4. PATHOPHYSIOLOGY OF THE BLOOD

Cells of the body communicate with the external world via various extracellular (EC) spaces, which constitute the external environment for cells. The main EC spaces include interstitial fluid (IF), plasma, lymph, cerebrospinal fluid (CSF), and the aqueous humor. These are in a dynamic balance with the intracellular (IC) spaces of different composition.

Supply of oxygen, nutrients, and basic materials required for specific functions of cells, transport of humoral factors influencing cell functions, as well as, clearance of the waste materials all occur via the EC space. The dynamic and effective interaction among the EC spaces, which preserves the composition of the interstitium in different tissues and organs and the specific transportation of certain substances, are ensured by the blood. Corpuscles of the blood take part mainly in this transport (e.g., red blood cell hemoglobin /Hb/ is important in transportation of oxygen), however, the corpuscles also have other functions (e.g., immunofunctions, clotting, etc). Proteins of the plasma also participate in transport processes, yet they are also important in the regulation of the oncotic pressure and pH. Other plasma components also participate in transportation processes to or from the tissues, while the coagulation system serves the protection of blood volume and the restitution of vessels.

Table 4.1.

Most important anorganic and organic components of the plasma

Anorganic components:	
Na^+	135-145 mmol/l
K^+	3.5-5.0 mmol/l
Ca^{++}	2.3-2.7 mmol/l
Mg^{++}	0.8-1.3 mmol/l
Cl^-	100-106 mmol/l
HCO_3^-	24-28 mmol/l
PO_4^-	1.0-1.5 mmol/l (adult)
Fe	7-27 mol/l, ill. 115 ± 50 µg/dl
Organic components:	
protein (total)	60-84 g/l
albumin	35-50 g/l
globulin	23-35 g/l
glucose (fasting)	3.9-6.0 mmol/l
lipid (total)	<1.7 mmol/l
cholesterol (total)	4.7-5.2 mmol/l
LDL- cholesterol	<3.4 mmol/l
HDL- cholesterol	>0.9-1.1 mmol/l
rest nitrogen	15-30 mmol/l
urea (CN)	2.9-8.9 mmol/l
creatinine	60-140 mol/l
bilirubin	<17 mol/l
hemoglobin (Hb)	140-160 g/l
organic acids, ketone bodies	
vitamins, enzymes, hormones	

4.1. BASIC CHARACTERISTICS OF BLOOD

4.1.1. PLASMA COMPONENTS, CHARACTERISTICS, ABNORMALITIES

The most important organic and inorganic components of plasma are shown in Table 4.1.

Osmotic concentration: 280-300 mOsm/kg (or mOsm/l).

Osmolality refers to the concentration of osmotically active substances in 1 kg solvent, while osmolarity expresses this concentration in 1 liter solvent. In the case of water, the volume of which greatly depends upon temperature, the use of osmolality is preferred.

Osmolality is determined primarily by Na^+ concentration: the osmotic concentration is decreased in hyponatremia and increased in hypernatremia. Its increase (but not decrease) can also occur independently of Na^+, e.g., due to glucose in diabetes or urea in uremia. With regards to osmotic pressure, the rest of the ingredients are less important [calculated osmotic pressure: ($2xNa^+$ + glucose + urea)]. ***Osmotic gap:*** the difference between the measured and calculated values, normally approx. 10-12 mOsm/l; its increase implies the presence of non-measured, osmotically active materials (ketone bodies, lactate, ethylene glycol, etc.). Colloid osmotic (oncotic) pressure is a fraction of osmotic pressure

originating from proteins (primarily from albumin) and other macromolecules. It is significant in determining the Starling forces in the capillaries, since the water-binding, osmotic effect of the macromolecules that cannot penetrate the capillaries will counteract the outward-pushing forces of the hydrostatic pressure, thus it will retain part of the plasma water within the capillaries. Its decrease (hypoalbuminemia) leads to edema formation.

Plasma pH: 7.36-7.42. If decreased: academia; if increased: alkalemia (acidosis and alkalosis refer to compensated forms, but are often used in manifest pH disorders, too).

Cell sedimentation: The albumin/globulin (A/G) ratio is normally between 1.5 and 2.0; decreased A/G ratio can occur in loss of albumin, or in relative excess of globulins (as seen in inflammation due to the increased fibrinogen and alpha-globulin fractions, occasionally in pathological globulin production). These will increase blood cell sedimentation (Westergreen, sedimentation rate) above the normal value of 3-10 mm/hours. Following loss of blood, the faster synthesis and replacement of albumin will result in an increased A/G ratio.

Plasma proteins:

Fibrinogen: Huge molecule with high viscosity, it plays a determinant role in clotting (clotting factor 1.)

Albumin: Largest protein fraction, it is important for the maintenance of colloid osmotic pressure, a source of tissue amino acid supply, and numerous transport proteins belong to this group (substrates compete partly for albumin binding sites). Albumin plays an important buffering role in the regulation of EC pH.

Globulins: Glycoproteins belong primarily to the alpha-globulin fraction which have important transport functions. The lipoproteins are members of the beta-globulin transport proteins. Mainly immune globulins (Ig-s) belong to the gamma globulin group.

Hypoproteinemia: Primarily the number of small-sized proteins can diminish significantly, which will result in decreased colloid osmotic pressure, defect in amino acid supply, insufficient transport functions, and, with a sequel of generalized hypoproteinemia (ch. 9.1.). Most frequent causes: insufficient protein intake/absorption, decreased production of plasma proteins (hepatic diseases), conditions with protein loss (burns, patients with renal diseases, nephrotic syndrome, albuminuria), increased protein catabolism (diabetes, steroid treatment, fasting). Lack of proteins with certain specific functions results in specific consequences even without general hypoproteinemia (e.g., bleedings in lack of coagulation factors, protein-deficiency anemia).

Hyperproteinemia: Relative hyperproteinemia is seen in dehydration. In the case of hyperproteinemias, accumulation of certain protein fractions occurs usually pathologically: e.g., in multiple myeloma, macroglobulinemia, lymphogranulomatosis, etc.

Dysproteinemia: The appearance of abnormal proteins, such as Bence-Jones paraproteins in plasmocytoma (multiple myeloma), alpha-fetoprotein (AFP) in liver and other tumors, and in pregnancies afflicted with genetic malformations. A change in the *level* of some proteins can be diagnostic: e.g., ceruloplasmin, elevated C-reactive protein (CRP) in inflammations and acute phase reactions (APR), increased lactoferrin and decreased transferrin in inflammations.

4.1.2. CORPUSCULAR COMPONENTS OF BLOOD AND THEIR CHARACTERISTICS

The blood consists of 45% cellular mass (hematocrit – Htc)

Red blood cell (RBC): 4-5 million/µl = 4-5 T/l (T = tera = 10^{12})

Reticulocyte: 1-2% of RBCs (immature, RNA residue-containing RBC, stainable with brilliant-cresyl-blue, following 24-48 h transforms to mature RBC in the circulation; increased ratio implies a higher RBC production)

White blood cell (WBC, leukocyte) (WBC-count = WCC) 4 000-11000/µl = 4-11 G/l (G = giga = 10^9)

Neutrophil granulocyte ~65%
Lymphocyte 30-35%
Eosinophil granulocyte 1-2%
Basophil granulocyte 0.5-1%
Monocyte 2-8%

Platelet 150-400 thousand/µl = 150-400 G/l (G = giga = 10^9)

4.2. PATHOPHYSIOLOGY OF THE RED BLOOD CELL SYSTEM

Origin of hematopoiesis

Red blood cells (RBCs) in fetal life are first produced in the yolk sac, then in the spleen, and later in the liver. In a mature newborn the hematopoiesis takes place in the active red bone marrow. Initially all bones produce RBCs, later red bone marrow is relocated only to

the flat and trabecular bones (hip, sternum, ribs, skull and vertebral bodies) and in the epiphyses of the long bones. Hence, in adults, bone marrow samples are usually collected from the sternum or from the crista iliaca. If needed (e.g., severe, prolonged hemolysis), red bone marrow can replace yellow bone marrow, moreover, extramedullary hematopoiesis can also occur. In bone marrow transplantation, hematopoietic stem cells can also adhere in the liver or in the spleen.

Control of RBC production

The proliferation of early progenitor cells, e.g., multipotent hemopoietic stem cells is stimulated by cytokines such as IL-3 and IL-6. The commitment and cell division to myeloid and megakaryocyte direction is promoted by granulocyte-macrophage colony stimulating factor (GM-CSF) and thrombopoietin (Tpo).

The most important regulator of RBC production is **erythropoietin**. This protein is mainly produced due to *hypoxia* in the peritubular interstitial cells of the kidney (90%), and to a smaller extent in the Kupffer cells of the liver. (The remarkable blood supply of the kidney serves mainly for maintaining its excretory function, and highly exceeds the oxygen demand of its own tissues. In normal conditions, kidneys have the lowest ΔavO_2 among our organs. Therefore, tissue hypoxia develops in kidneys only if the arterial blood contains insufficient amount of oxygen /hypoxemia, anemia/ a priori, or if circulatory shock develops.) Erythropoietin synthesis is enhanced by *androgens* (hence the RBC counts and Hb levels of males are higher), *cortisol*, thyroxine, and growth hormone.

There is a need for different substrates and vitamins in hematopoiesis: *protein* (globin synthesis), *Fe* (hem synthesis), *folic acid* and *vitamin* B_{12} (proliferation), vitamins $B_{1,2,6}$ (hem synthesis), and vitamin C (Fe-absorption).

The lifespan of the RBCs in the circulation is approximately 120 days. Old RBCs are eliminated by the spleen (or liver), and in the cells of the reticuloendothelial system (RES) albumin-bound bilirubin, which is produced from Hb, is transferred to the liver as indirect bilirubin, while Fe is almost fully recycled.

Structural characteristics of the RBCs

RBCs are biconcave (doughnut shaped, in cross section ladyfinger-form) discs without nucleus and other organelles. If all RBCs had nuclei and other cellular organelles, then each RBC could transport less Hb, and the higher RBC count would increase the viscosity of blood, which, to a huge extent, would increase the workload on the heart. Structural characteristics include the following:

- The diameter is 5-7 μm, and shows a normal distribution. A widening of the Gaussian curve, a.k.a. RDW (RBC distribution width), which depicts the distribution of RBCs' diameters, refers to

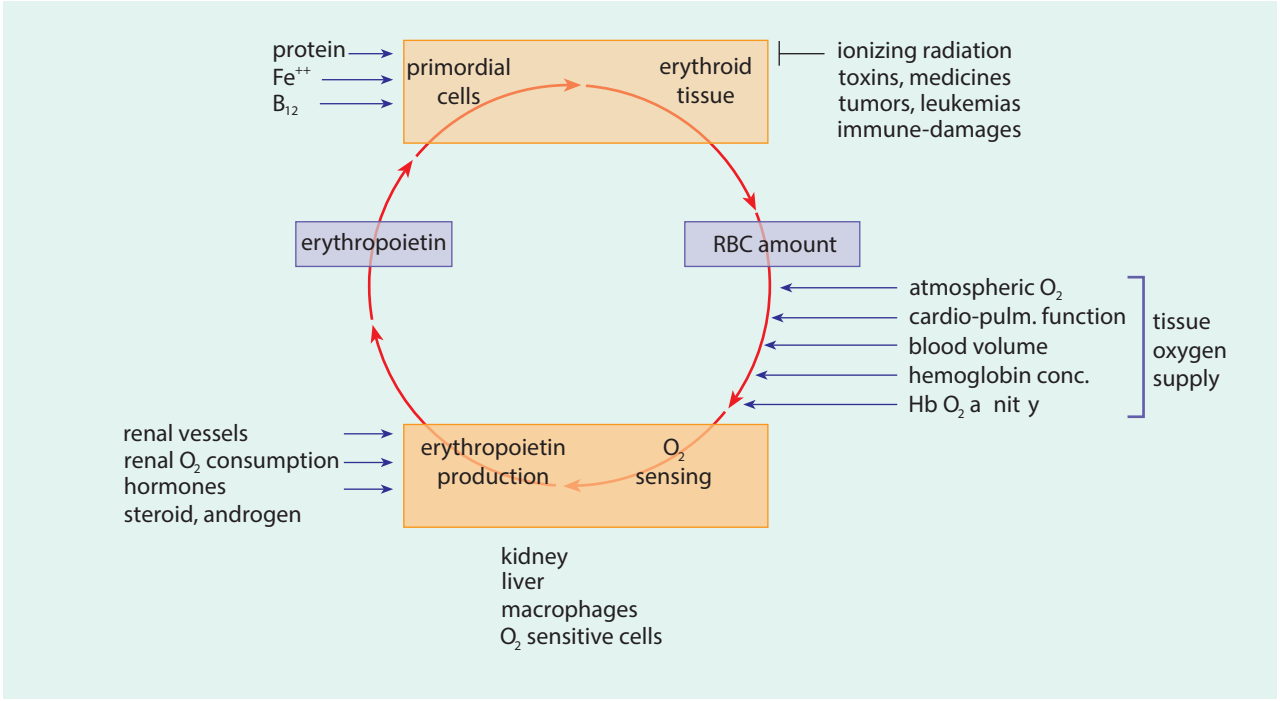

Fig. 4.1.: Control and disorders of hematopoiesis.

anisocytosis. If the shape of the RBCs differs significantly, it refers to *poikilocytosis*.
- Thickness is 1.7 μm on average.
- Mean cell volume (MCV): 90-95 fl (femto = 10^{-15})
- Surface: 130-140 μm^2
- Mean cell hemoglobin (MCH): 30 pg (pico = 10^{-12})
- Mean cell hemoglobin concentration (MCHC): 330g/l.

RBCs possess three important structural units:
1. special, high-plasticity membrane;
2. enzyme systems of glycolysis and direct glucose oxidation;
3. Hb.

The phospholipid bilayer of the RBC ***membrane*** is interconnected by a strong, yet flexible, two-dimensional protein network. Within this network the *spectrin* tetramers are connected by *actin* and *protein 4.1* units. This network is fixed to the membrane by *spectrin-ankyrin* bonds. *Band-3 protein* functions as an ion-channel (HCO_3^-/Cl^-). The special membrane of the RBCs enables them to pass through the capillaries with diameters smaller than their own, and more than 1000 times/day for approximately 120 days. While passing through the capillaries, the RBCs are significantly deformed, they take a parachute-like shape (see Fig. 4.2.), but in the postcapillary vein they regain their original shape. If the membrane is structurally damaged, then RBCs will be progressively injured over time, their lifespan shortens, may hemolyze within the vascular lumen, and will be eliminated in the spleen.

RBCs bear two important ***enzyme systems***: *Glycolysis* produces 2 moles of ATP by metabolizing 1 mol of glucose, which covers the energy demand of the RBCs. *2,3-diphosphoglycerol* (2,3-DPG), a by-product of glycolysis has an important role in the RBCs: if required, it can shift the Hb oxygen saturation curve to the right, thereby improving tissue oxygenation. *Direct oxidation of the glucose* (also known as pentose-phosphate path) serves regeneration of the antioxidant glutathione by producing $NADPH_2$ (this is important due to the high oxygen content in the RBC).

The most important component of RBCs is ***hemoglobin***. The molecular weight of HbA, consisting of 2α and 2β subunits, is about 64 kD, and it can bind 4 O_2 molecules. Characteristics of the Hb oxygen dissociation curve and the regulation of oxygen transport are described in chapter 3.4.1.

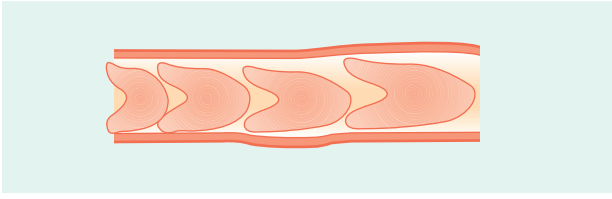

Fig 4.2.: Parachute-like deformation of RBCs in the circulation. Direction of flow: → It is easy to understand that even minimal decrease in plasticity of the membrane or larger RBC size will lead to fragility of the RBCs. Rigid RBCs can cause occlusions in the microcirculation with multiorgan hypoxia/ischemia.

4.2.1. ANEMIAS

4.2.1.1. DEFINITION OF ANEMIA

Despite of its word-by-word translation, anemia does not infer a lack of blood, but the decreased RBC count, thus, reduced hematocrit in the blood. The circulating blood is poor in RBCs, and the hematocrit is low. The extent of decrease in the number of the cells may be variable, which can be explained by the fact that the Hb content does not proportionally change with the cell count. The Hb content is a more important parameter physiologically, since this determines the main function of RBCs, namely the oxygen transport. A disproportional decrease between the cell count and Hb content can originate from the different size of RBCs in anemia together with the relatively constant cytoplasmic Hb density, even in pathological conditions, which can result in highly variable Hb content in the cells. Large RBCs contain more, while the smaller ones contain less Hb (macrocytic hyperchromic vs. microcytic hypochromic anemias). In the former case the RBC count can decrease extremely, e.g., in pernicious anemia it can be as low as 1 T/l, instead of the normal 5 T/l. A similar fall in the number of RBCs is not compatible with survival in microcytic hypochromic anemia, i.e. the common symptoms of iron deficiency at 3 T/l RBC count are almost equal to those of pernicious anemia with 1-1.5T/l RBC count. When the shape of RBC is changed (e.g., in spherocytosis = spherical RBCs instead of biconcave discs), the volume and Hb content of the small RBCs can be high.

4.2.1.2. GENERAL CONSEQUENCES OF ANEMIA

There are specific clinical signs of various forms of anemia, depending on their cause, however, there are general consequences as well, independently from the origin of anemia (Fig. 4.3.). Regardless from the cause of the

anemia, the low Hb content is characteristic in all forms (chapter 3.4.2.). According to the WHO classification, anemia is diagnosed if Hb concentration is under 130 g/l in males, and 120 g/l in females. When respiration is intact, alveolar gas composition is normal, and the alveolar diffusion is not compromised, the oxygen tension in the arterial plasma will be normal. Hb will be normally, practically 100% saturated with oxygen. *However, due to the low Hb content, the arterial O_2 content will be lower than normal.* In the case of intact capillaries and normal tissue oxygen tension (40 mmHg), the oxygen release of the Hb can be normal in %, but highly decreased in absolute measure. Normal oxygen release from Hb would be possible only if the tissue/capillary oxygen tension was significantly decreased (see also Fig. 3.18.). This would impair the oxygen uptake of the cells, therefore increased tissue perfusion would be needed, at least, as a partial form of compensation.

The circulation is increased in anemia (the resting cardiac output is high, chapter 2.1.4.5.). The primary cause of circulatory changes is generalized tissue vasodilation caused by tissue hypoxia. The initial decrease in blood pressure will lead to sympathetic activation via blood pressure regulating reflexes, which will result in elevation of heart rate (yet the peripheral vasodilation remains) and facilitate the increased venous return. The sympathetic tone would also increase the ventricle contractility, however, this latter compensatory step cannot be maintained due to the decreased oxygen content in the blood and myocardial hypoxia. The increased heart rate can temporarily increase contractility, but shortening of the diastolic time deteriorates the coronary circulation and myocardial oxygen supply. Circulatory redistribution can be observed, with relatively diminished renal, splanchnic, muscle, and skin perfusion and increased or maintained myocardial and cerebral supply. This increased perfusion is made possible by the decreased blood viscosity (decreased hematocrit) (Fig. 4.6.). In the case of similar tissue hypoxia, polyglobulia would limit the increase in cardiac output.

In ideal cases the heart can meet the significantly increased demand. In such cases, only the consequences of the compensatory mechanisms to maintain the permanently high cardiac output will appear, e.g. susceptibility for dyspnea triggered by increased preload and central venous congestion (see ventricular end-diastolic pressure, Starling mechanism) and susceptibility for hepatosplenomegaly and cardiogenic edema (anasarca) due to right ventricular involvement, however, signs of tissue hypoperfusion will also be notable, since the oxygen supply is not ideal even among the well-perfused organs.

If the increase in cardiac output does not meet the demands (although resting cardiac output is still higher than the normal 5 l/min), symptoms of high output cardiac failure will occur (approx. at 50% of the normal Hb level). The existing congestive and generalized hypoxic symptoms can grow increasingly worse, and the real signs of forward heart failure can occur: relatively hypoperfused organ disorders due to the redistribution of circulation. Tissue hypoxia also means decreased oxidative phosphorylation (ATP decreases), malfunction of the Na^+/K^+ pump, and possibly disorder of the neurotransmitter synthesis in the central nervous system. Although cerebral perfusion has the utmost priority and will be maintained even in this case, cerebral hypoxia will be inevitable due to the low oxygen content of the blood, resulting in a headache, somnolence, restlessness, inability to learn and concentrate. The generalized tissue hypoxia explains symptoms like generalized muscle weakness, endocrine dysfunctions, gastrointestinal motility and digestive disorders, moderately impaired urine concentration and dilution (hyposthenuria), polyuria during day and night, cold sensitivity, while myocardial hypoxia causes decreased ventricular contractility and angina pectoris in severe cases.

