involves the **rapid** uptake of **LDL particles present in excess**, especially **modified LDL** (oxidized LDL, acetylated LDL, glycosylated LDL, small and dense LDL) by **macrophages** via **non-specific** receptors and which are NOT subject to the regulatory process (through the negative feed-back mechanism); as such, they allow macrophages to **overload** with cholesterol and become *"foam cells"*.

c) HDL metabolism

Hepatocytes and **enterocytes** are the primary source of **HDL particles** that present **Apo-AI** on their exterior. After being released into the circulation, **HDL particles** are additionally loaded with **cholesterol** and **phospholipids** that are transferred from:

i) peripheral tissues: muscle, adipose tissue, tissue macrophages (including from vascular walls) that release free cholesterol

ii) other LPs, namely **VLDL** and **CHY** that release cholesterol and phospholipids during their catabolism under the action of **LPL**.

Free cholesterol on the HDL surface is esterified under the action of **LCAT** activated by Apo-AI, thus generating **mature HDL particles - large, spherical** containing *esterified* cholesterol in the central core.

Mature HDL thus transfer *esterified cholesterol* from **peripheral tissues to the liver** for metabolism, LP uptake at the hepatocyte level being mediated by the binding of **Apo-AI** on specific receptors for HDL - achieving the so-called *"reverse transport"* of cholesterol.

Remember!

- **LPL** deficiency is responsible for the decrease in the formation of **HDL** particles by decreasing the catabolism of VLDL and CHY (decreased transfer of cholesterol and phospholipids) - therefore patients with increased levels of TG secondary to their impaired clearance often also have reduced serum levels of HDL.
- **LCAT** deficiency is responsible for the decrease in the formation of **mature HDL** particles and the increase in ***free cholesterol*** in plasma.

HYPERLIPIDEMIAS / HYPERLIPOPROTEINEMIAS

- **DEFINITION**: **hyperlipidemias** or **hyperlipoproteinemias (HLP)** define a heterogeneous group of **metabolic diseases** characterized by **increased cholesterol (LDL)** and/or **triglycerides (CHY, VLDL) in plasma**
- **Etiopathogenic CLASSIFICATION:**
  A. Primary (familial) HLP
  B. Secondary HLP:
  - to *pre-existing conditions*: diabetes mellitus, chronic liver disease, hypothyroidism, chronic kidney disease
  - *drug-induced*: estrogen therapy, thiazide diuretics, beta-blockers, corticosteroid therapy

PRIMARY HYPERLIPOPROTEINEMIAS

Primary (familial) HLP are due to defects (mutations) in the genes that control the *synthesis, transport* or *catabolism* of lipoproteins.

Three main pathogenic mechanisms are described:

- impairment of *hepatic LP synthesis*
- *deficiency of cellular LP uptake* through mutations of genes encoding specific cellular receptors and/or apoproteins with a ligand role in the LP structure
- *deficiencies of enzymes involved in LP metabolism*

Classification:

- **The OLD classification - FREDRICKSON** - includes **5 phenotypes of HLP** that are presented below in correlation with the atherogenic risk, ***HLP types IIa, IIb and III being the most atherogenic*** (Tab. 40).

Table 40. Fredrickson classification of HLP.

Type	HLP name	LP class (ELFO)	Lipid type	Atherogenic risk
I	Familial hyperchylomicronemia	**CHY**	TG ↑↑↑↑	-
IIa	**Familial hypercholesterolemia**	**LDL**	TC ↑↑↑	+++
IIb	**Combined familial hyperlipidemia**	**LDL + VLDL**	TC ↑↑ TG ↑↑	+++
III	**Familial dysbetalipoproteinemia**	**IDL + CHY remnants**	TG ↑↑↑ TC ↑↑	+++
IV	Familial hypertriglyceridemia	**VLDL**	TG ↑↑	+
V	Mixed familial hypertriglyceridemia	**VLDL + CHY**	TG ↑↑↑↑	+

- **The NEW classification** – simpler and more clinically relevant is presented below and the *most common HLPs in practice are marked and briefly presented*.