The higher cardiac output does not mean proportionally higher perfusion in all organs: in the skin and mucosa, for example, perfusion will rather decrease (paleness of conjunctiva), occasionally perfusion is not sufficient also in the kidney (tubular hypoxia, hyposthenuria) and in the splanchnic region (non-obstructive mesenteric ischemia, malabsorption, decreased motility, and anorexia caused by liver hypoperfusion). The most obvious is that muscle perfusion does not increase sufficiently during exercise, thus the physical performance will be reduced.

Partly due to cardiogenic dyspnea, and partly due to hypoxia, ventilation will be increased - in reality this means alveolar hyperventilation that will not improve oxygen supply (since oxygen saturation of Hb is maximal even without hyperventilation), but will result in increased carbon dioxide washout (chapter 3.1.2.4.2). Many signs can be explained by hypocapnia, which indicate disorders of cerebral circulation due to hypocapnia-induced vasoconstriction (dizziness, ear tingling / tinnitus/, vision disorders, tendency to faint, etc.), which develop in addition to the anemic cerebral hypoxic dysfunctions.

Since the heart must provide a cardiac output that is equivalent to mild-moderate physical activity throughout the entire day, anemia (and its oxygen supply is also deranged, since one unit of blood contain less oxygen,

tachycardia increases the oxygen demand, while diastolic time is decreased), after a while the contractile function will deteriorate and the symptoms of classic congestive heart failure will develop.

In chronic, gradually developing anemia the tachycardia, which is characteristic in acute cases, will occur only upon exertion (barely in rest), and eventually myocardial hypertrophy, chronic oxygen deficiency, and overload-induced dilative cardiomyopathy will develop.

Whether in acute, or in chronic anemia, a dynamic, disproportionally high increase in frequency upon exertion will be observed (causing "pounding heartbeat", or increased palpitation, for the patient), since peripheral muscle perfusion must be excessively increased to the same extent of overall increase in oxygen consumption.

In summary, the general symptoms of anemia are originating partly from decreased oxygen tension in the tissues and partly from the pathological compensatory mechanism to maintain lastingly high cardiac output.

4.2.1.3. CLASSIFICATION OF ANEMIAS

1. Based on RBC size and mean corpuscular Hb concentration:
   - normocytic, normochromic (e.g., acute blood loss, hemolysis, erythropoietin-deficiency, aplastic anemia)
   - microcytic, hypochromic (e.g., iron deficiency, vitamin C deficiency, chronic diseases, thalassemia)
   - macrocytic, hyperchromic (pernicious type of anemia)
2. Based on way of development, anemias are likely due to the following:
   - insufficient production
   - increased loss (decreased lifespan)

INSUFFICIENT RBC PRODUCTION (see Fig. 4.1.)
The RBC production can essentially prove insufficient for three primary reasons:

1. lack of substrates or other factors (vitamins) in support of production;
2. diseases linked to the location of production (bone marrow);
3. disorders associated with the control of production.

IRON DEFICIENCY ANEMIA

In addition to *hemoglobin*, iron (especially Fe^{++}) is an important part of *myoglobin*, *cytochromes* and several enzymes e.g., *catalase*, *Pro-*, *Lys-hydroxylase*.

There is an estimated loss of nearly 1 mg of iron loss per day associated with desquamating skin and gastrointestinal cells. Bleeding during the menstrual cycle increases the iron loss among women of conceptive age, thus their iron need is higher, nearly 3 mg/day. Iron can be absorbed from the small intestines only in Fe^{++} (ferrous) form. In plants, most of the iron exists in ferric / Fe^{+++}/ form, and its conversion to ferrous iron is influenced by *gastric juice* and *vitamin C*. Nearly 10% of the iron originating from plants and dairy products is *absorbed*, in contrast to the absorption rate of hem-iron, originating from animals (black pudding, liver, meat), which can reach 25%. Considering absorption, *the recommended daily intake of iron is at least 10-30 mg, for children, and in pregnant or lactating women it is 40 mg.*

Absorption for Fe in the small intestine is precisely regulated. HFE gene (chromosome 6.), which is responsible for the development of hemochromatosis, encodes the "Iron Regulatory Protein" (IRP) which binds to the adequate "Iron Responsive Element" mRNA and stabilizes it, thereby facilitating the transcription of "Natural Resistance Associated Macrophage Protein" (NRAMP-2), also referred to as "Divalent Cation Transporter" (DCT-1), which transports Fe^{++} (ferrous iron) into the villus cells. IRP also facilitates production of the iron-transporting transferrin with a similar mechanism. However, the same protein via binding to the mRNA (reducing its activity) inhibits the translation of iron-storing ferritin.

Following absorption, in the blood the Fe^{+++} (transport form) is transported by transferrin, to different tissues (e.g., myoglobin production) and to the bone marrow, where it is used to produce hem, or it is stored as ferritin (in apoferritin bond). Iron, originating from the dying RBCs is also bound to ferritin in the RES, then it is mobilized into the plasma with transferrin, or the persistently stored ferritin is sequestered to hemosiderin with other metabolic by-products in the lysosomes, and becomes increasingly more difficult to access. If the amount of stored iron decreases, the average 10% absorption rate in the gastrointestinal system increases up to 16%.

In consideration of the requirement for Fe, intestinal cells absorb not only iron intensely, but other, high molecular weight metals as well, e.g., lead. This may result in complications, especially in large metroplexes in which lead-contamination of the air is considerably high. Chronic lead poisoning can cause nausea, vomiting, abdominal spasms, anemia, tachycardia, high blood pressure, acute tubular nephropathy, toxic encephalopathy, and confusion.

Frequent causes of iron deficiency:

- *insufficient intake*, e.g., monotonous diet among the elderly, strict vegetarianism, anorexia nervosa
- *inadequate absorption*, e.g., lack of gastric juice or vitamin C, diarrhea, malabsorptions (e.g., gluten enteropathy), plant-derived phytates and phosphate may bind iron in the gut, and in Crohn's disease the inflammation of the terminal ileum may impair the absorption of iron.
- *increased demand*: e.g., rapid growth in infants, children, and teenagers (boys can also suffer from iron deficiency in periods of rapid growth, especially due to increased muscle [myoglobin] development), pregnancy, lactation, in case of uremic or other types of hemolysis, and after treatment of pernicious anemia.
- *increased loss* due to regularly heavy periods or concealed gastrointestinal bleedings (e.g., in colon tumor the daily blood loss can be up to 10-20 ml).
- *in chronic inflammatory diseases* in which the production and effect of three types of acute phase proteins decreases the iron content of the blood. This phenomenon is part of the natural defense mechanisms, since bacteria, especially in a warmer environment (febrile host), have an increased need for iron to multiply. It is therefore advantageous for the host organism to decrease its iron levels. **1)** the 25 amino acid-containing **hepcidin,** which is produced by liver cells in inflammation (due to IL-6) and in case of high plasma iron levels, inhibits the iron release from macrophages and the ferroportin-dependent iron release of intestinal mucosa cells (decreasing thereby the iron absorption in the gut and its availability for the body). The production of hepcidin is facilitated mainly by high saturation of transferrin (via transferrin 2 receptors). **2) lactoferrin** released from granules of activated (IL-1 effect) neutrophil granulocytes is also able to bind iron. Complexes produced this way are taken up by macrophages and hepatocytes (but never released). **3)** In anemia associated with chronic inflammation, **ferritin,** an acute phase protein, can also play a role by binding iron, however, as a result, immature RBCs without proper receptors cannot take up and utilize the iron.

Iron deficiency is the most frequent of all deficiencies in the world; even throughout the developed countries its incidence is high (8%, even higher in big cities), while in developing countries it can be as high as 50%.

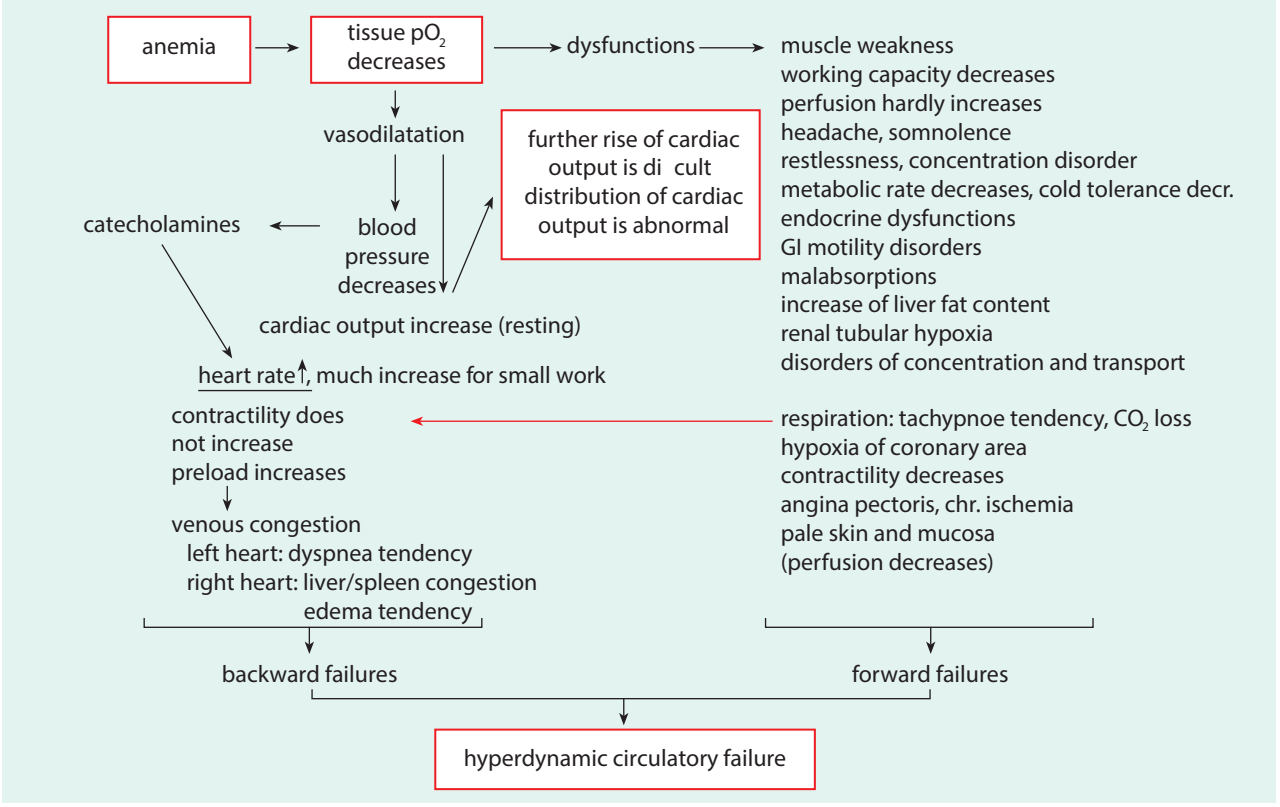

Fig. 4.3.: General sequel of anemia.

Consequences of iron deficiency:
At first, iron binding capacity only minutely increases (>360 µg/dl) along with a decrease in serum ferritin level (<20 ng/ml). *In moderately severe iron deficiency, serum iron levels will be lower than 7 µmol/l, or 50 µg/dl* along with higher Fe-binding capacity and with an additional decrease in the level of serum ferritin, the ratio of medullary sideroblasts decreases below 10%, the protoporphyrin content of the RBCs decreases. The characteristic hematological disorder is *microcytic hypochromic anemia* (MCV 70-90 fl, „target cells" in the smears with anisopoikylocytosis) which develops only in a *long-term, severe iron deficiency*.

Stomatitis angularis, glossitis (inflammation of the tongue), cheilitis (inflammation of the lips), dysphagia (difficulty in swallowing), hour-glass nails (concave, backward-bending nails) are characteristic for general iron deficiency.

Iron is essentially needed regarding several physiological functions, including cell division, since it is a component of certain enzymes, e.g., ribonucleotide reductase. Theoretically, the impairment of cell division results in inflammatory, degenerative lesions of the gastrointestinal mucosa which contain rapidly multiplying cells causing dysphagia and painful inflammation of tongue. The pathological changes can affect the skin and its accessories, such as the nails, which may become thinner, weakened, thin, and while using the hands bent backward ("hour-glass nails").

In the case of maternal iron deficiency, *premature birth* and *low birth weight* are both frequent.

Treatment of iron deficiency:
Ferrous sulfate (300 mg/day) administered orally or by injection. It has uncomfortable gastrointestinal side effects, e.g., obstipation, abdominal pain, black stool. The treatment is usually prolonged, since the bone marrow is hypoplastic due to the decreased cell division due to iron deficiency. Several weeks are required until the RBC count starts to improve – low grade increase of reticulocyte cell count occurs only after days. The recommended duration of the treatment is a minimum of 6 months. The iron stores which were depleted previously, must be also filled.

Iron intoxication, overdose:
In the presence of ferrous iron, the small quantity of hydrogen peroxide, which is always present in drinking water in small quantities, forms free hydroxyl radicals. Similar reaction can also occur between ferrous iron and small doses of vitamin C. In activated neutrophil granulocytes under oxidative stress, free radical formation is further increased due to an increased activity of $NADPH_2$ oxidase (oxidative burst). Acute intoxication (200-300 mg/kg intake) is accompanied by vomiting, diarrhea, spasms, and possibly death. In the case of chronic overdose, hemosiderosis develops. Iron deposited in various tissues induces damages and fibrosis.

Hemochromatosis is an inherited disease with increased iron absorption and deposition, inducing fibrosis in the lungs, liver, pancreas, heart, joints, and the pituitary gland. It may be the cause of sexual dysfunctions (homozygote: 3-4/1000, heterozygote 1/10!).

Bantu syndrome: In South Africa, bantus store homemade beer in iron dishes. Due to the unusually

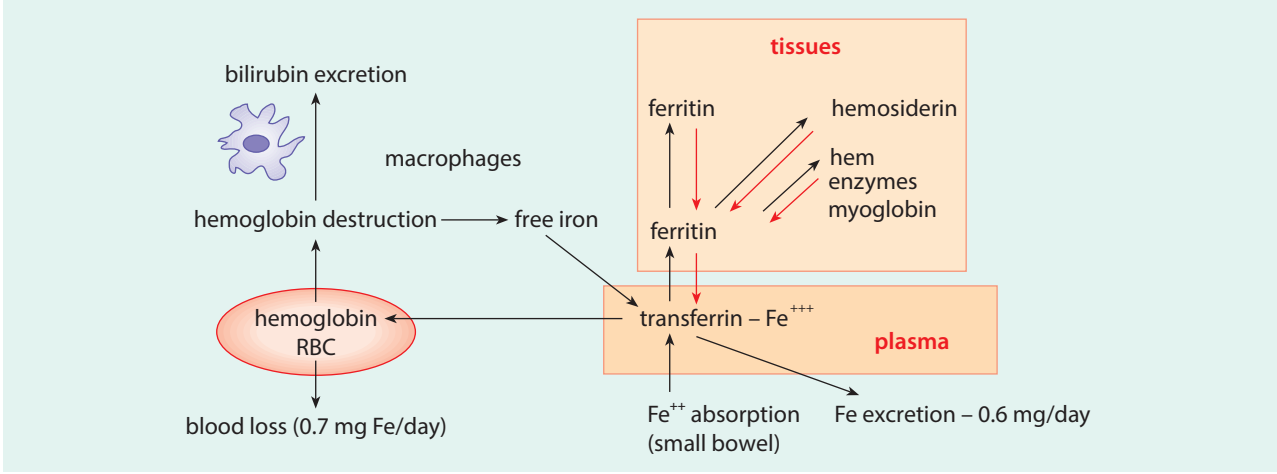

Fig. 4.4.: Transport and metabolism of iron.

high iron intake, *cirrhosis* and *liver cancer* are common in genetically susceptible persons (ferroportin, which is responsible for iron absorption, exhibits immense polymorphism).

ANEMIAS WITH MEGALOBLASTIC CHARACTER

A deficiency in folic acid or cyanocobalamine (vitamin B_{12}) causes symptoms via disturbed DNA synthesis, disorders in cell division, and inhibition of cell maturation, especially in the cells associated with high mitotic activity (hematopoietic organs and gastrointestinal cells).

FOLIC ACID carries single-carbon containing groups (methyl, formyl, methylene) in biochemical reactions, for example, methyl groups in the homocysteine-methionine transformation. It has an important role in *purine synthesis*, the recommended daily allowance (RDA) is 0.5-2 mg/day. In deficient states pernicious anemia develops. In pregnant women, an insufficient intake of folic acid can cause fetal *neural tube defects* (e.g., spina bifida with paraplegia, urinary and fecal incontinence).

Causes of folic acid deficiency

- *insufficient intake*: decreased intake and impaired metabolism in alcoholics
- *impaired absorption*: with chronic diarrhea, e.g., gluten enteropathy
- *increased demand*: first trimester of pregnancy (fetus), chronic hemolysis
- *folic acid antagonists*: e.g., methotrexate (antitumor, immunomodulant drug)

VITAMIN B_{12} is essential in the homocysteine–methionine conversion (tetrahydrofolate/THF/regeneration). In consideration of a lack of B_{12} "methyl-folate trap" develops, in which the active THF is depleted and *folic acid deficiency* occurs, and, in association to *pernicious anemia*, active regeneration of the gastrointestinal mucosa slows down, while levels of blood homocysteine increase, and *the risk* of *atherosclerosis* (enhanced free radical formation) and *thrombosis (insufficient protein C and S effect on activated V factor) rises.* This vitamin is required for the methyl-malonyl-CoA–succinyl-CoA conversion, which is a link between carbohydrate and fat metabolism.

A lack of B_{12} results in the accumulation of pathological (unpaired carbon chain) fatty acids with concomitant axonal demyelinization, and degeneration. Partially irreversible *peripheral* and central nervous system dysfunctions develop (e.g., subacute combined degeneration of spinal cord, funicular myelosis). Recommended daily allowance is 2-4 μg.

Causes of B_{12} deficiency

- *insufficient intake*: in strict vegetarians (animals take B_{12} of bacterial origin, humans ingest it with foods of animal origins, e.g., liver)
- *impaired absorption*: lack of glycoproteins produced by gastric parietal cells (chapter 7.3.3.), lack of R protein and intrinsic factor (Fig. 4.5.), e.g., in autoimmune mucous membrane atrophy (achylia gastrica – histamine-refractory achlorhydria), gastrectomy, disease of terminal ileum e.g., Crohn's disease or resection of the terminal ileum, helminthiasis (Diphyllobothrium latum) which may use up B_{12} prior to absorption
- *storage disorders*: severe, late hepatic cirrhosis (the liver stores B_{12} in the amount of several **mg**, while the daily allowance is 2-4 **μg**)

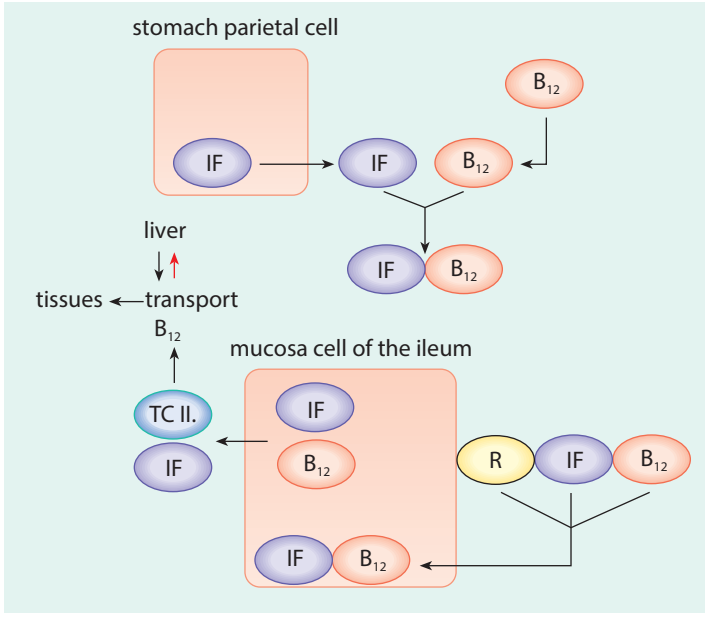

Fig. 4.5.: Intake and transport of vitamin B_{12}. The B_{12} with food binds to intrinsic factor (IF) and ileum receptors (R). It is absorbed and transported by the protein transcobalamine (TC II).

Diagnosis of B_{12} malabsorption, Schilling test:

The patient receives radioactively labelled vitamin orally, after that (about 20 min) B_{12} vitamin store is filled up by unlabeled intramuscular injection (1000 μg). Based on the quantity of the radioactive vitamin in 24-hour urine sample (min. 10%), the ability of absorption is assessed.

Consequences of folic acid and vitamin B_{12} deficiencies

Macrocytic, hyperchromic (large, strongly stained RBCs) anemia. This is also referred to as *megaloblastic* (due to the impaired cell division, the ratio of DNA/RNA increases, Hb production continues, and large megaloblasts are formed), or *pernicious anemia*, since the minimal number of RBCs can be very low (as low as 1 T/l). Erythropoiesis in the bone marrow is ineffective (instead of the normal 10-15%, 80-85% of RBCs succumb in the marrow prior to being released into the circulation). Vulnerable macrocytes appear on the peripheries with features including anisocytosis, ovalocytosis, poikilocytosis. The coagulated RNA (basophilic filaments) and DNA remnants (Howell-Jolly bodies) are seen in the cells, these cells perish early. Increased hemolysis and mild indirect hyperbilirubinemia occur (jaundice, see chapter 7.6.1.3.).

Division and maturation of other cells are also pathologic (*pancytopenia*): WCC decreases, *hypersegmented* neutrophil granulocytes (with multiple-lobed nucleus instead of 3-5 lobes) can be observed in the smear (indicating a disorder of nucleus maturation); the PLT count is also decreased.

In the case of cobalamine deficiency, along with hematological and *gastrointestinal symptoms* (red and painful Hunter glossitis, atrophic gastric mucous membrane, histamine-refractory achlorhydria, signs of malabsorption), *severe central nervous system disorders* are seen (demyelinization, spinal cord degenerations, sensory and motor disturbances), these can prevail over other symptoms and worsen upon folic acid treatment (folic acid will only alleviate hematological symptoms) and might be irreversible.

Pernicious type anemia reacts to treatment very rapidly with a *reticulocyte crisis* within days (percentage of reticulocytes can increase above 30-40%). The accelerated RBC production can result in iron deficiency anemia, which often requires treatment.

OTHER DEFICIENCY ANEMIAS

Production of Hb and RBCs is insufficient in generalized protein malnutrition: regardless of the cause in the background of prolonged protein malnutrition, the result is generally normo-, or microcytic anemia. Vitamin deficiencies can also cause deficiency regarding anemias. Particularly, the lack of vitamin B complexes (B_1, B_2, B_6, niacin) are important (as seen in chronic alcoholism!), although anemia also occurs in vitamin C hypovitaminosis (i.e., iron deficiency anemia).

ANEMIAS ASSOCIATED WITH CHRONIC DISEASES

In inflammatory states, the cause of iron deficiency is hepcidin produced in liver cells due to IL-6, thereby inhibiting the function of ferroportin – a transmembrane protein responsible for transferring iron – and, thus inhibiting macrophages and intestinal mucosa cells which decrease the absorption of iron in the intestines and the availability of iron throughout the body. Inflammatory cytokines also moderate the expression of ferroportin. Additionally, lactoferrin released by granules of activated neutrophil granulocytes and ferritin, which is also produced increasingly, can bind iron, thereby suppressing the availability of trace elements required for bacterial reproduction. In iron deficiency the bone marrow activity and the lifespan of the RBCs decrease as well. Inflammatory cytokines inhibit the division of erythroid cells in the marrow and suppress the release of erythropoietin from the kidneys and the response of the bone marrow to erythropoietin. The prevailing shift to WBC production and hemolysis both contribute to the development of anemia.

Anemia of renal patients develops due to the erythropoietin, iron, and protein deficiency, in addition to the suppression of the bone marrow and the increased hemolysis of the damaged RBCs due to the uremic toxicosis. Increased risk of bleedings can be also present.

In hepatic patients, cholesterol and phospholipids accumulate in the RBCs' membrane with concomitant membrane damage and formation of odd forms (spur cells, target cells), which facilitates increased hemolysis.

Endocrine disorders (hypothyroidism, Addison's disease, pituitary and gonadal insufficiencies) mainly decrease the effect or the production of erythropoietin.

In sideroblastic anemia, iron utilization is disrupted and is of secondary consideration when compared with the impaired hem production due to an enzyme defect (genetic enzyme disorder, δ-aminolevulinic acid synthase – /DALA/ loss). Unlike normally structured hem that inhibits iron uptake, deficient hem is unable to limit the excessive iron influx. The high amount of excess iron accumulates in the mitochondria as precipitated granules adjacent to the nucleus in a half-ring, or ring pattern (ringed sideroblasts). There is an increase

in medullary production, but only a few cells will enter the circulation, this is defined ineffective hematopoiesis. Peripheral microcytic, hypochromic anemia and increased hemolysis of the pathological cells is also possible. There is hemosiderosis in the liver and spleen. Its acquired, reversible form can be seen, e.g., in chronic lead intoxication or in vitamin B_6 deficiency.

APLASTIC ANEMIAS

This refers to severe decrease or the demise of proliferating bone marrow cells.

Causes of aplastic anemias

1. Inherited (rare) Fanconi-anemia[1]
2. Acquired
   a) *Idiopathic* 50-65%: Although this type of anemia is of "unknown origin", impaired activity of cytotoxic T lymphocytes and increased IFN-γ production is hypothesized in its etiology. The disease responds well to immunosuppressive (cyclosporin) therapy. It is also suggested that the disease results from an autoimmune reaction triggered by functionally and structurally altered stem cells.
   b) *Drugs (iatrogenic)*[2]*:*

- antibiotics, e.g., chloramphenicol, sulfonamides
- antitumor agents, e.g., methotrexate, cyclophosphamide, doxorubicin
- antiinflammatory drugs: phenylbutazone, indomethacin
- substances used to treat arthritis: gold compounds (earlier), colchicine, penicillamine
- analgetics: phenacetin, aspirin, salicylamide
- antithyroid substances: e.g., propylthiouracil
- antihypertensives, e.g., captopril, methyldopa
- anxiolytics: e.g., chlorpromazine, lithium

c) *Irradiation*: In addition to high-dose irradiation (e.g., Chernobyl nuclear power plant disaster in 1986, whole body irradiation prior to bone marrow transplantation), chronic application of small doses, e.g., ankylosing spondylitis and cancer therapy can all damage bone marrow.
d) Chemical substances: benzene, organic solvents (occupational risk among construction painters is about 5%), pesticides
e) Viral: hepatitis, HIV, Epstein-Barr
f) Others: various forms of leukemia, pregnancy, post-transplant immune reaction (graft versus host), methylene-dioxy-metamphetamine (MDMA), chronic administration of ecstasy can cause e.g. disseminated intravascular coagulation /DIC/, acute renal failure, hepatotoxicity, aplastic anemia
g) Along with insufficient RBC formation, differentiation of other cells can be also disordered, sometimes their loss is earlier diagnosed, since their lifespan is frequently shorter than when compared to RBCs. Lastly, if the depletion of the bone marrow is not complete, reproduction from the pluripotent stem cells is possible, however, in some of these cases, monoclonal acute leukemia might develop. Also, viral infections can selectively inhibit RBC production.

INCREASED RBC DESTRUCTION AND LOSS

Anemias of hemorrhagic origin

Following an acute loss, blood can be diluted with the fluid, mobilized from the interstitium, however, the decrease in the RBC count does not provide useful

[1] Fanconi-anemia is a very rare (5-10/10^6 births) disease with autosomal recessive inheritance, which results in panmyelopathy (aplastic anemia, thrombocytopenia) due to impaired DNS repair mechanisms (oxidative free radicals, cross-reacting harmful agents; e.g., the repair processes of the damages caused by mitomycin C, cisplatin). Earlier, the severe damage of the bone marrow resulted inevitably in death. Today, the one-year survival rate is still hovers at 50%. Aplastic anemia is often associated with other developmental disorders, low body height, smaller head diameter, thumb-, lower arm-, hip-, ear-, and heart deformations, horseshoe kidney, and skin pigmentation disorders. Patients are susceptible for acute myeloid leukemia (5-10%), and the incidence rate of other tumors is also higher. The disease can be cured by bone marrow transplantation. Other therapeutic methods (e.g., androgens) possess severe side effects (e.g., virilization among young patients, liver lesions).

[2] In the development of aplastic anemia, the pharmaceuticals are estimated to account for about 15-20%. In the most of the cases, medications bear a direct cytotoxic effect (alkylating cytostatic drugs, e.g., busulfan can cause chronic aplasia, in contrast to the transient effects of the folic acid antagonist methotrexate and antimicotics, e.g., daunorubicin). A big group of the non-cytotoxic agents can cause rare idiosyncrasy reaction (not dose-dependent, unpredictable, independent from the pharmacological effects of the drug, pathological bodily reaction) in susceptible patients.

information regarding the extent of blood loss: the hematocrit is nearly normal, since plasma and corpuscles are proportionally lost. Hematocrit and Hb concentration decrease hours or days after bleeding, and not immediately. In cases of unnoticeably developed, extremely low Hb concentration, occult bleeding must be always considered (concealed blood loss, e.g., in the case of a colon tumor): slow, but continuous blood loss results in anemia similar to iron deficiency.

Hemolytic anemias

The average lifespan of RBCs in the circulation is 120 days. This is influenced by the proper structure of the membrane (healthy RBCs are able to undergo extreme deformity without any damage when passing through the capillaries), the cell metabolism (sufficient ATP for the Na^+/K^+ transport, for keeping the SH-groups of the proteins in reduced form, and for ensuring the stable composition of the membrane lipids), and the normal structure of Hb. Abnormalities of the above factors constitute the intrinsic or ***"corpuscular"***, mainly congenital, disorders of the RBCs (sometimes the cause is acquired, e.g., secondary to metabolic disorders); in these instances, the in-vitro, osmotic resistance of the RBCs is decreased (it is not detectable *in vivo*).

A direct trauma of the originally intact RBCs, direct toxic effects, immunological disorders, or the hemolytic over-functioning of the spleen leads to acquired, from the RBCs' structure independent, ***extracorpuscular"*** disorders.

Depending on the extent and the speed of development of intravascular hemolysis, different severities of hemolytic icterus can be expected (indirect hyperbilirubinemia – jaundice), and this type of hemolysis makes the patient prone to hemoglobinuria (with concomitant toxic tubular nephropathy /ATN/, possibly with DIC), capillary obstructions, and tissue hypoxia. Following transiently elevated hemopoiesis (reticulocyte number increases), iron or sometimes folic acid deficiency occurs, or over the long term, the bone marrow will be expired.

Causes of corpuscular hemolytic anemias

MEMBRANE DEFECTS

Hereditary spherocytosis: Autosomal dominant disease, with spheric RBCs in the smear. The structure of multiple types of membrane proteins can be abnormal, including, e.g., spectrin or ankyrin synthesis. Spectrin forms the tetramer subunits of the protein mesh which stabilizes the RBC's membrane, ankyrin aids in the binding to phospholipid membranes). Most frequently, an impaired synthesis of ankyrin or spectrin lies in the background. Prevalence throughout Northern-Europe is relatively high (about 1:5000). The membrane elasticity and plasticity are decreased. Fragmentation occurs when a RBC passes through the capillaries, and takes on a spherical shape. Elimination of the pathologically shaped RBCs by the spleen is augmented. Hemolytic anemia, increased osmotic fragility, varying extents of jaundice, and splenomegaly are characteristic. The symptoms (e.g., occurrence of bilirubin gall stones occur in an unusually young age) may be alleviated by splenectomy. (It should be noted that as a side effect of splenectomy in children, the risk of certain infections, such as Pneumococcus or Hemophilus influenzae, is higher.)

Hereditary elliptocytosis: The RBCs have an elliptical shape. Among others, the disorder of the spectrin tetramer formation can lay in the background. Elimination of the pathologically shaped RBCs from the circulation is augmented in the spleen.

In hepatic diseases, cholesterol, phospholipids and pathological lipoproteins (LPX) accumulate in the RBC membrane, subsequently damaging the membrane, which facilitates the alterations of the cell's shape (spur cells, target cells, spiky cells), and predisposes to hemolysis.

Paroxysmal nocturnal hemoglobinuria: Acquired clonal corpuscular defect. The lack of a surface-binding molecule, glycosyl-phosphatidyl-inositol, leads to the deficiency of approx. 20 different proteins, such as decay-accelerating factor (DAF, CD55) and the membrane inhibitor of reactive lysis (MIRL, CD59). These proteins protect RBCs against activated complement factors. Hemolysis is continuous throughout the day, but dark-colored urine is noticed by patients typically in the morning hours. The disorder also affects platelets, which are activated by the complement system. Along with hemolytic anemia, increased susceptibility to thrombosis is characteristic, which leads to Budd-Chiari syndrome (splenomegaly, ascites, and abdominal pain) due to obstruction of the veins in the liver.

ENZYME DEFECTS:

Glucose-6-phosphate-dehydrogenase deficiency: Relatively frequent disease; it affects nearly 400 million individuals worldwide. It occurs with X-linked recessive inheritance. The direct oxidation of glucose is impaired, and the amount of $NADPH_2$ and reduced

glutathione (GSH) is decreased, oxidative damage of the RBCs can be expected, especially in states with increased oxidation stress, such as infections, acidosis, uremia, and ingestion of fava bean or certain drugs (nitrofurantoin, acetanilide). The damaged Hb precipitates in the form of Heinz bodies within the inner surface of the cell membrane, thereby increasing the rigidity of the membrane. The less elastic RBCs are susceptible to hemolysis. The disorder is limited to the older RBCs, while the young cells are not affected. The recessive form has some protective value against malaria, since the infected cells are eliminated more rapidly. It is more frequent in the Mediterranean region.

Pyruvate kinase and hexokinase enzyme deficiencies are rare, characterized with autosomal recessive inheritance, a selective disorder of glycolysis, and hemolysis of varying extent.

PATHOLOGICAL HEMOGLOBINS:

Sickle cell anemia/disease: Hereditary (autosomal recessive) disease. Heterozygotes are carriers, known as „sickle cell trait" (this genetic modality affects 8% of the Afro-American inhabitants of the USA, and 20-30% of the population in West-Africa), the homozygotes suffer from sickle cell anemia. In the background there is a mutation in the HbS β-chain (in position 6 Val is present instead of Glu). The solubility of deoxy-HbS is decreased, part of the pathological Hb precipitates in the venous circulation (at very low pO_2 in the arterial blood as well). The RBCs take on the shape of a sickle. Initially, this transformation is reversible, however, in the later phases it becomes irreversible. The pathologically shaped RBCs are eliminated by the spleen. During the time of *crisis*, the capillary obstructions (not only Hb is pathological, so is RBC membrane adhesion) lead to organ failures and necroses. In this febrile, extremely painful state, due to the capillary obstructions several organs can be affected, including the spleen (autosplenectomy may be observed – the spleen vanishes within the body due to repetitive necroses), the heart, the brain and the lungs (pulmonary hypertension), papillary necrosis in the kidneys, retinal detachment in the eyes, and ulcers in the lower limbs. In children, Salmonella osteomyelitis is more frequent, which can be accompanied by the abnormal growth of the affected fingers and toes and by the development of hand, foot and mouth disease (HFMD). The disease is more frequent in the Mediterranean region, where the innate heterozygotes are partially protected against malaria.

Thalassemia A: It is a genetic (autosomal recessive) disorder of the Hb α-chain formation. The α-chain is typically encoded by four genes. If all of these genes are defected, death occurs during intrauterine development (hydrops fetalis). HbH disease develops in the presence of three impaired alleles. In this case, the oxygen binding of HbH (4β) (30% of all Hb) is increased. The erythropoiesis is effective. In the case of two or three impaired alleles, the RBC count does not decrease, however, the diameter and Hb content of RBCs are reduced. Since the milder forms of this disease are advantageous in areas, where malaria is endemic, thalassemia minor is found in 20% of the inhabitants in the Mediterranean region and in regions of Asia and Africa.

Thalassemia B: It is a genetic (autosomal recessive) disorder regarding the formation of Hb β-chain. When homozygotes suffer from severe thalassemia major, they produce β-chains in small quantities or simply, not at all. In consideration of heterozygotes, a mild form of the disease develops (thalassemia minor). The four α-chains cannot form normal Hb, therefore Hb will precipitate. The erythropoiesis is severely impaired. The extremely high hemopoiesis leads to massive bone deformities (cruel face due to the distortion of the maxillary, mandibular, and skull bones). Widespread extramedullary hemopoiesis occurs. Various alterations are seen in the smear: microcytic, hypochromic anemia, aniso-, poikilocytosis, DNA and RNA remnants in the RBCs. Repeated transfusions are required. The chronic anemia leads to dilative cardiomyopathy. Patients suffering from thalassemia major are more susceptible to infections, which is explained by general malaise and by leukopenia, secondary to splenomegaly.

Most frequent causes of extracorpuscular hemolytic anemias

Mechanical trauma

- artificial valve, valvular stenosis (vitium)
- march hemolysis (marching, or jogging on a very hard surface)
- a-v shunts
- widespread endothelial damage[3]
- heat-stress, burns

[3] Vasculitides of immunological origin can cause endothelial damage, which destructs RBCs passing through the small vessels, and significantly shortens their lifespan. Such vasculitis develops in hemolytic uremic syndrome (HUS) in the kidneys, causing chronic renal failure, hemolysis, and, via contact with the injured endothelial cells on the irregular surface of small vessels, platelet activation.

Immune-hemolysis[4]

- alloimune (incompatible blood transfusion, Rh-incompatibility between mother and fetus)
- autoimmune diseases, like SLE (systemic erythe–matous lupus)
- drug-induced (penicillin, sulfonamides, phenothiazine, methyl-dopa)
- infection (EBV, mycoplasma)

Splenomegaly (hypersplenism): Stasis in the spleen prolongs the transit time of blood, which increases the sequestration of (functionally active) RBCs, WBCs, and platelets.

- portal hypertension, right-sided heart failure

Toxins

- uremic toxins
- snake venom (cobra), spider and plant venom and other forms of poisons, arsenic

Parasites

- malaria

Incompatible blood transfusions (AB0 and Rh incompatibility are the most important aspects considering the 500 antigens of more than 100 different blood groups) belong to the alloimmune hemolytic anemias. Following an incompatible transfusion, the patient will be immunized to blood group antigens, against which there is no automatic antibody production, e.g., Rh, which will damage the RBCs of an Rh-positive fetus already in intrauterine life in the subsequent pregnancy of a woman who was exposed to the Rh antigen during a preceding pregnancy[5]. In this case, IgG antibodies are produced, which have high affinity for antigens at normal body temperature and can pass through the placenta, thereby damaging the fetus of the pregnant woman. This damage is known as fetal hydrops or erythroblastosis fetalis.[6]

(During the natural immunization to the AB0 blood groups /which develops via carbohydrates found in food/, IgM antibodies are produced which type of antibody has low affinity to antigens at body temperature / its temperature optimum is around 4°C/ and does not pass through the placenta.)

[4] Antibodies are mainly IgM type, however, there are non-complement binding IgG and IgA forms, too. Drugs can cause immune hemolysis: some drugs, adhering to the RBC membrane (e.g., penicillin) can trigger antibody formation against the membrane, while kinins, phenothiazines, and sulfonamides all lead to hemolysis via complement activation by binding to plasma proteins. Others (e.g., heparin) trigger antibody formation against the RBCs as haptens by binding to the RBC membrane. Methyl-dopa acts on lymphocytes which produce antibodies against the RBCs, and facilitates the release of their suppression, which is normally present in a healthy organism.

[5] In Rh-negative mothers, who carry Rh-positive fetus, thus who can be immunized to the Rh antigen, the antibody production can be prevented if they receive exclusively group-identical blood, and if the antigens of the fetal blood mixed to the maternal blood during delivery or abortion are promptly (within 72 hours) neutralized with external antibodies. In general, preventive treatment with anti-D immunoglobulin is recommended for all pregnant women who are RhD negative and do not produce antibodies against the D-antigen. This is usually achieved by an injection between gestational weeks 28 and 30, or by two injections between gestational week 28 and 34.