1. HyperCHOLESTEROLEMIAS (HyperTC)
- **a) Familial hypercholesterolemia - HLP type IIa**
- **b) Polygenic hypercholesterolemia**
- c) Familial Apo-B100 deficiency

2. HyperTRIGLYCERIDEMIA (HyperTG)
- **a) Familial hypertriglyceridemia - HLP type IV**
- b) Familial LPL deficiency - HLP type I
- c) Familial apo-CII deficiency - HLP type I, HLP type V

3. MIXED (combined) hyperLIPIDEMIA
- **a) Combined familial hyperlipidemia - HLP type IIb**
- **b) Familial dysbetalipoproteinemia - HLP type III**

1. PRIMARY HYPERCHOLESTEROLEMIAS

- **GENERAL characteristics:**
  - **Clinical:**
    - Cholesterol deposits in macrophages: *xanthelasma, corneal arcus, tendinous xanthomas*
    - Accelerated ATS with risk of AMI at young ages: < 40 years for M, < 50 years for F
    - Frequent association with *obesity, DM and hypothyroidism*
  - **Paraclinical:**
    - Plasma aspect at 4°C after 24 hours: *clear*
    - Plasma lipid profile:
      - ✓ increased **total cholesterol (TC)** and **LDL-C**
      - ✓ normal TG

a) FAMILIAL HYPERCHOLESTEROLEMIA - HLP type IIa

- **Definition**: monogenic condition with autosomal dominant transmission determined by mutations in the **gene** encoding the **synthesis of LDL receptors (LDLr)** in most cases (95%), less often in the genes encoding the synthesis of ApoB100 or PCSK9 ("gain of function" type) with **increased LDL**.
- **Etiopathogenesis:**

– over 1500 mutations are known at the **LDLr gene** level which consist of:
  - ✓ lack of LDLr synthesis
  - ✓ decrease in LDLr membrane expression
  - ✓ deficiency in Apo-B100 binding to the LDLr
  - ✓ deficiency in endocytosis of the LDL/LDLr complex

  the consequence being in all cases a **decrease in LDL catabolism** with an **increase in LDL in plasma**

- **Characteristics**: moderate (heterozygous form) or severe (homozygous form) **hyperTC** - the latter is rare and requires intensive treatment (high doses of statins, PCSK9 inhibitors, LDL-apheresis)

b) POLYGENIC HYPERCHOLESTEROLEMIA

- **Definition**: **polygenic defects in hepatic cholesterol metabolism** and **LDRr expression** at the hepatic level which determine an **increase in LDL**
- **Etiopathogenesis**:
  - increase in hepatic cholesterol synthesis
  - decrease in LDLr expression due to the increased intracellular cholesterol deposits
- **Characteristic: hyperTC** triggered by hypercaloric/saturated fatty acid-rich diet that stimulates hepatic cholesterol synthesis

c) Familial Apo-B100 deficiency is a rare *autosomal dominant* defect in the **gene encoding the synthesis of Apo-B100 responsible for binding to LDLr** leading to **LDL elevation** (by decreased catabolism).

d) ApoB and PCSK9 gene mutations - Mutations in the ApoB gene impair LDLr binding and cause another relatively common monogenic disease, similar to familial hypercholesterolemia. Also, activating mutations in the PCSK9 gene cause a reduction in LDL receptors.

e) HDL disorders - Hereditary diseases of HDL metabolism are rare and recessively transmitted. These include diseases that cause low HDL-cholesterol levels, such as mutations in Apo A1, LCAT. They are associated with amyloid deposition and atherosclerosis.

2. PRIMARY HYPERTRIGLYCERIDEMIAS

- **GENERAL characteristics:**
  - **Clinical**:
    - Eruptive xanthomas (upper limbs, buttocks, back)
    - Recurrent episodes of abdominal pain
    - Risk of recurrent acute pancreatitis
    - Frequent association with *obesity, metabolic syndrome, diabetes mellitus, chronic alcoholism*

- **Paraclinical**:
  - Plasma aspect at 4°C after 24 hours
    - ✓ *opalescent* infranatant (↑ VLDL)
    - ✓ *creamy* supernatant (↑ CHY)
  - Plasma lipid profile:
    - ✓ **increased TG** (in severe forms > 1000 mg/dl)
    - ✓ TC and LDL-C N or slightly increased

a) FAMILIAL HYPERTRIGLYCERIDEMIA - HLP type IV

- **Definition**: condition in which an unknown genetic defect is responsible for **increased hepatic synthesis** and **decreased catabolism of VLDL**, which causes **increased VLDL.**

! It is the **most common primary HLP**.