[6] Hydrops fetalis (or erythroblastosis fetalis): severe fetal disorders often leading to spontaneous abortion, characterized by anemic hypoxia, indirect hyperbilirubinemia, fetal death and Kernicterus, if the fetus survives.

The incidence rate is 1:10[5]/year. Primarily, it affects middle-aged, 55-60 year-old individuals. Multiple pathological changes of growth factor receptors are in the background. In most cases, a mutation in the gene of the Janus kinase-2 (JAK-2, a type of Tyr-kinase) causes the disease (V617F). In some cases, a mutation of a tumor suppressor gene, the SHP-1 was demonstrated, which, mutation resulted in prolonged receptor sensitivity and effect in the myeloid precursor cells even at normal erythropoietin levels. In the case of other growth factors, such as insulin-like growth factor (IGF-I), IL-3, or the granulocyte-monocyte-colony-stimulating factor (GM-CSF), an increased responsiveness was observed.

Along with the drastic increase in RBC count (9-10 T/L, Htc 70%), the WBC and platelet counts usually also increase significantly, similar to that regarding the blood volume.

Susceptibility to thrombosis is increased with the significantly higher viscosity of blood (Fig. 4.6.). Sometimes thrombosis of the portal or mesenteric veins is seen, but two-third of the thrombi are formed in the arterial system and in small vessels: AMI and stroke are frequent. The increased TPR and hypervolemia lead to hypertension. Pulmonary hypertension may also develop (resistance in pulmonary microvessels increases). Tissue perfusion is decelerated due to the increased viscosity and the subsequent tissue hypoxia and high Hb content elevates the amount of the reduced Hb, therefore, in the acral parts (e.g., fingers) cyanosis is frequently seen. In the late phase, microcytes appear due to the relative iron deficiency. Massive splenomegaly is characteristic along with medullary hyperplasia, however, hyperuricemia and gout can be also among the relatively early symptoms.

A highly specific symptom is itching that develops during or after shower/bath, which is explained by the release of histamine from the numerous basophilic granulocytes and mast cells (aquagenic pruritus), which occurs in nearly 40% of the patients.

Following incorrect transfusion, the donor RBCs are hemolyzed due to antigen-antibody binding and immunoreaction. This can lead to life threatening consequences: 1) Hb-uria with subsequent acute toxic tubular nephropathy, to which the produced immune complexes also contribute; 2) DIC triggered by tissue factor which is released from the damaged RBCs; additionally, fever, hypotension, tachycardia, tachypnoe, and widespread tissue ischemia can be observed, and severe shock develops.

4.2.2. POLYCYTHEMIAS

The primary change, **polycythemia (rubra) vera** is a non-malignant myeloproliferative disease which develops due to an acquired clonal defect.

In the late phase of the disease, myelofibrosis often occurs, which leads to bone marrow damage, thus transfusions will be required.

Treatment comprises of frequent phlebotomies (in earlier centuries, medical leeches were used) and cytostatic drugs, such as hydroxyurea (which increases the risk of leukemia) among elderly patients and alpha-interferon in younger patients. Acetyl salicylic acid is recommended for the prevention of the platelet aggregation, while anagrelide is recommended towards decreasing platelet count.

The secondarily developing ***polyglobulia*** or ***polycythemia spuria*** is caused by an increased level of erythropoietin. In the background, there may be *chronic hypoxic hypoxia* (e.g., pulmonary diseases, heavy smoking, congenital genetic cardiac malformation, adaptation to high altitude), *methemoglobinemia*, *CO-hemoglobinemia*, *morbid obesity*, presence of high-affinity Hbs which strongly bind oxygen. In other cases, erythropoietin overproduction is the result of *renal diseases* (hypernephroma, polycystic kidneys, focal glomerulonephritis, renal transplant), or *endocrine disorders* (Cushing disease and syndrome, androgen overproduction, pheochromocytoma). The RBC count is lower than the extreme values measured in primary polycythemia, it hovers at or about 6-8 T/l. Pulmonary hypertension is more pronounced, however, the other polycythemic signs, including susceptibility to thrombosis, decreased tissue perfusion, cyanosis, systemic hypertension, all can be observed. Distinctly, treatment must aim at eliminating the primary cause.

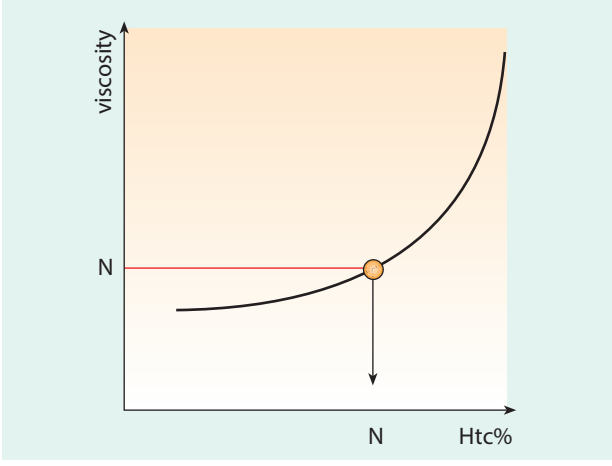

Fig. 4.6.: Association between blood viscosity and hematocrit. Decrease in hematocrit hardly decreases viscosity, but an increase is accompanied by large increase in viscosity.

4.3. PATHOPHYSIOLOGY OF THE WHITE BLOOD CELL SYSTEM

This chapter – without delving into full details – briefly summarizes, those characteristics and pathological alterations of the WBCs deemed important regarding the subject pathophysiology. Additional details of this topic can be found in the subjects of **immunology** and **pathology**.

WBCs can be functionally divided into two groups: (1) phagocytes, i.e., granulocytes and monocytes, and (2) immune cells, they originate from the identical multipotent hemopoietic stem cells in bone marrow as the erythroid (RBCs) and megakaryocyte/platelet cell lines.

4.3.1. GRANULOCYTES

Granulocytes are produced in the bone marrow over a span of 10-14 days. An average, 70-kg human produces 10^{10}-10^{11} neutrophil granulocytes per day. In regulation of their production, IL-3 and the (glycoprotein like) granulocyte-macrophage colony stimulating factors (GM-CSF) synthesized by circulating monocytes, tissue macrophages, and medullary stroma cells are the most important. Most of the CSF genes have basal activity until antigens, toxins, or inflammatory proteins stimulate T-cells to induce (usually via TNF and IL-1) higher production of GM-CSFs, which, together with IL-6, IL-11, and granulocyte CSF, controls the differentiation of precursor cells into granulocytes, as well as, the mobilization and proliferation of neutrophils.

In physiological conditions, only matured granulocytes are released into the peripheral circulation.

Neutrophilic granulocytes are cells with diameter of 12-15 μm, which have 3-5 segments and, as phagocytes, they play an important role in the defense against bacterial, viral and fungal infections. Their cytoplasm is transparent, since their granules are small and have light pink tint if stained using classic methods.

Eosinophilic granulocytes are cells with similar diameter to neutrophils and with 2-3 segments (often similar to motorbike goggles). The eosinophilic granulocyte number increases in parasitic infections (schistosomiasis, toxocariasis, trichinosis, echinococcosis, strongyloidiasis) and in allergic states, e.g., bronchial asthma. Their granules have eosinophil (acidophil, reddish) color.

Basophilic granulocytes are cells with diameter of 9-10 μm. Their nucleus is segmented (2-3), however, both the nucleus and the cytoplasm are covered by numerous, large, dark blue, basophil granules. These granulocytes possess IgE binding sites and their ratio increases in the peripheral smear in the presence of allergic and myeloproliferative diseases, and exoparasites (e.g., tick).

90% of the ***neutrophilic granulocytes*** are found in the bone marrow, 2-3 % in the blood, and the remainder among the tissues. Granulocytes in the blood can have two forms with a proportion of 50-50%: circulating cells (included in WBC count) and marginated cells on the vessel wall (not counted in blood samples). They spend only about 6-10 hours in the circulation, and the circulating cell count is 2.5-7 G/l. Their number can rapidly increase by mobilization from the marginal pool, e.g., adrenergic stimuli or cortisol weaken the marginal binding to the vessel walls and quickly cause granulocytosis (granulocytes with segmented nucleus), while viral infections enhance their margination, resulting in relative granulocytopenia. Matured neutrophils that are stored in the bone marrow constitute a huge reserve, their count can exceed that of the circulating cells about 10-15 times. For prolonged and extensive granulocytosis, increased cell production is necessary, in which case the more juvenile, less matured (less segmented, jugend or stab) forms are also released into the circulation (causing a „left-shift" in the smear).

When activated, the granulocytes degranulate, and many of their functions are associated with the substances released from the granules.

Their *primary* (azurophilic) *granules* contain acidic and neutral hydrolase, elastase, collagenase (lysosomal enzymes, which likely play a role in the formation of abscesses), proteases, e.g., cathepsin B, D, G, neutrophil myeloperoxidase (MPO) producing hypochlorous acid and chlorine gas from H_2O_2 and Cl^-, cationic proteins, bactericide/permeability increasing proteins (BPI), defensin (Cys-rich polypeptide), lysozyme degrading the

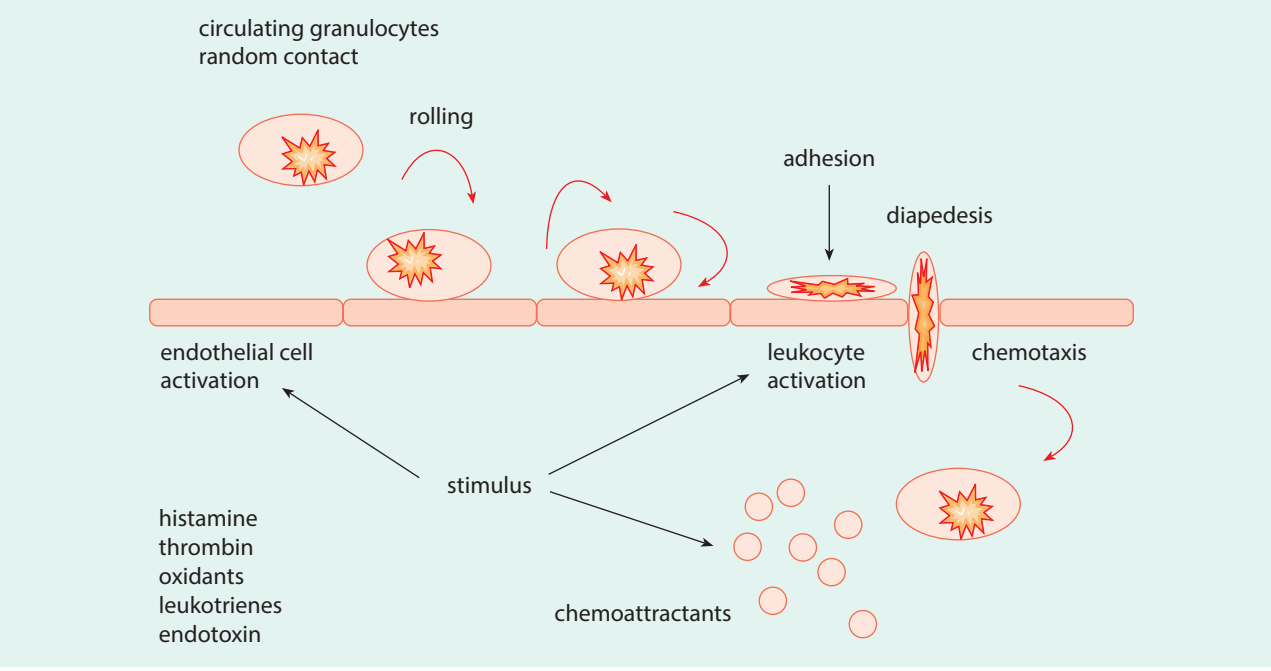

Fig. 4.7.: Granulocyte diapedesis from the vasculature towards environmental chemoattractans.

carbohydrate cell wall structures of microorganisms (muramidase). 10% of the granules in matured cells are such primary granules.

In the *secondary or specific granules* lactoferrin, B_{12}-binding protein, elements of the $NADPH_2$-oxidase system (e.g., cytochrome b558, which is an electron donor in the final phase of superoxide anion production), histaminase, chemoattractant receptors (leukocyte integrin CD11b/CD18), laminine receptor, and an adhesion increasing factor are found.

In their *tertiary granules* developing in the late phase of maturation (Jugend, Stab), in addition to lysozyme, cytochrome b558, and CD11b/CD18, the typical protease gelatinase (matrix metalloprotease-2 /MMP-2/ = gelatinase A and MMP-9 = gelatinase-B) can be found.

In the *secretory granules* located closest to the membrane and emptied first or purged upon activation, in addition to adhesion receptors, e.g., alkaline phosphatase and cytochrome b558 can be also found.

The most important function regarding granulocytes is *phagocytosis*, which happens mainly extravascularly. First, a weak-affinity connection is established between the granulocyte glycoprotein (e.g., L-selectin) and similar adhesion molecules of the endothelium (E and P-selectins) due to chemotactic stimuli (leukotriene B_4 = LTB_4, IL-8, bacteria-macrophage-neutrophil factors and several components of the complement system). A decelerating, twisting and turning movement of the granulocytes (rolling) commences, followed by „adhesion", i.e., a higher affinity, tighter connection by means of neutrophil integrins and endothelial intercellular adhesion molecules (e.g., ICAM). Granulocytes pass through gaps opening up between the endothelial cells by "diapedesis" (Fig. 4.7.), which involves platelet-endothel cell adhesion molecules (PECAM), and then they will undergo additional activation by chemokines (*chemo*attractant cyto*kines*), such as IL-8. During degranulation, receptors and adhesion molecules are expressed on their surface and they migrate to the location of the injury or infection. To accomplish this transit, i.e., migration, phagocytosis, and exocytosis, actin and myosin structures are needed.

During ***phagocytosis*** (a role for surface molecules such as fibronectin, or tuftsin [tetrapeptide] is presumed), neutrophils will tackle the particles with processes and then ingest them (endocytosis). Once inside the vesicles, which develop intracellularly, digestive enzymes, H^+, myeloperoxidase, antibacterial substances, e.g., defensins, complement activating factors, etc. are enclosed, which damage and destroy the ingested cells and bacterial fragments either enzymatically (***lysosomal enzymes***) or via ***free radicals***. (During the rapid increase of oxidative metabolism, referred to as ***"respiratory burst"***, $NADH_2$-dependent oxidases will produce free radicals and hydrogen-peroxide. These substances are cytotoxic *per se*, however, they also induce the production of highly aggressive hypochlorous acid /HOCL/ and Cl_2 as an effect of MPO in the presence of Cl^- ions. Activated granulocytes secrete inflammatory cytokines (TNF-α, IL-1, 6, 8), initiating the inflammatory cascade.

Neutrophil granulocytes destroy the bacteria not only through phagocytosis. Novel studies showed how these leukocytes can form, from their own DNA and bactericide granules, an extracellular bactericide trap (neutrophil extracellular trap = NET) during a specialized form of programed cell death. The elements of the trap are mixed already intracellularly, and then during the destruction of the cell this "mesh" is released into the extracellular space, where the bacteria are trapped and perish. Some bacteria defend themselves against such neutrophil attacks with DNA-degrading enzymes and with the production of an adhesion-inhibiting capsule.

4.3.1.1. GRANULOCYTE DISORDERS

GRANULOCYTOPENIA

It is defined as states with less than 2.5 G/l granulocytes. Mild symptoms appear below WBC count of 1.5 G/l, moderate symptoms below 1 G/l, and severe symptoms below 0.5 G/l.

Causes of granulocytopenia

Although the causes of granulocytopenia are nearly identical when compared with the causes of aplastic anemia (see 4.2.1.3.), certain inherited or autoimmune syndromes are only discussed here.

Decreased production (inhibited bone marrow activity)
Granulocytopenia caused, e.g., by antibiotics or NSAIDs occurs due to idiosyncrasy (see aplastic anemia).

Drug-induced forms: cytotoxic agents (cyclophosphamide, methotrexate), antibiotics (chloramphenicol, sulfonamides, penicillin), non-steroid anti-inflammatory drugs (NSAID)

Ionizing radiation

Uremia (uremic toxins inhibit the activity of the bone marrow)

B_{12} vitamin and folic acid deficiency

Infections (viral, hepatitis, HIV)

Genetic disorders

Severe congenital neutropenia (Kostmann syndrome): granulocytopenia which is typically lethal in the first year of life, characterized by autosomal recessive inheritance and a neutrophil count below 100/μl). In most cases, the genetic defect of neutrophil elastase causes the disease, which responds well to administration of G-CSF.

Idiopathic cyclic neutropenia (Autosomal dominant disease, characterized by 3-week cycles. The neutrophil count fluctuates between 0.1 and 1.5 G/l in 21-day, and severe neutropenia lasts for 3-6 days. In certain cases, the mutation of the neutrophil elastase was found in the background. The cycles are also present in the production of RBCs, platelets, and monocytes. Simultaneously with the decrease of neutrophil count, the monocyte count usually increases.)

Inherited immunodeficiency syndromes

Wiskott-Aldrich syndrome: in this X-linked recessive inherited disease, the gene encoding the Wiskott-Aldrich syndrome protein is damaged on the short arm of the chromosome. Functions of the affected protein include the regulation of actin polymerization (cell movement, phagocytosis), however, a role in signal transduction and apoptosis is also implied. This genetic defect leads to the development of thrombocytopenia, neutropenia, and eczema, all of which are characteristics of the disease.

Hyper IgM syndrome: In this rare, inherited disease, a communication disorder develops between lymphocytes (mainly associated with the gene encoding CD40), in addition to the absence of neutrophil granulocytes. Consequently, the normal maturation of antibody production is absent. While the primary IgM-type antibody response is normal, the production of higher-affinity, more efficient, IgG class antigen-specific antibodies (IgM-IgG switch) is impaired.

The *Severe Combined Immunodeficiency* (SCID) and *Swiss type agammaglobulinemia* are also inherited immunodeficiency syndromes, in which the humoral and cellular immunity are both impaired, mainly due to the damage of lymphocytes.

Increased cell-death

Splenomegaly (hypersplenism, see by extracorpuscular hemolytic anemia)

Autoimmune diseases (Felty syndrome[7], rheumatoid arthritis, SLE[8])

Drug-induced immune response against neutrophil granulocytes (e.g., paracetamol, acetaminophen)[9], methyl-DOPA, phenylbutazone, penicillin, cephalosporins, barbiturates, etc.)

Wegener granulomatosis: Presumably of autoimmune origin, a necrotizing granulomatous vasculitis affecting mainly the lower and upper airways and also the small vessels of kidneys. It is characterized by widespread granuloma formation and vasculitis. The sinuses, lower and upper airways are often ”affected” by therapy-resistant runny nose and prolonged sinusitis. Glomerulonephritis develops in the kidneys, arthritis in the joints, and conjunctivitis

in the eyes. Occurrence of antineutrophil cytoplasmic antibodies may be in the background of the neutropenia.

Peripheral accumulation (transient neutropenia)

Extremely severe infections and interventions accompanied by complement activation, e.g., hemodialysis, or cardiopulmonary bypass, can lead to granulocytopenia.

CONSEQUENCES OF GRANULOCYTOPENIAS

Levels below 1.5 G/l leukocyte count, repeated, severe bacterial infections are present in the skin, middle ear, and upper and lower airways, furthermore, osteomyelitis is also more common. Aphthae (recurrent, viral,

[7] Felty syndrome is a rare and severe type of rheumatoid arthritis (1-3% of cases), which is associated with chronic polyarthritis, splenomegaly, neutropenia, and frequent infections. More than 90% of the patients is HLA-DR^4 positive. In the background of neutropenia, the increased sequestration of neutrophil granulocytes, covered by immune complexes and antibodies against neutrophil surface antigens, in the spleen, are all suspected.

[8] SLE: In more than half of the patients suffering from systemic lupus erythematosus, antineutrophil antibody can be detected in the circulation and the apoptosis of neutrophils is accelerated by several ways (e.g., CD95 or TNF-related apoptosis-inducing ligand /TRAIL/).

[9] Severe granulocytopenia (agranulocytosis), as an adverse effect of acetaminophen, also named paracetamol, has been known for many years. In 1922, Schultz described “angina agranulocytotica” (squeezing sensation in the throat): severe pharyngeal inflammation and high fever, extremely severe neutropenia. The active ingredient of the drug triggers the immune reaction by binding to proteins and acting as a hapten, which then destroys the neutrophil granulocytes. Paracetamol is the active ingredient of the most popular over-the-counter painkillers.

grey, not purulent, painful ulcers) on the mucous membranes with frequent gingivitis and other parodontal infections may develop. Levels below 1 G/l, the severity of bacterial and fungal infections increases dramatically. Levels below 0.5 G/l, the resident bacterial flora (oral cavity, intestine) cannot be controlled, and below 0.2 G/l, there will be absolutely no inflammatory response (the cause of death is sepsis).

GRANULOCYTE DYSFUNCTIONS („*lazy leukocyte*")

Causes and consequences of impaired granulocyte function

Steroid treatment

Neutrophil count is elevated (increased mobilization, decreased marginal pool), however, the neutrophilic functions are impaired following prolonged treatment.

In chronic granulomatosis

The oxidative burst and free radical formation are impaired due to the absence of a $NADPH_2$ oxidase subunit. Due to the decreased protection against catalase-positive (i.e., able to degrade hydrogen-peroxide) bacteria (e.g., Staphylococcus aureus), repeated purulent infections develop, occasionally with potentially lethal severity. Eosinophil granulocytes and monocytes are also affected by the defect.

In uremia

Several uremic toxins can decrease phagocyte function. In addition, phagocytes are also damaged during hemodialysis.

Myeloperoxidase deficiency

Autosomal dominant disease, the prevalence is 1/2000. Bactericide activity is decreased, yet not completely lost. Independently, it does not cause symptoms, but together with other immune suppressing diseases (e.g., diabetes) it increases the risk of, e.g., candidiasis and other fungal infections.

Deranged granulocyte-adhesion (leukocyte adhesion deficiency = LAD)

In the background of the disease, the mutation of, e.g., CD18 (LAD 1) can lie, which is the beta subunit of various leukocyte adhesion surface proteins, including an integrin, in which its loss impairs the neutrophil-endothelium adhesion. Despite the high neutrophil count (15-20 G/l results in no marginalization), the immune reactions are impaired. Staphylococcus aureus, Gram negative, and fungal infections in the skin, as well as, in the mucosa of the respiratory and gastrointestinal system are frequent. Progressive tissue death, slow wound healing, and defective scar formation are characteristic. LAD 2 causes adhesion defects via the defect of CD15s (Sialyl-Lewis X), a glycoprotein which binds to the endothelial selectin.

Chédiak-Higashi syndrome

This autosomal recessive disease is characterized by suppressed chemotaxis and impaired phagolysosome fusion since the defect in the regulator gene of lysosomal transport (lysosomal trafficking regulator gene, LYST). In addition to the neutrophil granulocytes, the mutation is also present in several other cells, e.g., melanocytes, connective tissue cells of the skin, and neurons. Recurrent purulent inflammations, oculocutan albinism, progressive peripheral neuropathy, and, occasionally, mental disorders are characteristic for the disease.

Decreased chemotaxis – high IgE - recurrent infection syndrome ("Job syndrome")

In addition to the decreased neutrophil chemotaxis, mild eosinophilia and suppressed late hypersensitivity reaction can be detected. Recurrent sino-pulmonary and skin infections (mainly Staphylococcus aureus) develop. The inflammatory response is impaired, leading to the occurrence of "cold abscesses". The abscesses heal with consequen scare formation, which can occur in the skin (causing face distortion), the lungs, and the joints. The general condition of the patient is „too good" compared to the severity of the infections. The characteristic „sickness behavior" (fever, apathy, sleepiness, loss of appetite, hyperalgesia) does not develop. The high IgE level is caused by insufficient IFN-γ response to IL-12 (T_{H1}) and subsequent IL-4 (T_{H2}) dominance. Coarse facial features (hyperostosis frontalis externa), a larger distance between eyes – hypertelorism, kyphoscoliosis, osteoporosis, and eczema are characteristic regarding the clinical picture.

Mycobacterium-sensitivity

Decreased protection against intracellular microorganisms (e.g., decreased IFN-γ production, IFN-γ and IL-12 mutations, pathological genetic changes in Signal Transducers and Activators of Transcription-1 /STAT-1/ and NFκB essential modulator /NEMO/), which leads to severe, disseminated infections by Bacillus Calmette-Guérin (BCG, attenuated, live strain of Mycobacterium bovis used in vaccinations against tuberculosis). Nontuberculous mycobacteria can also cause disease among these patients.

(NEUTROPHIL) GRANULOCYTOSIS

The number of the neutrophil granulocytes is between 10-25 G/l. Persistent granulocytosis with a WCC of 30-50 G/l (but below 100 G/l) is referred to as leukemoid

reaction. The latter usually occurs secondarily due to severe infections, tumors, hemolysis, and toxic megacolon.

Causes of granulocytoses

Acute infections, endotoxin effect

Increased production, medullary release, mobilization from the marginal pool. There is typically „left shift" in the smear (in acute infection, many young, immature wand- or horseshoe-shaped /stab, jugend/, nucleus-containing neutrophils can be seen in the peripheral smear) with eosinopenia, and relative lymphocytopenia. It is accompanied by fever, inflammation, tissue damage, and, in the long term, fibrosis.

Stress, trauma, intensive sport

Due to the effect of catecholamines, part of the neutrophils will enter the circulation from the marginal pool (there is no "left shift").

SIRS (systemic inflammatory response syndrome)

Severe traumas, sepsis, acute pancreatitis can cause life-threatening, widespread inflammatory reaction, in which the increased neutrophil granulocyte count and activity can also contribute to the development of multiple organ failure. The apoptotic activity of neutrophils, characterized by increased oxidative burst and CD11b expression, is inhibited.

Corticosteroids

Granulocytosis is caused by increased mobilization of the marginal pool, however, the positive effect of glucocorticoids was also shown on neutrophil production (increased effect of G-CSF, enhanced expression of LTB_4 receptor expression which improves survival and chemotaxis), and on inhibition of apoptosis. On the other hand, decreased functions, e.g., reduced migration ability to the site of inflammation (augmented MMP-induced L-selectin detachment from the neutrophil membrane), and suppressed superoxide anion production can be also observed among the effects of steroids.

Acidosis

The acidic pH which often accompanies inflammations, activates the neutrophil granulocytes via a transient increase in the IC Ca level, while it also increases the expression of CD18 (the beta subunit of different leukocyte adhesion surface proteins, including an integrin), and inhibits the apoptotic processes of the neutrophils.

Hypovolemia

The volume of migrating neutrophils typically increases (35-60%). Swelling of the cells due to different reasons promotes the migration of neutrophil granulocytes, based on observations. Hypovolemia and subsequent hypotonicity (due to compensatory ADH activation) also lead to swelling of the cells, thus facilitate the migration of these leukocytes, while in old patients, the hypovolemia-associated hypertonicity inhibits the migration due to cell contraction.

Metabolic diseases

Among others, neutrophil cell count and activity also increase in diabetic ketoacidotic coma (acidosis), acute renal failure (acidosis) and gout attack (acute inflammation).

Myeloid leukemias (discussed in greater detail in the subject of pathology)

Early maturation forms can be present in an enormous number in the circulation, while the number of mature forms is insufficiently low. Often other lines of hemopoiesis are also affected (megakaryocyte, RBCs). Leukemias are classified based on the nature of the involved cell types and on the progression of the disease. In chronic myeloproliferative diseases "-cytic", in acute forms "-blastic" forms predominate.

EOSINOPHIL GRANULOCYTES

The ***eosinophil leukocytes*** are morphologically and functionally, in part, similar to the neutrophils. However, several of their features differ from neutrophils. The production and function of eosinophils is primarily influenced by IL-5. Their chemoattractant is eotaxin. Eosinophils are present in the blood for 8-12 hours, however, their peripheral lifespan is longer: 8-12 days. Moreover, tissue eosinophils are able to re-enter the circulation. Their primary importance is not related to the defense against bacterial, viral or fungal infections, but rather to parasitic and allergic diseases. Thus, their granules also differ from those of the neutrophils'.

There is a crystalline nucleus in the middle of eosinophil granules, formed by the Arg-rich **major basic protein (MBP)**. This protein is not only the endogenous inhibitor of heparanase (which degrades the heparan-sulfate component of the basal membrane) which plays an important role in tumor metastasis formation, but it also induces the histamine release from mastocytes and basophil granulocytes, activates neutrophils and alveolar macrophages, and its role was confirmed in the development of epithelium damage in asthma, exfoliation, and bronchial spasm. Eosinophil peroxidase produces HOCl from H_2O_2 and Cl^- similarly as MPO. Eosinophil cationic protein (ECP) damages the epithelial cells of the airways (or the intestinal mucosa), thus the submucosa remains unprotected against external impacts (irritant pollutants, allergens). Eosinophil desquamative inflammation, which is characteristic regarding allergies, is augmented, and both

cilia and the brush border are damaged. Their granules can also contain Charcot-Leyden crystal proteins (lysophospholipase), and neurotoxin. Acid phosphatase is typically found in smaller granules, but from different microgranules, the release of leukotrienes, interleukins (IL-3, IL-5), platelet activating factor (PAF) and GM-CSF has also been also shown.

Causes of eosinophilia (> 0.5 G/l)

Helminthiasis (worms)

Allergies (bronchial asthma, hayfever)

Loeffler syndrome: Eosinophil pneumonia characteristic in parasitic infections.

Eosinophilic endocarditis (Loeffler-endocarditis): A rapidly progressing form of secondary restrictive cardiomyopathies, in which myocardial inflammation and its fibrotic remodeling are induced by eosinophil accumulation.

Rheumatoid arthritis *(= polyarthritis chronica primaria, PCP)*

Rheumatoid arthritis is often associated with eosinophilic pneumonia. Also, in the blood of patients suffering from bronchial asthma with severe plasma eosinophilia, the rheuma factor was detected, the level of which correlated with the extent of the eosinophilia (and with the severity of asthma).

Churg-Strauss syndrome

Although its exact mechanism is not yet known, it develops in association with allergic diseases. In the patients typically allergic rhinitis develops first, then sinusitis, and, later, allergic asthma. Factually, this disease is an allergic granulomatous vasculitis which causes tissue and serum eosinophilia and affects the small and medium arteries and veins. The symptoms can occur in the skin, lungs, heart, kidneys, and in other internal organs. It leads to pulmonary fibrosis, the progression of which can be delayed by steroid therapy.

Idiopathic hypereosinophilic syndrome

Severe cardiac and central nervous system damage, in which the kidneys, lungs, gastrointestinal tract, and skin are also affected.

Eosinophilia-myalgia syndrome

Eosinophilia exceeding 1 G/l. Severe, disabling muscle pains, pneumonitis, myocarditis, neuropathy, respiratory failure, and encephalopathy are characteristic. The extensive tissue damage is presumably caused by deposition of eosinophil-derived toxic proteins. The possible role of IL-5 and transforming growth factor beta (TGF-β) are also suggested in the development of the disease. More than 1500 new patient were diagnosed in the USA between 1989 and 1991. In these cases, a certain, not properly prepared, L-Trp-containing nutritional supplement caused the disease with a mortality of nearly 5%. Since then, such patients continue to be diagnosed worldwide, however, in much lower numbers.

In the treatment of the eosinophilias, glucocorticoids are rather efficient, however, certain cytostatic agents (e.g., hydroxyurea) or interferons (IFN-α) can be also applied.

Causes of eosinopenia

Glucocorticoid treatment

Acute bacterial infections

In such cases, eosinopenia is independent from the endogenous cortisol release, since it can be also observed in adrenalectomized animals).

Stress

Eosinopenia has not been associated nor is identified with adverse effects on the body.

BASOPHIL GRANULOCYTES

Basophil granulocytes possess a diameter of 9-10 μm, and they are characterized by dark purple-blueish granules, which also cover the nucleus which has 2-3 segments. As the cells enter into the tissues they transform into mastocytes. Their granules contain heparin, serotonin, and histamine. They are an important source of IL-4, yet they are also able to produce TNF-α, IL-6, and IFN-γ. Their lifespan is 1-2 weeks. Their number rarely increases above 0.1 G/l, however, if it does, then most frequently myeloproliferative diseases (e.g., chronic myeloid leukemia or polycythemia vera) lie in the background. Reactive basophilia was described occasionally in hypothyroidism associated with myxedema, in chicken pox, and in ulcerative colitis. They may have a role in parasitic and allergic diseases. This is supported by the fact that high-affinity IgE receptors can be found upon their surface, in addition to activated complement-binding (C3a, C5a) receptors.

A decreased basophil count was described in chronic urticaria (this is difficult to diagnose due to the generally low cell count).

4.3.2. MONOCYTES (MONONUCLEAR PHAGOCYTE SYSTEM)

Monocytes are the largest cells in the smear, with oval, kidney-shaped nucleus. Their diameter is nearly 16-20 μm. The cytoplasm is characterized by azurophil granulation and small vacuoles. Their production in

the bone marrow is regulated by GM-CSF and monocyte-CSF (M-CSF). After spending 1-3 days in the circulation and throughout the tissues, they transform to tissue macrophages, and their lifespan can last for several months or even years. Tissue macrophages are referred to as Kupffer cells in the liver, alveolar macrophages in the lungs, intraglomerular mesangial cells in the kidneys, microglias in the central nervous system, and osteoclasts in the bones.

The main functions of the monocytes are similar to neutrophils: chemotaxis, phagocytosis, and the destruction and digestion of internalized microorganisms.

Among the causes of monocytosis (above 0.8 G/l), are chronic bacterial infections (tuberculosis, Brucellosis, subacute bacterial endocarditis), mononucleosis infectiosa (Epstein-Barr virus infection in teenagers), chronic inflammations (liver cirrhosis, Crohn's disease, ulcerative colitis), autoimmune diseases (SLE, rheumatoid arthritis), chronic neutropenia, acute myeloid leukemia and myelodysplasia (especially chronic myeloid leukemia). Chronic and persisting monocytosis (with mild anemia) can indicate myeloproliferative diseases or the possibility of myelodysplasia.

In the background of monocytopenia (< 0.2 G/l), can be the effects of drugs which also inhibit neutrophil granulocyte production in the bone marrow. A transiently decreased cell count was observed in stress and after glucocorticoid treatment. It can also be characteristic in hairy cell leukemia.

4.3.3. LYMPHOCYTES AND THEIR DISORDERS

In addition to granulocytes, **lymphocytes** are also important elements of the WBC system. Their unique characteristics are antigen-specificity and immunological memory. Lymphocytes, the fundaments of cellular and humoral immunity, include T cells originating from the thymus, and B cells originating from the bone marrow and the lymphoid organs. Their detailed discussion can be found in the subject, immunology and, in relation with lymphoproliferative diseases, pathology.

Absolute or relative lymphocytosis (viral infections, chronic inflammations) and lymphocytopenia (immune deficient conditions) can be mentioned in general pathophysiological processes, however, they have only limited importance in the pathogenesis of disorders subject to pathophysiology studies.

Lymphocytes are cells with 6-9 μm in diameter (9-10 μm under light-microscope) (although they are the smallest among the WBCs, they are still bigger than RBCs), transparent cytoplasm, and a relatively big, round nucleus. Their count in the blood is 1.5-4 G/l. These small lymphocytes account for 90% of all circulating lymphocytes. In contrast, reactive lymphocytes following antigen recognition are larger, with a diameter of 9-15 μm (15-20 μm under a light-microscope) and with a plenty of basophil cytoplasm, in which azurophil granules and vacuoles are also present (large granular lymphocyte = LGL). In the background of the transformation lurk be viral infections, drug side effects (e.g., antiepileptics, phenytoin), immunoreaction, irradiation, stress, and autoimmune diseases (rheumatoid arthritis, Addison's disease). These cells are comprised of natural killer cells (NK cells) in 75%, and of cytotoxic T lymphocytes in a smaller proportion.

Following birth, the bone marrow and the thymus are the principal lymphoid organs, where the production and development of the lymphocytes take place. The antigen-lymphocyte communication occurs in secondary lymphoid organs, lymph nodes, spleen, lymphoid cell groups of the respiratory and gastrointestinal system (MALT = mucosa-associated-lymphoid tissue: BALT = bronchoalveolar lymphoid tissue in the lungs and Peyer plaques in the intestines), and the skin (SALT = skin-associated lymphoid tissue).

B lymphocytes are matured in the bone marrow. During their development, IgM complexes occur gradually on their surfaces, which is characteristic for immature B cells. Hence, antigen specificity of the B lymphocyte is determined, and the antigen-independent maturation is finished. During B lymphocyte maturation, selection processes are dominant, 90% of cells are destroyed within the bone marrow, without ever being released into the circulation. The remainder of the B cells emigrate to secondary lymphoid tissues via the circulation, where their antigen-dependent maturation is achieved. During this process, certain regions in the immunoglobulin-coding genes of the cells undergo frequent mutations (somatic hypermutation, 10^5-10^6 times more frequently than the genetic material in other cells), the result of which is restricted to the affected cell. Therefore, the number of recognizable antigens substantially increases. The membrane receptor of B cells contains the immunoglobulin characteristic for the cell, which will be produced and secreted following the activation of the cell. When the specific antigen-recognizing domain of B cells is activated (by binding an antigen), cell proliferation is initiated, part of the activated B cells will transform to memory cells,

while another part will turn into Ig producing plasma cells. The matured B cells also express IgD on their surface, in addition to IgM.

Antigen-dependent maturation of B cells can occur through various means. As a first step in *T cell-dependent activation*, antigen-presenting cells (macrophages or dendritic cells /antigen-presenting cells with dendrites on their surface, previously known as Langerhans cells/) present the ingested and digested pathogen molecules bound to MHC II molecules on their own surface. The macrophage activates the antigen-specific T cell by paracrine mechanism, which consequently proliferates and transforms to effector (CD4+) and memory cells. The T_{H2} effector cell promotes the activation of the antigen-specific B cell (to which the antigen itself has already bounded). These two effects, the parallel binding of the antigen and T_{H2}, induce maximal antibody response of the B cell. Most antigens trigger this type of reaction. T cell-dependent antigens also contain proteins, of which, by binding to the MHC II complexes of B lymphocytes, activate the proper T_{H2} cell, which reversely activates the B cell via cytokine production (e.g., IL-4), and stimulates its IgG, IgA or IgE production instead of IgM. If this change occurs (Class Switch Recombination = CSR), B cells will no longer produce original IgM or IgD antibodies.

Many antigens are able to activate B lymphocytes by *T cell-independent mechanism*. Certain types of these antigens, e.g., bacterial endotoxin (part of the outer membrane in Gram-negative bacteria, lipopolysaccharide = LPS) similarly as mitogens, can initiate B cell proliferation, basically by acting as polyclonal B cell activators. Another subtype of these antigens is a domain-repeat-containing polymer with a large molecule weight, such as polysaccharides in the bacterium wall and in the viral capsid. These antigens are able to form cross-bindings between antigen receptors on the surface of B cells, thus B cells can produce IgM antibodies without T cells' interaction. B cells account for 25% of the lymphocytes.

Humoral immunity mediated by B cells is maintained by different types of immunoglobulins. **IgM,** found on the membrane of immature B lymphocytes, is a multimer, containing 5 subunits. Since an IgM molecule includes 10 antigen-binding sites, its agglutination tendency is high (resulting in precipitation of pathogens). **IgD** (1% of membrane proteins), which is also expressed on the membrane of immature B lymphocytes, is produced via alternative splicing. Its blood level is low and its function is not yet known. **IgG** is a monomer immunoglobulin, which consists of 2 heavy and 2 light chains. Each molecule can bind 2 antigens. This immunoglobulin is present in the largest amount, which is distributed in 50-50% between the blood and the nterstitial space. It mediates humoral defense against bacteria, viruses, and fungi. It is able to activate complements and to increase the efficacy of phagocytosis by binding to antigens of pathogens (opsonization). Different subtypes (IgG_{1-4}) can be distinguished, which possess distinct complement-activating, phagocytosis-augmenting effects. IgG can easily penetrate the placenta, thus, e.g., in case of Rh incompatibility between mother and the fetus (due to the pathological immunization, the Rh-negative mother can produce IgG antibodies against the Rh-positive fetus), it damages the RBCs of the fetus after crossing, thereby causing severe or even lethal hemolysis.

IgA, also referred to as secretory immunoglobulin, accounts for 20% of circulating immunoglobulins in the blood, although its primary importance is in the defense of mucosa (oral cavity, intestines, airways), and it is secreted by breast milk and saliva. In the secretion, it is typically present in dimer form, while in the circulation it occurs as a monomer. It has a role in neutralization of inhaled, swallowed, and body surface (of any means) pathogens.

IgE is a monomer immunoglobulin, which binds so strongly to the surface receptors of basophils and tissue mast cells, that it generally, only can be detected from the blood; its plasma concentration is very low. The most important role of IgE is in type I hypersensitivity and immunity against parasites.