- **Etiopathogenesis:**
  - increased hepatic synthesis of VLDL
  - decreased VLDL catabolism due to LPL and/or Apo-CII deficiency
- **Characteristic**: *frequent association with obesity, metabolic syndrome and type 2 diabetes* - hyperinsulinism (on the background of insulin resistance) stimulates hepatic production of VLDL.

b) FAMILIAL LIPOPROTEIN LIPASE (LPL) DEFICIENCY is a rare defect with *autosomal recessive* transmission in which the **absence/deficiency of LPL** determines the **increase in CHY** (HLP type I) by decreasing their catabolism. The characteristics are: HyperTG > 1000 mg/dl in a child presenting hyperchylomicronemic syndrome with: eruptive xanthomas, hepato-splenomegaly, lipemia retinalis (lactescent appearance of retinal vessels due to CHY deposition) and major risk of acute pancreatitis - but Apo-CII is present.

c) FAMILIAL Apo-CII DEFICIENCY is a rare defect with *autosomal recessive* transmission at the level of the gene encoding **Apo-CII**. The deficiency/absence of **Apo-CII** causes the decrease in **LPL** activity which determines the **increase in CHY and VLDL** (HLP type I or V) with HyperTG > 1000 mg/dl in the child.

3. MIXED (COMBINED) HYPERLIPOPROTEINEMIAS

- **GENERAL characteristics:**
  - **Clinical:**
    - Accelerated ATS - family history of early cardiovascular disease
    - Frequent association with *DM, obesity*
  - **Paraclinical:**
    - Plasma lipid profile:
      - ✓ **Increased TG**
      - ✓ **Increased TC and LDL-C**

a) COMBINED FAMILIAL HYPERLIPIDEMIA - HLP type IIb

- **Definition**: condition that causes through unknown (polygenic?) defects the **increase of VLDL** and **LDL** = **LP that contain Apo-B100**
  It is the **most common mixed hyperlipidemia**.

- **Characteristics**:
  - Frequent association with: *obesity, metabolic sdr., diabetes mellitus, HT and hyperuricemia*
  - Family history of early cardiovascular disease: < 55 years in M, < 60 years in F
  - Decreased HDL
  - *Specific test*: **serum Apo-B100** level is **increased**

b) FAMILIAL DYSBETALIPOPROTEINEMIA - HLP type III

- **Definition**: *autosomal recessive* defect in the **gene encoding Apo-E synthesis** and which determines the **increase in CHY remnants** and **IDL**
- **Etiopathogenesis**:
  - there are 3 isoforms of Apo-E in humans - E2, E3, E4 - with different affinity for hepatic receptors for LDL
    - Apo-E3 is the *normal* isoform, the *most frequently found in the population*
    - **Apo-E2 has the *lowest affinity for LDLr*** → individuals who are **homozygous** for **ApoE2 develop the disease**
    - Apo-E4 has the *highest affinity*, but homozygous individuals have **increased LDL** (by competition with remnants at the level of the LDLr), **accelerated ATS and increased risk of Alzheimer's disease**
- **Characteristics**:
  - Decreased hepatic uptake of CHY remnants and IDL leads to increased remnants in plasma - "remnant" disease
  - **Palmar xanthomas** ("yellow palm disease") and **tuberous xanthomas** (elbows, knees) are pathognomonic
  - *Specific test*: identification of the **homozygous Apo-E2/E2 genotype**

SECONDARY HYPERLIPOPROTEINEMIAS

1. HLP from DIABETES MELLITUS (DM) ⇒ Secondary HYPERTRIGLYCERIDEMIA or mixed HLP

a) HLP in type 1 DM

- **Characteristic**: plasma lipid levels depend on the **degree of glycemic control**
  - *if type 1 DM is controlled* → plasma lipid levels are **normal**
  - *if type 1 DM is poorly controlled* → absolute insulin deficiency causes **TG to increase** due to **VLDL** (+/- CHY) **increase**
- **PATHOGENIC mechanisms:**
  - in **ACUTE insulin deficiency**: **lipolysis increases** in the **adipose tissue** (via the activation of **lipase**) which causes the mobilization of FA that will be used by the liver for the production of TG and **VLDL** (and ketogenesis)
  - in **CHRONIC insulin deficiency**: decreased **LPL** activity causes decreased **VLDL and CHY catabolism**

Remember!

- Increased plasma LDL levels are **NOT** characteristic in type 1 DM, but when present they contribute to **accelerated ATS**.
- Insulin administration **corrects** hypertriglyceridemia.

b) HLP in type 2 DM

- **Characteristics**:
  - HLP is **constantly** present, even if there is good glycemic control
  - It is characterized by the **TRIAD**:
    - **Increased TG (VLDL)**
    - **Decreased HDL**
    - **Increased LDL with increased atherogenicity**: modified LDL - oxidized, glycosylated, small and dense LDL
- **PATHOGENIC mechanisms**: insulin resistance/relative insulin deficiency causes:
  - increased **lipolysis in the adipose tissue** $\Rightarrow$ increases the release of FA into plasma that will serve for hepatic synthesis of TG that will be incorporated into VLDL
  - **decreased LPL activity** $\Rightarrow$ decreased VLDL and CHY catabolism which secondarily determines decreased **HDL** particle formation by decreased cholesterol and phospholipid transfer with reduced "reverse transport" of cholesterol
  - decreased **LCAT** activity is responsible for **decreased formation of mature HDL** particles and in part, for increased **free cholesterol** and **LDL** in plasma.