T cells develop from lymphocytes migrated in the thymus, while they wander from the cortex to the medulla. In this maturation process, the role of thymus-derived growth factors, e.g., thymosin, thymulin, thymopoietin or thymic humoral factor, has not been fully clarified. During maturation, defective or autoreactive strains are eliminated by apoptosis (negative selection). In the cortical region, those T lymphocytes stay alive, which are able to connect to the major histocompatibility complex (MHC) molecules of epithelial cells (positive selection), while the rest of the cells are eliminated. While migrating to the medulla, the maturing cells interact with dendritic and macrophage cells of the thymus. Those cells which can connect with too high of an affinity to the host's antigens will be destroyed in this phase. Elimination of the autoreactive cell lines promotes the development of autotolerance. During this migration, the maturing T cells start to express CD4 (T helper = T_H) and CD8 (cytotoxic or T suppressor = T_S) surface molecules. In the

medulla, only matured, CD4 (CD4+) or CD8 (CD8+) expressing T lymphocytes can be found which possess T cell receptor (TCR) and the attached CD3 complex. During the selection processes, 95% of cells are destroyed in the thymus, and only 5% of them survive and exit the thymus as matured T cells. The αβ TCR subunit-expressing T cells, which react with protein antigens, account for the majority (97%) of the whole cell population, while the remaining minority of the cells (2-3%) possess the δγ TCR subunit (e.g., in intestines, thymus medulla, and follicular regions of lymph nodes) can recognize non-protein antigens. Additional special features of these cells are that they can recognize heat shock proteins, and likely play a role in the immune response to certain tumors, as well as, in the protection against mycobacteria.

CD4+ cells can be further divided into T_{H1} subtype, producing mainly IFN-γ, IL-2, IL-3, TNF-α, and GM-CSF, and into T_{H2} subtype, producing mainly IL-4, IL-3, IL-5, IL-6, IL-10, IL-13, and GM-CSF. The former plays a role mainly in defense against pathogens and strong antigens (IL-12 is important in their activation), while the latter is essential in allergic, parasitic diseases and in defense against weak antigens (IL-4 is important in their activation).

The **natural killer (NK) cells** are CD8+ and do not have TCR. These are large cells with cytoplasmic granules, and, upon their surface they express receptors (Fc = CD16) capable of binding complement. NK cells destroy mainly tumor cells and virus-infected cells, which weakly express antigens that belong to the HLA (human leukocyte antigen, MHC I) group. HLA receptors occur on the surface of NK cells, and other cells that can bind to these receptors inhibit the NK cell activity. If the HLA antigen is missing, the cytotoxic effect is activated. In addition, the NK cells are also capable of antigen-dependent, cell-mediated cytotoxicity, because they are able to destroy antibody-labeled cells by recognizing the Fc part of an antibody which was previously bound to the target cell surface. Their ratio is nearly 7-10% of all lymphocytes.

The number and function of lymphocytes are influenced by numerous physiological factors (e.g., physical activity, stress, pregnancy, and aging).

Insufficient functionality regarding the lymphocyte system results in immune-deficiency syndromes which increase the incidence and severity of bacterial and viral infections. Augmented lymphocyte function indicates activation of host's defense mechanisms, however, in pathological conditions, it is part of autoimmune processes.

4.3.3.1. DISORDERS OF THE LYMPHOCYTE SYSTEM

4.3.3.1.1. IMMUNE-DEFICIENT CONDITIONS

Lymphopenia: lymphocyte count is under 1.5 G/l. The main primary and secondary causes of immune-deficient conditions include the following.

PRIMARY FORMS

Disorders of B cells (disorders of antibody production)

X-linked agammaglobulinemia *(Bruton syndrome)*

Severe or complete loss of immunoglobulins (IgG, IgM, and IgA) causes the disease. In the background, the mutation of Bruton-like tyrosine-kinase gene was shown, which presumably, causes the disorder of B cell maturation, since the early B cell count is normal. Frequent bacterial infections are characteristic.

Variable immune defect *(common variable immune deficiency = CVID)*

Deficiency of the innate immune system is characteristic. Disease severity differs among the patients, and it can vary over time in the same patient. B cells do not differentiate into immunoglobulin-producing plasma cells, which subsequently results in hypogammaglobulinemia.

Selective IgA deficiency

It is the most frequent immune deficiency syndrome (1:400). The plasma IgA level is low. The clinical picture consists of recurrent bacterial infections affecting mainly the mucosa. In most cases, the airways, nose, pharynx, and the urogenital and gastrointestinal systems are affected. Malabsorption can also occur. Autosomal recessive inheritance is the most frequent. Patients are susceptible to allergic diseases, they may produce antibodies, e.g., against IgA. Thus, in these patients, upon *transfusions*, *anaphylactic shock* can occur, which can be prevented by group-identical blood transfusion of donors, also suffering from IgA deficiency. Among the B lymphocyte precursors of the bone marrow, forms expressing surface IgA antibodies can be detected, which indicates disorder of the terminal maturation of B lymphocytes. Currently, IgA cannot be supplemented (the administration of breast milk may be an option).

Disorders of T cells

Developmental disorder of pharyngeal arches 3 and 4, accompanied by congenital vitium, parathyroid insufficiency (hypocalcemia), and lack of thymus. Lympho-

cyte count is normal, but T lymphocytes are absent or their count is decreased. Cellular and humoral immunity are both impaired. Patient cannot be vaccinated with living pathogens (BCG), because the risk of sepsis is high due to the lack of cellular immunity. (Nezelof syndrome: lack or hypoplasia of the thymus is not associated with other developmental diseases.)

Purine nucleoside phosphorylase (PNP) deficiency
Inhibition of ribonucleotide-reductase is presumed to result from an accumulation of deoxy-GTP due to the enzyme deficiency. The impaired DNA synthesis in this disease leads to selective T cell deficiency. The amount of immunoglobulins is normal, yet their function is impaired.

Complex disorders of B and T cells
Severe combined immunodeficiency (SCID) (or Swiss type agammaglobulinemia)

Here, both humoral and cellular immunity are damaged due to defective lymphocytes. Inheritance can be autosomal recessive or X-linked. In the X-linked type, a shared component of interleukin receptors (e.g., IL-2, IL-4, IL-7, IL-9), the structure of the common γ-chain is defective. In other cases, the cause is the lack of the adenosine deaminase (ADA) enzyme, which inhibits DNA synthesis in the common precursors of B and T cells. Lymphocyte count decreases and the amount of immunoglobulins is low.

Wiskott-Aldrich syndrome (see 4.3.1.1.)
T lymphocytes count is decreased and the plasma IgM level is low due to accelerated protein catabolism. Production of IgG and IgA increases proportionally, thus compensates for the increased degradation.

Bloom syndrome

Inherited immune deficiency which impairs mainly cellular immunity. It is associated with recurrent airway infections, higher risk of cancer, teleangiectasia, and intrauterine or postnatal growth disorders. DNA repair mechanisms (DNA-helicase) are impaired.

Ataxia teleangiectasia

A disease characterized with a higher risk of infections and cancer, also associated with severe mental retardation, cerebellar ataxia, and oculocutaneous teleangiectasia. An autosomal recessive inheritance was found. DNA repair mechanisms are impaired, chromosomes are highly instable, thus sensitivity to ionizing radiation, e.g., X-ray, is increased.

Reticular dysgenesis

Multipotent erythropoietic stem cell deficit, leading to severe combined immune deficiency. Inherited, rare, and nearly always a fatal disease, involving impairment of most immune functions. The majority of patients expire early in life as infants. Lymphocyte, granulocyte and platelet precursors are also affected by the stem cell deficit. Severe bone marrow hypoplasia or aplasia is characteristic. Development of the thymus and lymph nodes is incomplete or absent. Severe, often "opportunistic" infections (Candida, Cytomegalovirus, and Pneumocystis carinii), loss of appetite, fever, diarrhea, and weight loss occur as well. Bone marrow transplantation can be successfully applied in some cases.

Hyper IgM syndrome: (see 4.3.1.1.)

SECONDARY FORMS
Secondary disorders of the lymphocyte system are much more frequent than the inherited primary lymphopenias.

Disorders of B cells (disorders of antibody production)

Protein losing conditions (e.g., nephrotic syndrome, protein-losing enteropathy)

Loss of immunoglobulins by urine in nephrotic syndrome or through the intestines in protein-losing enteropathy cannot be compensated properly with increased production.

Myeloma multiplex

Impaired immunoglobulin production together with accelerated immunoglobulin elimination cause hypogammaglobulinemia and increased risk of infections. A quarter of patients suffer from recurrent infections, while among three-quarters of the patients, severe infections occur during the disease.

Disorders of T cells

AIDS (Acquired immune deficiency syndrome)
The human immunodeficiency virus (HIV) results in quantitative and qualitative insufficiency of CD4+ T_H cells. This condition leads to the occurrence of opportunistic infections (e.g., Pneumocystis carinii). As part of the disease, other hematological disorders may occur as well. Severe anemia, requiring erythropoietin treatment and transfusions, occurs, which can be explained partly by impaired bone marrow function, effects of opportunistic infections, and side effects of drugs used in AIDS (e.g., zidovudine). In the background of the accompanying neutropenia and thrombocytopenia, pathological immune processes and bone marrow disorders are suggested. The risk of lymphomas is also high.

Lymphomas

The biggest part of lymphomas (90%) originate from the B lymphocyte cell line.

Cushing disease and syndrome

Characteristically, the count of lymphocytes and eosinophil cells are decreased. In particular, the reduced number of CD4+ cells (increased ratio of CD8+ cells) was observed.

Drugs

Steroids and certain cytostatic drugs (cyclosporine, azathioprine) may decrease the T cell count.

Complex disorders of B and T cells

Drugs (steroids, cytostatic agents)

Decreased production in the bone marrow

Irradiation (radiation injury) impairs lymphocyte functions. In the first year following bone marrow transplantation, the incidence of certain viral infections (e.g., cytomegalovirus, herpes zoster) substantially increases due to disorders in the regulation of the immune system.

Chronic lymphoid leukemia

See a detailed explanation in the subject of pathology.

4.3.3.1.2. CONDITIONS WITH LYMPHOCYTOSIS

Lymphocytosis: lymphocyte count is above 3.5 G/l.

Mononucleosis

Acute (occasionally prolonged) infection (glandular fever) caused by the herpes virus, Epstein-Barr. The B lymphocytes are infected by the virus, which results in the characteristic T lymphocyte reaction. Young adults, under 15 years of age, are mostly affected, while it rarely occurs above the age of 40 years. Fever, pharyngitis, enlargement of the lymph nodes, nodes, the spleen, and, occasionally, the liver occurs. The increased WBC count, lasting 1-2 weeks (10-30 G/l), results from the accumulation of mononuclear elements, and that of the typical and atypical lymphocytes. Cytomegalovirus and Toxoplasma gondii infection can cause a very similar alteration as the infectious mononucleosis.

Lymphocytosis due to acute infection

Among children and young adults, lymphocytosis can occur instead of neutrophilia during acute infections.

Lymphocytosis due to chronic infection

Tuberculosis, syphilis, brucellosis, and toxoplasmosis all characteristically result in an increased lymphocyte count.

Thyrotoxicosis

Absolute, or, in many cases in association with general leukopenia, relative lymphocytosis is characteristic. Thyroid hormones increase mitogen-induced lymphocyte proliferation and their IL production.

Autoimmune diseases

In the development of autoimmune diseases, the lymphocyte system also takes part. In autoimmune diseases, lymphocyte infiltration can be shown in the affected tissues. It is also known in which diseasethe lymphocyte system may have a key role in the pathomechanism of autoimmunity. Toll-like receptors (TLR) of B lymphocytes can respond to the host's antigens directly with antibody production, i.e., without preceding T lymphocyte activation. Consequently, the activation of T lymphocytes also occurs. In autoimmune diseases, the activity of natural regulator T cells (CD4+CD5+, Treg), which normally inhibit the function of autoreactive lymphocytes, may be also be defective.

Malignant diseases

This includes acute lymphoblastic leukemia and certain forms of non-Hodgkin lymphomas. A more detailed discussion can be found in the subject of pathology.

4.4. Pathophysiology of hemostasis

In the case of vascular damage, local mechanisms are initiated to prevent blood loss. These are: **1**) factors of vascular origin**, 2**) platelet derived mechanisms, and **3**) clotting factors of the plasma. Intravascular clotting may develop due to certain pathological effects, which is normally counteracted by the effects of an anticoagulant-antithrombin activity. Fibrinolysis is a mechanism **4**), which results in lysis of the thrombus within 48-72 hours.

Injury-induced reflex neurogenic vasoconstriction, which, in the injured endothelium, involves an increased production of vasoconstrictor mediators, e.g., endothelin-1, thromboxane A_2 (TXA_2), including a decreased production of vasodilator mediators (e.g., NO, PGI_2), which contribute (as humoral factors) to the partial or complete closure of the injury in the damaged small vessels within seconds. At the site of the injury, due to the impaired antithrombic endothelial function (e.g., decreased activity of the platelet activation-inducing endothelial ADP degrading enzyme and decreased production of NO and PGI_2), platelets accumulate and form a "plug", thus they seal the injury far more strongly (platelet phase, 5-7 minutes). At the

site of the injury, tissue thromboplastin and serotonin along with aggregation-increasing other humoral, vasoactive substances (TXA_2, ADP) will be released from the aggregated platelets, in addition to platelet derived thromboplastin and Ca^{++} – and these will be required in clotting. In the event of a larger injury, in which even the combination of these two coagulation mechanisms is not sufficient, only a larger clot can prevent bleeding, yet for this, the clotting factors of the plasma are essential (secondary hemostasis, 7-10 minutes). At last, the clot retracts (retraction), then the fibrin content will be dissolved (fibrinolysis, 48-72 hours), and the vessel becomes passable again (recanalization).

Abnormalities of hemostasis include disorders due to insufficient hemostasis (or exaggerated fibrinolysis), leading to increased bleeding, including disorders of increased coagulation on the basis of hypercoagulability which result in abnormal clot formation, or thrombophilia caused by insufficient fibrinolysis (thrombosis).

4.4.1. BLEEDINGS OF VASCULAR ORIGIN

Hereditary or acquired connective tissue disorders may increase the fragility of small vessels on the basis of decreased capillary endothelial function and reduced stability of the capillary basement membrane and perivascular connective tissues, thereby leading to vascular purpuras (small bleedings of a few-mm diameter in the skin and the mucosa). Inherited vascular malformations can also cause small bleedings of vascular origin, e.g., teleangiectasia (red spots in the skin caused by dilated small vessels).

VASCULAR PURPURAS

Hereditary

Teleangiectasia (Osler-Weber-Rendu syndrome)

Teleangiectasia is a pathologically dilated peripheral capillary-postcapillary vascular malformation (occasionally arterio-venous shunt). These, unlike other purpuras, turn colorless under a glass pressed against the skin. In the background of this autosomal dominant malformation, there is the genetic disorder of the TGF-β receptor, an important factor in tissue regeneration and angiogenesis. It is characterized by familial accumulation, frequent epistaxis (nose bleeding), and teleangiectasias. Arterio-venous malformations (shunts) may be present tissues, which can result in high output cardiac failure.

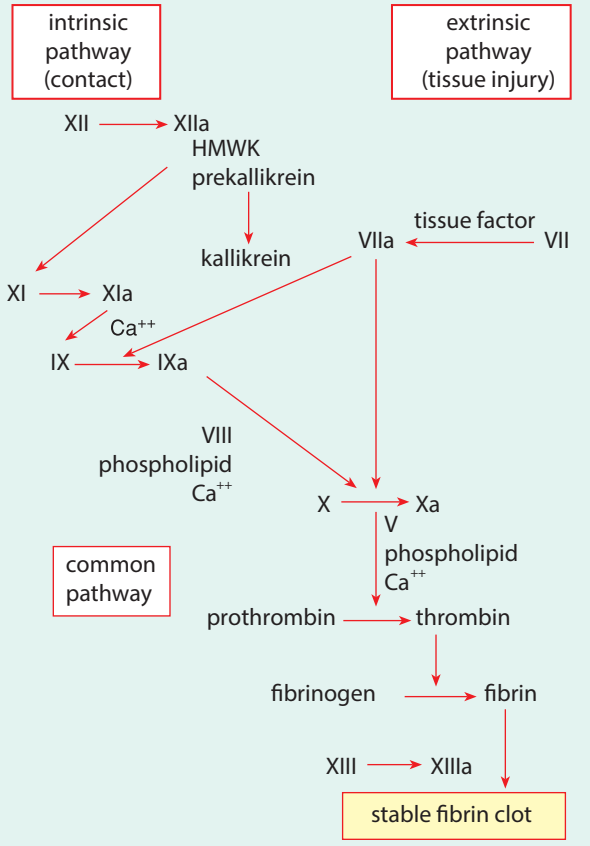

Fig. 4.8.: The clotting cascade.

Ehlers-Danlos syndrome

Autosomal dominant, complex disease, that is caused by pathological collagen formation. It is accompanied by an increased elasticity of the tissues, resulting in vascular purpuras, increased flexibility of the joints (this condition is frequent among flexible circus acrobatic artists and performers). Large skin folds can be formed even in the region of the elbows and the knees, which flexibly retract to the original form. Similar elasticity is found in other organs too, which explains the higher risk for development of varicose veins and mitral valve prolapse.

Acquired

Senile purpura

It is characteristic even among the healthy elderly: the weakening of the vascular wall structures is in the background. It can complicate symptoms and consequences of other wall-structure disorders (e.g., small bleedings in the brain).

Vitamin C deficiency

The tertiary structure of collagen is changed second-

arily to insufficient activity of the Lys and Pro-hydroxylase enzymes, which are responsible for the posttranslational hydroxylation of the collagen (vitamin C keeps the iron, found in the center of the enzyme, in reduced, ferrous form). The stability of the β-helix, consisting of three chains, is decreased, and a pathological condition develops, involving fragile vessel walls, purpuras, and gingival bleedings.

Cushing disease and syndrome (see 10.8.4.)

In high cortisol levels, increased protein catabolism (due to augmented gluconeogenesis) and decreased collagen production can be observed, the basement membrane of the vessels is also weakened.

Henoch-Schönlein purpura

It develops secondarily to a post-streptococcal, viral, or drug-induced immune reaction. During childhood, it generally presents itself as a sudden development of purpuras on the extensor surface of the extremities following an upper airway infection. Hematuria (see 5.3.3.2.) indicates renal involvement (acute diffuse glomerulonephritis, see 5.5.3.4.) which may result in chronic renal failure the long term.

Sepsis

Development of meningococcal sepsis can be indicated by the unexpected appearance of skin purpuras during airway infection. Sepsis-induced damage of endothelial cells is presumably lurking in the background.

Allergy

Allergic vasculitis can trigger small vessel injuries, causing purpuras in the skin and the mucosa, as well as, purpuras leading to articular, gastrointestinal, and renal symptoms.

Rheumatoid arthritis

There is a vasculitis of immunological origin in the background. Purpuras due to small vessel vasculitis can occur as brownish dots in the cuticle, microvascular damages can be demonstrated, among others, in synovial membranes, lungs, intestines, liver, and spleen.

In all cases dotted, capillary-like, purpural-petechial bleedings, and prolonged bleeding time can be found[10]. The capillary resistance tests[11] are positive, however, the clotting time[12] is normal.

Today, additional clotting parameters are also measured, however, these change similarly as the classical clotting time. **Prothrombin time** (PT): duration between tissue administration of thromboplastin o the plasma and clot formation. Since the PT reference values differ among laboratories, the WHO introduced the use of INR (International Normalized Ratio) to standardize the results among patients, who receive anticoagulant therapy. This test can help to control whether the dosage of anticoagulant drugs is correct, and to diagnose bleeding disorders. In patients on anticoagulant therapy, the INR is adequate between 2.0 and 3.0. Calculation: INR = (patient PT / control PT)^{ISI} (ISI = International Sensitivity Index /sensitivity value shown on all reagents by the law, which indicates the reagent's sensitivity to thromboplastin compared to the WHO's reference preparation/). The INR in a healthy patient is 1, if the blood coagulation is normal.

Activated partial thromboplastin time (APTI): following incubation of a negative surface-providing contact activator together with plasma, the duration measured between administration of Ca^{++} and the appearance of the clot. Thrombin time: duration between administration of thrombin to the plasma and the development of the clot (it indicates the speed of the fibrinogen-fibrin transformation).

4.4.2. HEMOSTATIC DISORDERS OF PLATELET (TCT) ORIGIN

Platelets (TCTs), produced by megakaryocytes are not real cells, however they are able to recoup their energy needs by producing ATP from glycogen in their mitochondria. They are also able to secrete and store biolog-

[10] Bleeding time: tested by the Ivy-method: a blood pressure cuff is inflated on the upper arm to a pressure of 40 mmHg. An 8-10-mm long and 1-mm deep incision is made on the palmar surface of the lower arm, and bleeding is tested by a filter paper, by contacting the lower edge of the wound every 30 seconds. Normal: bleeding stops within 3-7 minutes.

[11] Capillary resistance (Rumpel-Leede) test: the blood pressure cuff is placed on the upper arm, and its pressure is set between the diastolic and systolic values, at or about 100 mmHg. After 15 minutes, the number of purpuras, which developed in a designated circle with 2.5 cm in diameter, 4 cm down from the elbow, is counted. This count is normal below 10, moderately abnormal if it is 10-20, and abnormal above 20. Vacuum disks (e.g., on the neck) can cause similar effects.

[12] Clotting time is the duration until the first fibrin filament appears in the blood placed into an hour-glass. The normal value is 5-10 minutes.

ically active substances, adhesion molecules, clotting and growth factors, nucleotides, and enzymes (e.g., ADP, Ca^{++}, serotonin /5-HT/, thromboxane, thrombosthenin, von Willebrand factor) in their cytoplasmic granules, the membrane of which contains phospholipids. Their lifespan is about 8-10 days in the circulation. Their normal count is 150-400 G/l, however, this can rapidly change due to the mobilization of platelets „stored" in the spleen.

Main platelet functions: in the presence of endothelium-derived ADP, they adhere to the injured collagen fibers of the vessel wall (adhesion), during which the glycoprotein of the platelet membrane is attached to the collagen fibers with von Willebrand factor. The shape of the adhered platelets changes, their granules are depleted, ADP is released, which will trigger further adhesion. Thrombocyte-factor-3 is also released, which triggers the aggregation of thrombin and platelets (this is inhibited by endothelial prostacyclins, yet induced by TCT-derived thromboxanes). ADP induces a loose adhesion of fibrinogen to the thrombocytes, resulting in a „white thrombus". A stable bond will only develop later, once fibrin is produced from fibrinogen, enclosing the corpuscles and creating the "red thrombus". At the same time, vasoconstrictor serotonin and contractile thrombosthenin are released from the TCTs, the latter being responsible for retraction of the clot. Due to the effects of the released lysosmal enzymes, an inflammatory reaction is initiated.

Thrombocyte-insufficiency develops if the TCT count drops below 50 G/l (**thrombocytopenia**), or if their count is normal, but they are dysfunctional (**thrombasthenia**). It is commonly characterized by prolonged bleeding time (insufficiency of the production of primary clot), *purpural, petechial bleedings (small, not bigger than pinpoint size bleedings)*, however, in severe cases, *suffusions,* low blood volume extending to large surface, may likely develop. In severe cases, the attachment of the fibrin net (developing without primary clot) to the vascular wall also becomes abnormal, and in part due to this, and in part, due to the lack of TCT-derived coagulation factors, bleeding will be pronounced. Additionally, the retraction of the clot is disordered. *In vitro clotting time is normal*, the capillary resistance tests are negative.

Overactivity (thrombocythemia) is defined as too high TCT count and results in an increase in the tendency to develop thromboses. In myeloproliferative disorders the TCT count can exceed 400 G/l, which exaggerates the development of intravascular thrombi, occasionally paradoxical mucosal bleedings, due to microinfarctions. This may also be found in polycythemia. Reactive thrombocytosis is known as part of the acute phase reactions in systemic inflammations.

THROMBOCYTOPENIAS

Decreased TCT production

Hereditary

Wiskott-Aldrich syndrome
Deceased cellular and humoral immunity (IgM deficiency), along with thrombocytopenia, characterized by atopic dermatitis (eczema), and increased susceptibility to autoimmune and hematological diseases. The inheritance is linked to the X-chromosome.

Fanconi syndrome (see aplastic anemias)

Acquired

Bone marrow diseases, for specific causes, please see aplastic anemias.

Increased sequestration

Splenomegaly
If spleen congestion develops, due to portal hypertension or right heart failure, the filtration of the blood corpuscles is increased.

Idiopathic (autoimmune) thrombocytopenic purpura (ITP)

Platelets are destroyed by viral, bacterial or drug-induced immune reaction (IgG autoantibodies against TCTs).

In childhood, it presents itself in an in acute form following an upper airway infection, or viral rash disease. The patients **usually** recover within 1-6 months. In adults, the chronic form (lasting several years) is more characteristic, which develops as a consequence of the occurrence of antibodies against IIb-IIIa, and Ib-IX surface-glycoproteins. Thrombocytopenia of immunological origin may appear in hepatitis, cytomegalovirus infections, furthermore, HIV infection lies in the background with increasing frequency. These aspects may all likely lead to the first symptoms of autoimmune diseases, such as systemic lupus erythematosus (SLE) accompanied by the appearance of an antinuclear antibody.

ITP can be induced by drugs, e.g., penicillin, which bind to the TCT membrane, and the antibodies produced against them destroy the TCTs. Other drugs, e.g., heparin, also bind to the membrane and induce immune

reaction together with the TCT surface antigen in the form of a hapten, which then leads to the destruction of TCTs. Definite autoimmune hemolytic anemia and thrombocytopenia (anti-TCT antibodies occur which can independently bind to TCTs) is induced by the administration of methyl-DOPA, however, the exact role of the drug is unclear regarding the pathogenesis. The thrombocytopenia-inducing drugs also include, e.g., sulfonamides, digoxin, and certain anxiolytics.

As part of disseminated intravascular coagulopathy (DIC)

Consumption coagulopathy: TCTs are consumed due to extensive systemic microthrombus formation (see below).

Vasculitides

In *hemolytic-uremic syndrome* (HUS), the development of the disease is preceded by bacterial diarrhea (e.g., E. coli 0157:H7, Shigella, Salmonella, Yersinia) and sometimes by upper airway infection. Bacterial toxins destroy the small renal vessels, resulting in renal failure, uremia, and endothelial damage of small vessels with uneven surfaces, thereby leading to the destruction of RBCs and TCTs with hemolysis, thrombus formation and thrombocytopenia.

THROMBASTHENIAS

Hereditary

von Willebrand disease

Von Willebrand factor, produced in the endothelial cells, helps the adhesion of TCTs to the endothelium. It is also essential for the activity of factor VIII. If it is absent, then adhesion disorders and coagulopathy develop.

Bernard-Soulier disease

Lack of GPIb-IX surface glycoproteins impairs adhesion.

Glanzmann disease

Lack of GPIIb-IIIa surface glycoproteins inhibits TCT aggregation.

Secretion disorders

The lack of secretion granules, insufficient ADP production, abnormal enzymatic synthesis of thromboxane, insufficient production of thrombocyte-3-factor, and disorders of clot retraction can also occur.

Acquired

Aspirin

It belongs to the nonsteroidal anti-inflammatory drug (NSAID) group and inhibits the constitutive cyclooxygenase (COX) enzyme, activated in inflammatory states. COX is found in most tissues and in TCTs. Once aspirin is bound to the enzyme, its regeneration requires 5-6 days. In the lack of COX-produced thromboxanes, the TCT aggregation disordered. A regular intake of small-dose aspirin (81-160-320 mg/day) proved to be useful in the primary and secondary prevention of AMI (about 25 % decrease in incidence). Decreased TCT aggregation inhibits the development of thrombotic-vascular obstruction on the grounds of atherosclerosis.

Glycoprotein IIb-IIIa inhibitory drugs

Thrombasthenia can be also induced by inhibition of the TCTs' GPIIb/IIIa cation-dependent adhesion molecules (e.g., abciximab, eptifibatide or tirofiban), which belong to the integrin group. These intravenously applicable drugs, which, as of yet, have no appropriate antidotes, are used in percutaneous coronary interventions and stent implantations to inhibit TCT aggregation.

Toxins

Uremic toxins or other toxins accumulating in hepatic failure inhibit not only TCT functions, but also the proliferation of the megakaryocyte cell line in the bone marrow.

DIC

In this complex hemostasis disorder, not only is the consumption thrombocytopenia (see below) problem-problematic, but it also also includes the inhibitory function of the fibrin degradation products (FDPs) on the TCT functions.

Antibiotics

Some antibiotics (e.g., penicillin) may cause thrombasthenia.

4.4.3. COAGULOPATHIES

Plasma clotting system disorders lie in the background of **coagulopathies**. The essence of clotting is the precipitation of fibrin from fibrinogen, however, this is based on a chain reaction (cascade). The cascade can start ***intrinsically*** or ***extrinsically,*** however, the final steps (Fig. 4.8.) are common. Fibrin must be stabilized, then retracted, and followed by fibrinolysis. Insufficiency of individual factors results in different types of coagulopathies, yet these are commonly characterized by ***normal bleeding time and prolonged clotting time***, and they typically result in incomparably ***larger bleeding*** than those of thrombocytic or vascular origin.

To prevent the unnecessary intravascular thrombus formation, endogenous anticoagulants and intact endothelium are required. On the luminal surface of the intact endothelium, there is no TCT adhesion or activa-

tion of coagulation processes, but, anticoagulant factors (such as PGI_2, thrombomodulin, heparan-sulphate, and plasminogen activators) are predominant. However, in reference to contraluminal surface of the endothelium, procoagulant factors (collagen, tissue thromboplastin, fibronectin, proteoglycans, and von Willebrand factor) correspondingly act. Additionally, procoagulant factors can also originate from the activated TCTs.

The lack of plasma clotting factors results in decelerated clotting, or clotting may not even commence. Such injuries, which cannot be controlled by the primary hemostasis (vascular and platelet factors), may result in immense, even lethal, external or internal forms of bleeding. The bleeding time is normal, however, the clotting time is significantly prolonged. Not all factor deficiencies develop *in vivo*: a decreased level of factor IV (Ca^{++}) *in vitro*, may prevent coagulation, yet there is no such extent of drop in Ca^{++} levels *in vivo*, which inhibits the coagulation cascade. Consequently, when considering hypocalcaemia, tetany or arrhythmias dependent upon the severity, may unfortunately result in premature death. The lack or insufficiency of the different factors can be either acquired or inherited.

4.4.3.1. INHERITED COAGULOPATHIES

Hemophilia A: (lack of factor VIII)
It shows an X-chromosome linked recessive inheritance, with a prevalence of 1/10.000 newborn boys. It is consequence of a relatively *frequent chromosome mutation*, and the stable incidence (number of newly diagnosed cases) refers to continuous appearance of new mutations. Mothers are carriers, the disease manifests in boys, while the extremely rare homozygote girls expire in the intrauterine life. The essence of the disorder is the lack or dysfunction (1-5 % activity instead of the normal 50-200%) of factor VIII, produced in the endothelial cells of the hepatic sinusoids. Factor VIII deficiency affects the common part of the coagulation pathway, therefore, the consequences are considerably severe. Large musculoskeletal bleeding (hematomas), iliopsoas and retroperitoneal bleeding, bloody cysts, bleeding in the joints (= hemarthros – knee,) all can lead to severe movement disability), spontaneous hematuria, and intracranial bleeding can be observed. Iliopsoas bleeding can damage nerves, and hemarthros results in cartilage loss and arthrosis. Since the bleeding time is normal, massive bleeding occurs hours following a routine tooth extraction.

Due to the short biological half-life of the factor, bleeding patients are treated with fresh blood products (or lyophilized concentrates). Today, modern therapy is based on recombinant and plasma-derived factor VIII concentrates. Ideally, patients can administer factor VIII intravenously according to their own specific needs in the privacy of their homes. This modern treatment is unfortunately not widely used as of yet. Risks and perils regarding various forms of treatment include the following: production of antibodies against factor VIII and infections (hepatitis B, C, AIDS).

Haemophilia B: (lack of factor IX /Christmas factor/)[13]
Another X-chromosome linked disease, caused by the lack or abnormal structure of factor IX (Christmas factor). Based on the clotting cascade, it can be understood why the consequences are similar to those associated with hemophilia A. Different blood products are used in the treatment (half-life of factor IX is longer than that of factor VIII); the dangers are due to the presence of activated clotting factors, with subsequent thrombosis, in addition to the risks of polytransfusion.

Haemophilia C: (lack of factor XI)
The lack of factor XI is a rare and mild disease. It is a part of the intrinsic pathway, and clotting can be activated extrinsically.

von Willebrand disease: (lack of factor vWF)
Autosomal (co)dominant (equally present in males and females) inheritance, involving the lack or abnormality of the endothelium-derived vWF. The vWF is produced as monomer, yet the high molecular weight multimers (connected by disulfide bridges) are much more effective. The mean prevalence of the different forms with variable severity is nearly 1% in the population. Apart from TCT dysfunction, factor VIII is unable to bind to and undergo transportation by vWF, therefore, the plasma levels of factor VIII are relatively low. The symptoms are comprised of coagulopathy (prolonged clotting time), thrombasthenic purpuras, and prolonged bleeding time. In mild forms, bleedings can be observed only in special circumstances, such as major traumas or surgeries, while severe forms result in spontaneous epistaxis even without trauma and in heavy periods (menorrhagia).

[13] Historical notice: Hemophilia B was frequent among the family relatives of Queen Victoria, thus Alexei Tsarevich suffered from the condition as well – and this was the basis of Rasputin's influence.

Rare, inherited coagulopathies:
Afibrinogenemia is a rare (1:10^6) bleeding disorder associated with autosomal recessive inheritance caused by the lack of fibrinogen. Its less severe form, hypofibrinogenemia (fibrinogen level is less than 25% of normal) results in severe symptoms as well. Dysfibrinogenemia (appearance of pathological fibrinogen forms) is an autosomal dominant disease causing coagulopathies (40-50%), can be asymptomatic (40%), yet can facilitate the manifestation of venous or arterial thromboses (nearly 10-15%). The inherited insufficiency of factor XIII or VII is very rare. The lack of factor XII usually does not cause bleeding disorders, while factor VII deficiency shows milder symptoms than when compared with classic hemophilias (nasal and gingiva bleeding and/or bleeding after trauma). The inherited lack of factor XIII (transglutaminase, which stabilizes the fibrin clot) is a very rare disease, these patients are susceptible to umbilical bleeding following birth and, later in their life, are prone to intracranial bleeding.

4.4.3.2. ACQUIRED COAGULOPATHIES

These are based on disordered synthesis of clotting factors, or on appearance of antibodies against certain factors, or even on drug effects.

Vitamin K deficiency: The liver is unable to produce the γ-carboxy-Glu (Gla) containing, posttranslationally modified, Ca^{++}-binding II, VII, IX and X factors. The ***anticoagulant*** coumarines and warfarins inhibit the regeneration of the epoxy form of vitamin K. These drugs are administered in atrial fibrillation or in the case of akinesis, following myocardial infarction in order to prevent the clot formation, as well as, in the prophylaxis of deep venous thrombosis among the elderly and/or infirmed patients).

An overdose of newly developed anticoagulants (rivaroxaban, apixaban – **activated factor X inhibitor**, dabigatran – **specific thrombin inihibitor**), **which have different mechanism of action from coumarines**, can cause coagulopathy as well, and in particular, since they do not yet have antidotes.

Hepatocellular disorders: These cause multiple clotting disorders. Hepatic failure may result in insufficient production of vitamin K-dependent clotting factors (II, VII, IX, X, including C and S proteins) along with dysfibrinogenemia, a decreased level of antithrombin III, thrombocytopenia (decreased production and increased sequestration induced by splenomegaly), increased fibrinolysis, and decreased clearance of activated clotting factors.

Fibrinolytic treatment (***streptokinase***), or primarily accelerated fibrinolysis can cause coagulation disorders due to the augmented fibrin dissolution. Streptokinase is the plasmin-activating agent of beta-hemolytic Streptococcus, which binds to plasminogen, thus it can transform other plasminogen molecules to activated plasmin. Endogenous urokinase, found in large amount in pelvic tissues, or its recombinant forms, and other non-tissue-derived plasmin activators are often used as well. An overdose of these substances results in acquired coagulopathies.

Heparin (or its synthetic pentasaccharide analogue) decreases clotting by inhibiting the effect of thrombin and activated factor X (Xa) (by binding to antithrombin, changes its conformation and increases its effectivity). Unfractionated heparin consists of a mixture of materials of 4-30 kD molecular weights, which bind to antithrombin, resulting in its higher activation, thereby, leading to equal inhibition of serinproteases, such as thrombin and Xa. Fractionated, low molecular weight heparin (LMWH) components with 5 kD inhibit factor Xa 2-4 times stronger than thrombin, by binding to antithrombin. However, in contrast with unfractionated preparations, they can be safely administered based on body weight without close labor control. Consequently, mainly the latter form is used in clinical practice. Overdosing, which is rare, can increase the risk of bleeding.

Appearance of autoantibodies against factor VIII was described in postpartum women, susceptible to autoimmune disease. In systemic lupus erythematodes (SLE) patients, these antibodies can appear, although antibodies against cell nuclei are characteristic in this disease. SLE is one of the autoimmune diseases with the most variable symptomatology, in which, in addition to classical erythema, multiorgan manifestation with, e.g., fever, arthritis, anemia, and renal failure, can be observed. Antibodies against factor VIII can be present in the elderly and in polytransfused (hemophilic) patients as well.

Disseminated intravascular coagulopathy is a complex clotting disorder.

DISSEMINATED INTRAVASCULAR COAGULOPATHY (DIC)
Definition
A life-threatening disease, characterized by widespread microthrombus formation, vascular fibrin precipita-

tion, obstruction of the small vessels, tissue hypoxia, bleeding, tissue damage and simultaneous organ failures, due to a rapid activation of the clotting system. Due to the massive clot formation, platelets and clotting factors are consumed, in parallel with the activation of the fibrinolytic system, inevitably leading to inefficient clotting.

Phases

1. In the *thrombotic phase* there is massive microthrombus formation due to systemic activation of the coagulation system, which results in fibrin precipitation and obstructions in small vessels, tissue hypoxia, suffusions, tissue damage, and multiorgan failure.
2. *Phase of consumption coagulopathy* develops when, due to the extensive clot formation, the thrombocytes and clotting factors are consumed, leading to the development of the characteristic bleeding disorder.
3. In addition to the coagulation cascade, the fibrinolytic system (plasmin) is also activated, which further exaggerates the susceptibility for bleeding. Plasmin cleaves fibrin (and fibrinogen), and the fibrin degradation products (FDPs) and substances cleaved from the stable fibrin (D-dimers) appear in the circulation. These products further impair clotting – *fibrinolytic phase*.
4. Tissue damage in DIC (cerebral infarcts, renal cortical necrosis, lung infarcts, widespread, superficial gastrointestinal erosions, skin necroses) and hemorrhages (hemorrhagic strokes, hematuria, gastrointestinal bleeds, and skin petechias) appear simultaneously and characterize the clinical picture.

DIC is a potentially lethal disease, characterized usually by dramatically rapid progression, leading to circulatory shock and multiorgan failure. Rarely (in 5-10%), e.g., in the case of extended adenocarcinomas, a milder, permanent, chronic form occurs, which is characterized by moderate microthrombus formation. Vasospasm of small vessels due to endothelial damage and microthrombotic obstructions lie in the background of the developing acrocyanosis (bluish discolorations of the fingers, nose, and genitals, progressing later to pregangraenosus lesions).

Pathogenesis

The primary activation of the intrinsic, extrinsic, and fibrinolytic systems can play a role in the pathogenesis (Fig. 4.9.).