Remember!

Increased plasma LDL levels are **NOT** characteristic of type 2 diabetes, and when they exist they usually reflect the coexistence of **primary hyperTC** and contribute to **accelerated ATS**.

2. HLP from CHRONIC LIVER DISEASES $\Rightarrow$ Secondary HYPERTRIGLYCERIDEMIA or MIXED HLP

- **Characteristic**: HLP depends on the **etiopathogenesis of the liver disease**:

  a. CHRONIC hepatitis (infectious, toxic):

  = hepatocellular lesions + chronic inflammation + increased oxidative stress determines:
  - decreased β-oxidation of FA ☞ use of **FA** for **TG** synthesis $\Rightarrow$ **increased VLDL production**
  - increased **Apo-B** production $\Rightarrow$ **increased VLDL production**

  b. CHOLESTATIC hepatopathies:
  - decreased cholesterol excretion in bile
  - increased i.c. deposits cause the decrease of LDLr expression $\Rightarrow$ **increased LDL by decreased catabolism**

3. HLP from CHRONIC ALCOHOLISM $\Rightarrow$ secondary HYPERTRIGLYCERIDEMIA

- **PATHOGENIC mechanisms**: alcohol **inhibits hepatic β-oxidation of FA** and **increases** through metabolization the **supply of acetate and hepatic [NADH + H^+]**, with the following consequences:
  - the excess of FA will be esterified into endogenous TG $\Rightarrow$ increased **VLDL** synthesis

- the increased supply of acetate and hepatic [NADH+H$^+$] ⇒ increases the *de novo* synthesis of FA
- increased synthesis/storage of TG ⇒ **hepatic steatosis** (alcoholic "fatty liver")

Remember!
Patients with **primary (familial) hyperTG** may develop **acute pancreatitis** & eruptive xanthomas under conditions of alcohol consumption.

4. HLP from ESTROGEN THERAPY ⇒ secondary HYPERTRIGLYCERIDEMIA
- **Characteristic**: occurs in the case of using preparations with a **high content of ESTROGENS**
- **PATHOGENIC mechanism:**
  - *increased hepatic synthesis* of **ApoB-100** ⇒ increased **VLDL** production
  - decreased **LPL** activity ⇒ decreased **VLDL** catabolism

Remember!
- Secondary HyperTG is **significant** in women with **pre-existing** (possibly undiagnosed) **primary (familial) HyperTG**, with the risk of triggering **acute pancreatitis**.
- The use of preparations with a **low estrogen content** has a minimal effect on lipoprotein metabolism.

5. HLP from HYPOTHYROIDISM ⇒ secondary HYPERCHOLESTEROLEMIA
- **Characteristics**:
  - **Frequent** secondary HLP - second in frequency after DM!
- **PATHOGENIC mechanisms:**
  - **Main**: **increased HMG-CoA reductase activity** with the following consequences:
    - **increased cholesterol synthesis** and intracellular deposits with reduced **LDLr expression**
    - decreased LDL catabolism and **increased LDL** in plasma
  - **Secondary**: **decreased LPL activity** causes decreased VLDL (+CHY) catabolism and **increased TG** in plasma

Remember!
- **Increased LDL** is present in the **subclinical stages of the disease**.
- In patients with **elevated LDL**, **thyroid function** must be evaluated.
- Correction of hypothyroidism **corrects** the hypercholesterolemia.

6. HLP from CHRONIC KIDNEY DISEASE ⇒ MIXED HLP
a. In NEPHROTIC syndrome
- **Characteristics**:
  - the most common cause of secondary HLP in CKD
  - is proportional to the *severity* of hypoproteinemia
- **Pathogenic mechanisms:**
  - **increased HMG-CoA reductase activity** *and* **decreased LDLr expression** ⇒ **increased LDL** by decreased catabolism

- **renal loss of albumin** causes the **compensatory hepatic synthesis of globulins**, including Apo-B100 ⇒ **increased VLDL** by increased hepatic production
- **urinary loss of LPL** ⇒ **increased VLDL** + **increased CHY** by decreased catabolism

b. In the stage of RENAL FAILURE (chronic uremia):

- LPL deficiency ⇒ **increased VLDL (+CHY)** by decreased catabolism
- increased oxidative stress ⇒ increased production of **oxidized LDL** with increased ATS risk.

10. PATHOPHYSIOLOGY OF PROTEIN METABOLISM ABNORMALITIES

PROTEINEMIA - Brief physiology overview

- **Definition**: total plasma protein concentration, normally comprised between **6.7 - 8.4 g/dL**.
- **Main plasma protein fractions:**
  - albumins = 3.5-5.2 g/dL
  - globulins = 2-3.5 g/dL
- **Site of plasma protein synthesis:**
  - **liver**: albumins (100%) & globulins (80%)
  - **plasma cells**: immunoglobulins
  - kidneys: renin, erythropoietin
  - intestine: apo-B48
  - endocrine glands: polypeptide hormones

DYSPROTEINEMIAS

- **Definition:** pathological conditions characterized by **abnormal values of plasma protein fractions, with or without changes in proteinemia.**
- **Classification:** Tab. 41.

Table 41. Classification and characteristics of **dysproteinemias.**

Type of dysproteinemia	Characteristics	Etiology	Pathogenic mechanisms
1. Dysproteinemia from ACUTE INFLAMMATION	- ↓ **albumins** - ↑ **α_1-globulins** - ↑ **α_2-globulins** ○ normal proteinemia	– bacterial infections: pneumonia, pyelonephritis, etc. – acute myocardial infarction – burns, polytrauma – post-surgical interventions – exacerbations of chronic inflammatory diseases: rheumatoid arthritis, SLE, Crohn's disease – necrotic/ulcerative malignant tumors	– **increased synthesis of acute phase proteins** that migrate on ELFO with **α1-globulins** (α1-antitrypsin, α1-anti-chymo-trypsin, α1-orosomucoid) and **α2-globulins** (α2-haptoglobin, α2-ceruloplasmin) – **decreased hepatic synthesis of albumins**
2. Dysproteinemia from CHRONIC INFLAMMATION	- ↓ **albumins** - ↑ **γ - globulins** **Possibly also:** - ↑ **α_1-globulins** - ↑ **α_2-globulins** ○ normal proteinemia	– chronic infections – collagenosis – malignant tumors	– **increased synthesis of Ig** that migrate on ELFO with **γ-globulins** – **increased synthesis of acute phase proteins** may

			be associated - especially in exacerbations – **decreased hepatic synthesis of albumins**
3. Dysproteinemia from HEPATIC DISORDERS	**- ↓ albumins** **- ↑ β-globulins** **- ↑ γ-globulins** and characteristic **"cirrhotic dome"** appearance (merging of β and γ peaks on ELFO) **Possibly also:** - ↑ **α_2-globulins** *in chronic ethanolic hepatitis* o in severe/chronic forms it is associated with *hypoproteinemia*	– chronic hepatitis: = chronic active hepatitis = chronic ethanolic hepatitis = liver cirrhosis	– decreased **hepatic synthesis of albumins** – increased production of **Ig (IgM, IgG, IgA)** that migrate on ELFO with **γ-globulins** in the context of the inflammatory-immunological syndrome – increased production of **Apo-B100** and VLDL and LDL that migrate on ELFO with **α2-** and **β-globulins** may be associated in the context of ethanolic/cholestatic hepatopathies
4. Dysproteinemia from NEPHROTIC SYNDROME	**- ↓ albumins** **- ↓ γ-globulins** **- ↑ α_2-globulins and β- globulins** o it is associated with *hypoproteinemia*	– **Causes:** proteinuria > 3,5g/ 24 h	– **increased urinary albumin and γ-globulin losses** (selective/non-selective proteinuria) – compensatory increase in hepatic synthesis of **α2-** and **β-globulins** (e.g., apo-B100 from the structure of VLDL and LDL) – low urinary loss rate of **α2-macroglobulin** causes **increased α2-globulins**

PARAPROTEINEMIAS

- **Definition**: the presence of **pathological proteins** in the blood, **with or without changes in proteinemia.**
- **Classification**: Tab. 42.

Table 42. Classification and characteristics of **paraproteinemias**.