During the activation of the ***intrinsic pathway*** (e.g., due to extensive endothelial damage in shock, or due to endothelium-damaging factors), the endothelial damage or the endotoxin activates factor XII (Hageman) first. Then, factor XII will 1) initiate the intrinsic cas-

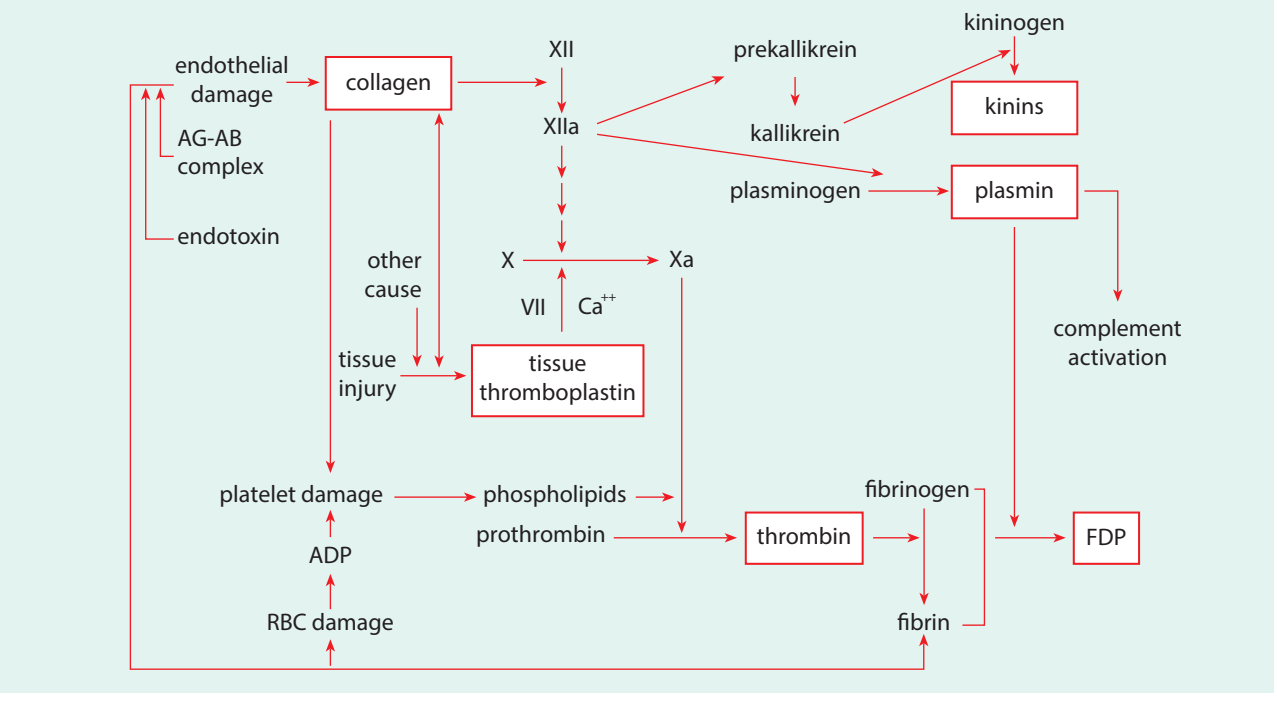

Fig. 4.9.: Pathomechanism of disseminated intravascular coagulopathy (AG-AB = antigen-antibody).

cade, 2) activate the kallikrein-kinin system, resulting in the production of bradykinin, which causes vasodilation and, subsequently, hypotension, 3) cleave plasmin from plasminogen, thereby initiating fibrinolysis. During the development DIC via the intrinsic pathway, hypotension and fibrinolysis are seen relatively early. Endothelial damage will also activate the TCT system, and decrease the effect of endogenous anticoagulant factors.

As a first step in the activation of the ***extrinsic pathway***, a large amount of tissue thromboplastin enters the circulation due to significant tissue damage. This will activate factor VII first, which, in turn, activates factor X (the first factor of the common phase in the clotting cascade) and factor IX (the last step of the intrinsic pathway prior to merging into the common part). In contrast to the activation of the intrinsic pathway, fibrinolysis and hypotension occurs relatively later, only following the thrombotic phase, when circulatory shock develops.

Primary activation of the fibrinolytic pathway will not cause a bleeding disorder initially, however, the final picture resembles DIC. During this process, pathologically activated plasmin cleaves fibrinogen instead of fibrin, thereby, producing fibrin degradation products (FDPs). The FDPs inhibit coagulation and TCT functions, thus leading to a bleeding disorder. In such situations, even blood withdrawal, insertion of an i.v. cannula, or fresh (even surgical) wounds result in severe forms of bleeding. This immense loss of blood leads to circulatory shock and to activation of the intrinsic pathway.

Causes of DIC

Activation of the intrinsic pathway (initiated by widespread endothelial damage)

- Circulatory shock
- Sepsis: proteoglycans of Gram+ bacteria, endotoxins of Gram– bacteria (e.g., meningococcal sepsis with Waterhouse-Friderichsen syndrome
- Extreme cold or heat (severe hypothermia and heat shock)
- Severely abnormal pH
- Aortic aneurysm: chronic, non-dissecting form
- Hemolytic-uremic syndrome
- Acute glomerulonephritis

Activation of the extrinsic pathway (factor VII is activated either by widespread tissue injury or by substantial release of tissue thromboplastin)

Tissue damage

- Burns, frostbite, extensive tissue-damage in trauma
- Severe head injury (e.g., subdural hematoma, contusion hematoma)
- Polytrauma (a combination of injuries that affect multiple organs, or organ systems, in which one of the injuries, or the common effect of more injuries, is directly life-threatening, e.g., injury involving two body cavities, or a body cavity and two long bones. It is very frequent in cases of, e.g., vehicle accidents. Due to the extensive tissue damage, tissue thromboplastin is released into the circulation.)

Obstetric syndromes

During placenta injury in different obstetric syndromes, amniotic fluid, which is highly rich in tissue thromboplastin, enters the venous circulation through the opened venous sinuses.

- Amniotic fluid embolism
- Abruption of the placenta (placenta previa)
- Dead fetus syndrome
- Abortion in the 2nd trimester

Fat embolism (polytrauma, e.g., fracture of the femur)

Malignant diseases

- Promyelocytic leukemia
- Extended adenocarcinoma
- Other cancers, such as lung, stomach, breast

Intravascular hemolysis

- Particularly the immune hemolyses (e.g., incompatible transfusion), - which also damage the endothelium

Heat stroke (ch. 8.5.2.3.)

Acute pancreatitis (ch. 7.5.1.)

Primary activation of the fibrinolytic system

- Prostate cancer (The gene of urokinase becomes activated in the tumor tissues due to demethylation, and its product, urokinase, as a protease, can promote tumor extension and metastasis formation. It activates plasminogen as well.)
- Urogenital inflammation and various forms of surgery (release of urokinase, tissue activators). In the pelvic tissues, there is abundant endogenous plasminogen-activator urokinase. This creates an increased risk in pelvic surgeries, tumors, and inflammations.
- Hepatic cirrhosis (due to the decreased liver activity, decreased elimination of tissue plasminogen activators and fibrinolytic agents).
- Snake venom (e.g., toxin of *Vipera lebetina* contains fibrinolytic lebetase [Zn metallo-endopep-

tidase] and fibrinogen-degrading proteases, toxins of other vipers contain plasminogen activator /serinprotease]/).

The course of DIC

In ***acute*** DIC, the widespread tissue damage may evoke fever, inflammation, even systemic inflammatory response syndrome (SIRS). The bleeding, endothelial damage, and systemic inflammation lead to the development of circulatory shock (hemorrhagic and distributive types), or they aggravate the progression of a pre-existing shock. Tissue ischemia and lactate acidosis develop. Consequently, multiple organ dysfunctions (MOD), and inevitably ***multiple organ failure*** (MOF) develops. The most severely affected organs: lungs, kidney, brain, gastrointestinal tract. In severe forms, the mortality rate is high.

The rare, ***chronic*** forms of DIC are typically associated with tumors, in which the formation of microthrombi is slow and moderate. The characteristic bleedings and fever are absent, and in their place, ***acrocyanosis*** develops. The acral parts (nose, fingers, genitals, etc.) are severely ischemic, moreover, pregangrenosus lesions may develop. The laboratory parameters of coagulation are abnormal (prolonged prothrombin time, low fibrinogen level, presence of FDP and D-dimers). Spontaneous bleeding is not characteristic; the bleeding disorder develops only in cases of severe trauma.

Treatment of DIC: in many cases, attempts are made to replenish the plasma volume and clotting factors by the administration of fresh/lyophilized plasma. If the TCT count is very low (<50 G/l), it should be also normalized. Heparin is rarely used, if and when microthrombus formation is predominant (mainly in chronic forms). It is important, however, to treat the primary pathogenic cause of DIC, and to replace volume, if necessary.

4.4.4. ENDOGENOUS ANTICOAGULATION AND FIBRINOLYSIS DISORDERS

Minimal microthrombus formation may be normally present in the vascular system. Neither this, nor any kind of larger coagulation should extend substantially beyond the injured area. Progressive clot extension (or in case of no injury, clot formation) is prevented by different mechanisms:

1. Endogenous inhibitors neutralizing the activated factors (endogenous heparin, antithrombin III, thrombomodulin, protein C and S, and other endothelium-, PLT- or plasma-derived factors);
2. Intermediary products of coagulation (thromboplastin, fibrin monomer) are removed by the RES and leukocytes, and diluted by the circulation;
3. The fibrinolytic system dissolves even the activated and stabilized fibrin.

Protein C is a vitamin K-dependent plasma glycoprotein (protein S has the same dependence, it supports the function of protein C), which binds to about one half of the circulating thrombin, and decreases the affinity of thrombin for binding fibrinogen, thereby inhibiting fibrin formation. Thrombomodulin is an endothelial receptor, which binds thrombin (thus removes it from the circulation), and enhances the activity of protein C, which in turn, acts as a protease and neutralizes the activated factors V and VIII.

Removal of intermediary products and activated coagulation factors (liver) obviously decreases clotting functions.

Fibrin-degradation products (FDP, D-dimer) accelerate fibrinolysis and inhibit clotting.

Fibrinolysis is achieved by plasmin, which is derived from plasminogen upon the effect of tissue or urokinase activators, or exogenous substances (streptokinase). Normally, plasmin's action is limited to the thrombus, however, its enhanced activation is also known (e.g., in DIC), and suppressed physiological inhibition of the fibrinolytic system is also possible (normally, e.g., the menstrual blood). Deficiency of physiological plasminogen activators, including the appearance of abnormal plasminogen, or augmented production of plasminogen activator inhibitors (PAI) (in the adipocytes of obese patients) can lead to a tendency for thrombosis (conversely, impaired hepatic degradation of PAI in liver failure contributes to coagulopathy).

4.4.5. THROMBOSES

The development of thrombosis is typically preceded by an imbalance between the different pro- and anticoagulatory factors, which leads to increased coagulation. Very rarely, it can occur in the arteries (white), yet most commonly it is a venous disorder (red). Occasionally, wall thrombus can develop in the heart chambers in atrial fibrillation and/or following myocardial infarction.

Most important causes of the development of thrombosis (Virchow's triad)

1. ***Decreased blood flow, turbulent circulation***

Inactivity

In prolonged sitting (immobilization), e.g., during (several hours) long journeys by car or plane, when venous blood-pumping effect of the lower-limb muscles are absent and the veins are „folded", thrombus formation can develop.

Chronic immobilization (bed-rest, e.g., in stroke)

Varicosity

If superficial and deep veins of the lower extremity are pathologically dilated, the function of the venous valves to normally prevent blood backflow is blunted, the blood flow becomes slower and turbulent.

Obesity

The accumulation of visceral fat inhibits venous return in the veins of the legs.

Pregnancy

The uterus in pregnancy prevents free blood flow in the pelvic veins.

Polycythemia

High RBC count leads to hyperviscosity and decelerated circulation.

Urogenital compression

Pelvic, mainly gynecological, tumors lead to iliac vein compression, which results in acute or chronic venous insufficiency of one or both lower limbs. All factors promoting venous congestion, also exaggerate the development of thrombi.

1. ***Vascular injuries***

Mechanical injuries

Sclerosis

Rupture or superficial injury of the atherosclerotic plaque in the arterial system (e.g., coronaries, carotids) will result in thrombus formation.

Inflammation

Thrombophlebitis typically leads to thrombosis, which, in turn, results in the inflammation of the given section of the vessel.

Toxins (e.g., allylamine and beta-aminopropionitrile)

Circulatory shock

In the late, refractory phase of circulatory shock, ischemic damage of the endothelial cells occurs, which is worsened by the sludge formation due to slow circulation and by the developing microthrombi.

Vascular injury results in local release of thromboplastin; endothelial damage can cause an insufficiency of the endogenous anticoagulant factors (thrombomodulin – protein C system).

2. ***Increased coagulability, decreased fibrinolysis***

Leiden mutation of factor V:

It is responsible for 25% of all inherited thrombophilia cases. In position 506 of the peptide chain, Arg is replaced by Glu. The activated factor V is resistant to protein C. The risk of thrombosis is seven times higher in heterozygotes and fifteen times higher in homozygotes, when compared to that of the healthy population. Other thrombophilia factors e.g., pregnancy, oral contraceptives, can further increase the risk for thrombosis.

Antithrombin III deficiency

A decrease in the function of antithrombin III causes a proportional increase of thrombophilia. The heterozygous form is relatively frequent: 1:2000.

Protein C deficiency

Protein S deficiency

Dysfibrinogenemia

Abnormal plasminogen

Increased levels of factors VII and IX

Polycythemia vera

Increased responsiveness of the bone marrow to erythropoietin, thrombopoietin, and other growth factors results in very high RBC count (9-10 T/l), which, at this level, exponentially increases the blood viscosity. Thrombocytosis, accompanied with increased coagulability, is also part of the disease. Even arterial thrombus formation can occur.

Estrogen therapy

The newest, low-dose, oral estrogen treatment increases 2-3 times the risk of venous thrombosis. Several beneficial cardiovascular effects of estrogen were formerly described, e.g., decreased myointimal and smooth muscle hyperplasia, increased endothelium-dependent venous vasodilation, decreased production of the atherosclerosis-promoting, platelet-derived growth factor in the TCTs, nevertheless orally (yet not transdermally) administered estrogen is thrombogenic in the coagulation system. It has been demonstrated that the levels of antithrombin III and of the anticoagulant protein S were decreased and protein C resistance developed in women using oral contraceptives (orally administered drugs occur in the liver in higher concentration compared to transdermal application). The thrombogenic effect of estrogen may further increase the risk in genetic thrombophilias.

Paroxysmal nocturnal hemoglobinuria

A binding molecule, glycosyl-phosphatidyl-inositol, and binding proteins are missing from the surface of PLTs and RBCs, as a consequence, the responsiveness to activated complement is augmented, leading to the activation of PLTs and increased thrombophilia.

Obesity

Abnormally activated (for details, see consequences of obesity) visceral fat cells produce inflammatory cytokines, hormones, and insulin resistance-inducing substances, as well as, inhibitor of plasminogen activator (PAI-1), thereby preventing fibrinolysis. Additionally, originating from adrenal androgens these cells produce estrogen, which has its own thrombogenic effect.

Diabetes mellitus

Levels of fibrinogen, factors VII, VIII, XI, and XII are increased in DM, while the level of protein C is decreased. Thrombocyte functions are exaggerated. Hyperglycemia and subsequent hyperosmolality results in increased expression of P-selectin induced by thrombin receptor-activating peptid (TRAP) in the PLTs via superoxide anion production, and leads to increased TRAP-induced fibrin binding via the protein kinase C (PKC) signal transduction pathway. In addition to the increased coagulation, fibrinolysis is impaired, as indicated by the higher plasma PAI-1 levels. Endothelial injuries, resulting from the hyperglycemia, also increase the risk of thrombosis.

Homocysteinemia

Homocysteine is the sulfur-containing degradation product of the amino acid methionine in humans (see 9.1.2.2.). Through impaired endothelial NO production (it promotes the production of a NOS inhibitor, asymmetric dimethyl-arginine, and decreases the activity of the antioxidant glutathione-peroxidase), it increases the oxidative damage of the endothelium and decreases endothelium-dependent vasodilation. It facilitates vascular smooth muscle proliferation, TCT activation and aggregation (platelet-derived TXA_2 production increases). Through reduced DNA methylation, certain genes are activated, which can influence the function of both endothelium and vascular smooth muscle. Free radicals produced in homocysteine metabolism and its metabolite can both modify LDL, which accelerates atherogenesis.

Homocysteine has several effects on coagulation, e.g., it increases the activity of factor V and VII and the production of thrombin, while it suppresses the inactivation of factor V, the production of plasmin, and the expression of thrombomodulin. In summary, by damaging endothelial antithrombotic mechanisms, promoting coagulation processes, and decreasing fibrinolysis, homocysteine accelerates the risk of venous and also arterial (especially due to atherosclerosis) thrombosis (it is an important pathogenic factor in nearly 10% of all thromboses).

Stress

The aggregation of TCTs is enhanced by catecholamines. Activation of alpha-2-adrenergic receptors, and subsequently increased expression of P-selectin on the TCTs' surface, changes in the GPIIb/IIIa conformation lead to degranulation, thus consequently resulting in TCT, leukocyte, and endothelium activation.

The increased activity of coagulation factors and decreased activity of fibrinolysis elevate the risk of thrombosis.

Consequences of thromboses

Consequences of ***venous thrombosis*** are, in part, local: circulatory and ischemic disorders (swelling, cyanosis) of the affected area due to the occlusion; in part, distant caused by the effects of an embolism released from the thrombus. Thrombi formed in the lower extremities (mainly deep venous) are filtered in the pulmonary circulation. A major portion of pulmonary embolisms (PE) is not successfully diagnosed even today (especially among the elderly the diagnosis is difficult), while the mortality without treatment is nearly 30%. The diagnosis is complicated by the fact that, the characteristic symptoms: dyspnea, pleuritic, sharp, localized chest pain, blood in the sputum, and cough are present only in a small percentage of the patients. Physical signs are not entirely specific either: tachypnea (>16/min), rales and ronchi, tachycardia, fever, acute muscle weakness, confusion, sweating, lower extremity edema, and cyanosis, however, even these signs are not clearly present in all patients.

Total pulmonary embolism may result in sudden circulatory arrest, pronounced venous congestion also in the upper body, cyanosis and it leads to death within minutes. Subtotal occlusion can cause severe pulmonary hypertension and acute right-heart failure, which can rapidly improve due to the activation of the anticoagulation system and fragmentation of the embolus. Smaller emboli lead to the necrosis of a given lung lobe (sharp chest pain, hemoptysis), to the development of pulmonary hypertension, and, over the long term, to the formation of abscesses in the necrotized area. Multiplex microembolisms progressively diminish the diameter of the pulmonary vessels, resulting in pulmonary hypertension and gradually developing chronic right-heart failure.

Thrombosis can develop ***in the arterial*** system as well. Thrombi developing on ruptured atherosclerotic plaques in the carotids and other arteries (coeliac, renal, femoral arteries, etc.) and, occasionally, poly-

cythemia/polyglobulia or myeloproliferative diseases can often be in the background. Emboli released from arterial thrombi can lead to the development of transient ischemic attack (TIA), ischemic stroke, and limb gangrenes.

Thrombus formation is also possible in the heart chambers, e.g., atrial thrombi in the case of atrial fibrillation or flutter ("ball" thrombi), wall thrombi following myocardial infarctions and aneurysms. Thrombi released from the right side of the heart cause pulmonary embolism, while those originating from the left side of the heart cause embolism in the systemic circulation, primarily in the carotid area.

Elimination of predisposing factors is the most efficient method in the prevention of thrombosis. For short-term pharmacological prophylaxis of thrombosis, heparin (LMWH) can be administered before and after surgery, while in consideration of the long term, the administration of coumarine and its derivates can be used. Anticoagulants of different action mechanisms are discussed in bleeding disorders (see above).

4.5. HEMATOLOGICAL CHANGES IN THE ELDERLY

Although the ratio of malignant hematological tumors is significant among senior patients, anemia and coagulation disorders have much more significance in consideration of the quality of life issues among the elderly.

The ratio of anemia is extremely high among seniors, which is important since the burden on circulation and also due to the subsequent tissue hypoxia. Anemia among elderly patients has a complex etiology, but most often it originates from insufficient RBC production. Anemia can also occur due to iron deficiency (insufficient intake, malabsorption, iron binding in chronic /inflammatory/ diseases), vitamin deficiency (especially, lack of B_{12} caused by gastric or ileum mucosa disorders), protein deficiency, and general feeding disorder. Decreased production can be caused by bone marrow disorders, either by decreased erythropoietin production or by more general, "idiopathic" atrophy of the bone marrow (in this case, production of other cells is also impaired – granulocytosis can be absent in infections among elderly patients). Occult bleedings, originating especially from the GI tract or prostate, are common causes of anemia in the elderly, but other tumors are also regularly followed by anemia.

Coagulation disorders are caused mainly by slow peripheral circulation and increased viscosity (exsiccosis is common). Thrombosis is frequent, especially in the veins. This can be exaggerated by immobilization. Thromboses are important due to their thromboembolic consequences. Insufficient pump function of the heart and impaired atrial functions result in the development of "ball" thrombus, while wall thrombus can occur following myocardial infarction. Arterial thrombi, causing ischemic tissue damages, can also develop easier due to the sclerotic lesions of the vessels.

Further readings

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