Type of paraproteinemia	Characteristics	Etiology	Pathogenic mechanisms
1. Paraproteinemia from MONOCLONAL GAMMOPATHIES	- ↑ γ-globulins - possibly, ↑ β-globulins (IgM migrates with the β fraction) ○ associates with: – *HYPER-proteinemia* and Bence –Jones paraproteinuria (presence of Ig light chains in the urine in multiple myeloma)	– multiple myeloma – Waldenstrom macro-globulinemia	Increased synthesis of paraproteins = pathological immunoglobulins of the classes: IgG, IgM, IgA or only their light chains (κ or λ) produced by a clone of malignant plasma cells
2. Paraproteinemia from CRYO-GLOBULINEMIA	- ↓ albumins - ↑ γ -globulins - ↑ α_1-globulins - ↑ α_2-globulins ○ normal proteinemia	– chronic viral hepatitis C – lymphoproliferative diseases: lymphoid leukemias, lymphomas – collagenoses – neoplasms	Increased synthesis of Ig from the IgG or IgM classes = γ-globulins which precipitate at a temperature < 37°C and become soluble again at normal blood temperature. May be associated with: – *increased synthesis of acute phase proteins,* especially in exacerbations – *deficiency of hepatic synthesis of albumins*

IMPAIRMENT OF PURINE NUCLEOTIDE METABOLISM

Purine nucleotide metabolism - Brief biochemical overview

1. Purine nucleotides (AMP, GMP, IMP) come from **3 sources:**
   - *exogenous intake* (purine-rich diet)
   - *de novo biosynthesis* (from ribose-5-phosphate)
   - normal turnover of nucleic acids with *reuse of purine bases through the "salvage pathway"*
2. The final product of catabolism of endogenous and dietary purines is **uric acid**

a) *De novo* biosynthesis of purine nucleotides (Fig. 3, top)

Purine nucleotides are synthesized by all tissues, but the main site is the **liver**. The *key* steps of de novo biosynthesis are:

- synthesis of **5-phosphoribosyl-1-pyrophosphate (PRPP)** from ribose-5-phosphate and ATP under the action of ***phosphoribosyl-pyrophosphate (PRPP) – synthetase***:

  Ribose-5-phosphate + ATP → ADP + PRPP
- synthesis of **phosphoribosyl-1-amine** from PRPP under the action of ***amido-phosphoribosyl-transferase*** ⇒ the step that regulates the rate of purine synthesis through the *negative feed-back effect* of IMP, GMP and AMP on the enzyme (their increase causes enzymatic inhibition)

b) The "salvage pathway" of purine nucleotides (Fig. 3, bottom)

Ensures the recycling of *purine bases* (adenine, guanine) and *nucleosides* (guanosine, inosine, adenosine) resulting from the degradation of purine nucleotides:

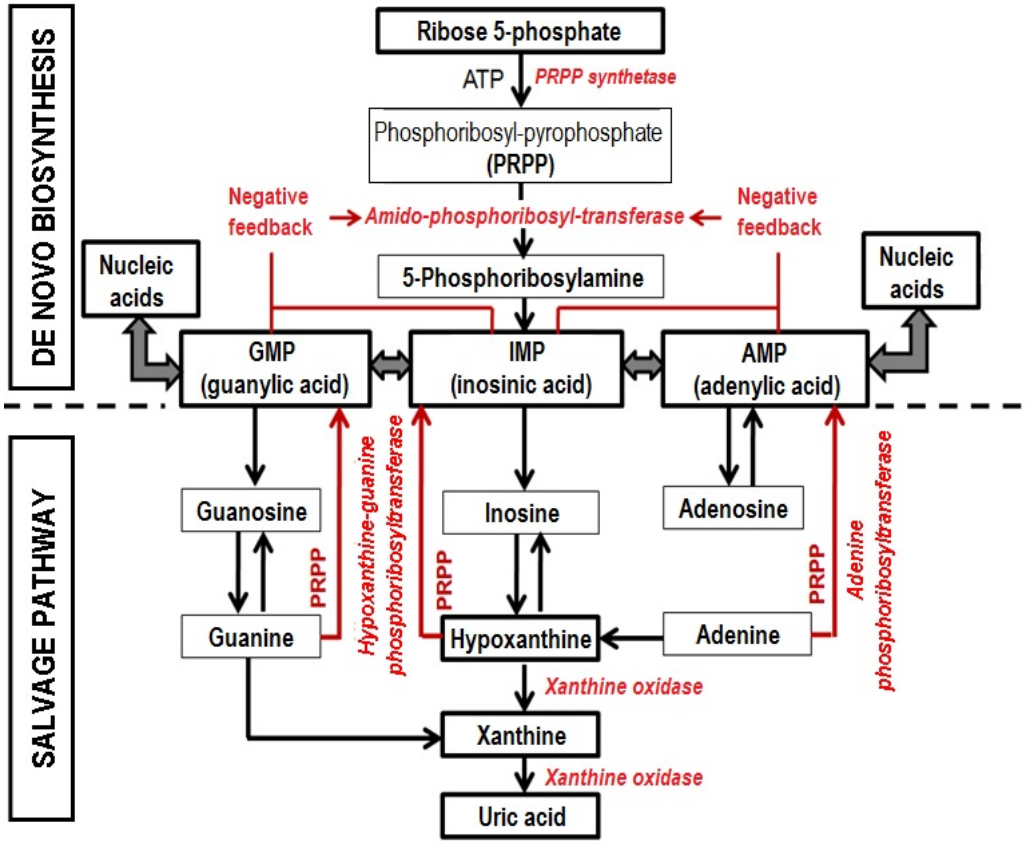

Figure 3. *De novo* biosynthesis and the "salvage pathway" of purine bases.

- **90%** of the amount of purine bases is reused through the intervention of **2 enzymatic systems** and **PRPP**:
  - **adenine-phosphoribosyl-transferase** with the "salvage" of adenosine

Adenine + PRPP → AMP + Ppi

- **hypoxanthine-guanine-phosphoribosyl-transferase** with the "salvage" of hypoxanthine and guanine

Hypoxanthine + PRPP → IMP + PPi
Guanine + PRPP → GMP + PPi

- **10%** of the amount of purine bases is converted into **xanthine** and, subsequently, into **uric acid**, by ***xanthine oxidase***.

HYPERURICEMIA

DEFINITION: increased plasma uric acid level **> 7 mg/dL**, a value from which the formation of monosodium urate crystals can occur in the peripheral joints (deposition being favored by the decrease in temperature and pH in the extremities).

Observation!
Hyperuricemia is most frequently asymptomatic, but the presence of arthrosis at the joint level favors the occurrence of gout attacks.

Etiopathogenic CLASSIFICATION:

1. **RENAL hyperuricemia** – caused by the **decrease in uric acid EXCRETION** (90% of cases)
2. **METABOLIC Hyperuricemia** – caused by the **excessive increase in endogenous uric acid production** determined by:
   a) genetic enzymatic defects: **primary hyperuricemia** (less than 1% of cases)
   b) conditions associated with increased purine metabolism: **secondary hyperuricemia** (approx. 10% of cases)

- **Consequences**: uric acid and its salts, mainly monosodium urate, are relatively insoluble molecules in aqueous solutions, e.g. urine and synovial fluid, and are deposited in the kidney and joint synovium, the deposition being favored by acidic pH (uric acid is more soluble in alkaline than in acidic environments).

1. RENAL Hyperuricemia

- **Definition**: hyperuricemia determined by **decreased uric acid excretion**
  The mechanisms of urinary excretion of uric acid involve:
  - glomerular filtration and total reabsorption (100%) at the level of the proximal convoluted tubule
  - active tubular secretion (50%) at the level of the distal convoluted tubule (process dependent on the plasma level of uric acid) and presecretory reabsorption (40-45%), so that normally about 5-10% of the glomerularly filtered quantity will be eliminated
- **Pathogenesis**:
  - decreased glomerular filtration rate (decreased GFR)
  - increased tubular reabsorption
  - decreased tubular secretion
- **Causes**: causes and mechanisms of renal hyperuricemia – Tab. 43.

Table 43. Etiopathogenesis of renal hyperuricemia.

Causes	Pathogenic mechanism
▪ **Chronic kidney disease**	– decreased GFR (but clinically manifest gout is rare)
▪ **Volume depletion** in: – excessive diuretic treatment – adrenal cortex failure – diabetes insipidus – vomiting/diarrhea	– decreased GFR + increased tubular reabsorption
▪ **Metabolic** acidosis from ketoacidosis – type 1 diabetes ▪ **Alcohol consumption, intense physical exertion, starvation** – lactic acidosis ▪ **Aspirin chronic treatment / overdose** – metabolization to salicylic acid	– decreased tubular secretion through competition between uric acid and organic acids (keto acids, lactic acid, salicylic acid) for transport proteins at the renal tubular level

2. METABOLIC Hyperuricemia

- **Definition**: hyperuricemia caused by **excessive uric acid production**
- **Pathogenesis**:

a) Enzymatic genetic defects, responsible for **primary hyperuricemia** mediated by:

1. **Increased *de novo* synthesis of purine nucleotides**
   - increased concentration of PRPP (phosphoribosyl pyrophosphate) in conditions of an X-linked genetic defect characterized by increased ***phosphoribosyl synthetase*** activity
   - decreased GMP and AMP with increased ***phosphoribosyl transferase*** activity (negative feed-back mechanism)
2. **Decreased reuse of purine bases on the "salvage pathway"**
   - **hypoxanthine-guanine-phosphoribosyl transferase** deficiency ⇒ X-linked genetic defect called **Lesch-Nyhan syndrome** characterized by mental retardation, self-mutilation, nephrolithiasis

b) Increased turnover of purines, responsible for **secondary hyperuricemia** from:

- lymphoproliferative diseases: leukemias, lymphomas
- myeloproliferative diseases: polycythemia vera
- carcinomas: post-chemotherapy or post-radiotherapy tumor lysis syndrome
- hemolytic anemias (with erythropoiesis stimulation)

GOUT

- **Definition**: **inflammatory arthritis** induced by **hyperuricemia** (regardless of cause), more common in men
- **Causes**: decreased excretion (90%) or increased production (10%) of uric acid
- **CLINICAL Forms:**

a) ACUTE MONOARTICULAR inflammatory arthritis (gouty arthritis) characterized by:
- accumulation of monosodium urate crystals in the synovium and joint cartilage causes **acute joint and periarticular inflammation (gout attack)**
- the **most frequent location** (75%) is at the **metatarsophalangeal joint** of the **hallux** ⇒ reduced blood flow to the extremities and increased local mechanical pressure causes a decrease in local pH which favors the deposition of urates
- other locations (25%): at the tarsal joints, ankle, knees, elbows, fingers, etc.

b) CHRONIC GOUT characterized by:
- gout attacks overlap against the background of a persistent chronic inflammation ("low-grade"), with the appearance of **joint lesions**

c) CHRONIC POLYARTICULAR TOPHACEOUS GOUT characterized by:
- large masses of monosodium urate crystals deposited atthe level of: i) *joints* with the appearance of *structural lesions*: *bone destruction and joint deformation* and ii) *soft periarticular tissues*, at the level of fingers, ears, Achilles tendon, in the form of whitish-yellowish deposits that can ulcerate
- gout attacks are more frequent and prolonged, polyarticular, joint pain becomes chronic, determining the limitation of activity

- **Pathogenesis of the GOUT ATTACK:**
  - **Triggering factors**
    - diet rich in purines: red meat, organs, shellfish ⇒ increases uric acid production
    - increased alcohol consumption (red wine, beer and soft drinks with high fructose content) ⇒ renal elimination of uric acid decreases (tubular secretion decreases)
    - dehydration (e.g., diuretic medication) ⇒ renal elimination of uric acid decreases (GFR decreases + tubular reabsorption increases)
  - **Sequence of the PATHOGENIC MECHANISM**:
    1. Increased uric acid concentration favors urate deposition in the articular synovium ⇒ **gouty micro-tophi**
    2. Urate crystals are phagocytosed by local phagocytes and cause rupture of lysosomal membranes (phagocytosed urate crystals cannot be digested) ⇒ release of **lysosomal proteases** into the synovial fluid with local destruction
    3. Phagocytes release **proinflammatory cytokines** (IL-1b, TNFα)
    4. Activation of the complement system ⇒ **acute local inflammation**

- **CLINICAL Manifestations:**
  - painful attacks usually begin at night (after exposure to a triggering factor) and have a duration of between 7 days to several weeks
  - local inflammatory signs: pain, redness, swelling, increased local temperature, lymphangitis
  - systemic inflammatory signs: leukocytosis, fever, increased ESR

Observation!
- In the initial stages of the disease, after the initial gout attack has subsided, the person becomes asymptomatic and there are no changes in the joints = ***intercritical gout*** stage
- After the first gout attack, **months** or **years** may pass before a new attack appears
- The increase in the frequency of attacks causes **permanent** joint changes

Other manifestations of HYPERURICEMIA

1. ASYMPTOMATIC hyperuricemia: sometimes lifelong

2. Uric Acid NEPHROLITHIASIS

- **Characteristics**:
  - clinical form of **renal lithiasis** caused by hyperuricemia and favored by dehydration
  - concentrated urine (reduced fluid intake) + low pH (protein-rich diet) causes **urate precipitation** at the tubular level
    - the size of the calculi varies from the appearance of grains of sand to coral-like stones
    - the content of the calculi: monosodium urate ± calcium oxalate, calcium phosphate

3. Uric Acid NEPHROPATHY

- **Characteristics**:
  - clinical form of **acute tubular injury of postrenal origin** usually associated with secondary hyperuricemia from leukemias and lymphomas
  - renal tubular obstruction causes increased tubular pressure with a significant decrease in renal blood flow and GFR

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