

ence in peripheral blood. The erythrocytes are normochromic and moderately macrocytic. Marked reticulocytopenia is present. The thrombocytopenia and neutropenia must be confirmed through repeated examinations. The bone marrow examination reveals the hypocellularity and replacement of bone marrow tissue by adipose tissue.

Patients with severe aplastic anaemia may be treated by bone marrow transplantation requiring multidisciplinary approach. The blood transfusion represents rather a risk than a method of treatment. Except for hepatitis and haemosiderosis, the blood transfusion can cause an undesirable sensibilisation before the bone marrow transplantation. The bone marrow responses sometimes favorably to the androgen application. GM-CSF (granulocyte-macrophage colony stimulating factor) is effective in pancytopenia developing during AIDS, during myelodysplasia, or under the influence of myelotoxic substances.

2.2.12.2 Primary bone marrow disturbances

The aplasia of erythrocyte line represents a selective disorder in production of erythroid cells. Granulopoiesis and megakaryopoieses remain intact. Patients have normochromic normocytic anaemia, but with associated reticulocytopenia. The underlying causes of this condition are not known. A very similar clinical picture of aplasia is observed with frequent findings of thymoma and of myasthenia gravis. In these case the erythropoiesis may be inhibited by IgG affecting selectively the erythroblasts in bone marrow. Erythropoietin inhibitors have been observed in some patients.

The myelodysplastic syndrome is considered to be an independent clinical unit. It is the refractory anaemia associated with neutropenia and thrombocytopenia.

2.2.12.3 Myelophthisic anaemia

Infiltration of bone marrow by tumors, fibrosis and granulomas may lead to anaemia development. Among the solid tumors prevail the metastatic deposits from breast carcinoma, stomach, prostata, lung and thyroid gland.

The bone marrow fibrosis is usually associated with myeloid metaplasia. In tuberculosis, but also in metabolic disturbances e.g. osteoporosis, anaemia may develop.

Myelophthisis results in normochromic normocytic anaemia. In the peripheral blood normoblasts may occur. At the beginning the number of reticulocytes moderately elevated.

2.3 Haemostasis and haemocoagulation

Normal haemostasis is the result of complicated relations between the vessel wall, thrombocytes and coagulation and fibrinolytic system. If the mutual proportions are not impaired the whole system is balanced. The balance is continuously maintained between the systems supporting the local haemostasis and those inhibiting the disseminated thrombosis.

The inner surface of vessels – endothelium – is a nonthrombogenic barrier inhibiting the interaction of blood components with subendothelial structures. If these substances have been in contact the thrombus formation would be initiated. This inhibitory function of endothelium is its primary function and is called the non-thrombogenicity.

The thrombocytes circulate in an inactive form. They do not contain nucleus and have irregular discoid shape. Their surface is covered with phospholipid membrane and the contractile filaments are situated below it. They contain three types of granules (dense, alpha and lysosomal) and the system of minute channels through which the content of granules is released into the plasma.

If the endothelium is damaged, the thrombus is formed immediately. First the thrombocytes adhere to the subendothelial components. The thrombocyte adhesion is an interaction between the membranous thrombocytic receptor (glycoprotein Ib) and the subendothelial collagen with participation of the plasmatic cofactor (von Willebrand's factor – vWF). To this reaction contributes a further plasma protein – fibronectin (glycoprotein present in cell membranes and is a component of plasmatic proteins, it is produced in fibroblasts, in endothelial cells and in macrophages). To the impaired site adhere several thrombocytes simultaneously. Such a thrombo-

cyte aggregation becomes activated. **This activation begins** with membrane receptor activation by several agonists especially by collagenous fibrils, ADP, adrenaline, serotonin, thrombin and some arachidonic acid metabolites. The thromboxan A₂ is a potent vasoconstrictive agent. Some agonists are released from the thrombocytes themselves, the others from the vessel wall. ADP is released also from erythrocytes occurring in near surroundings. The activation of thrombocytes triggers the action of thrombocyte contractile system. Thrombocytes change their shape from irregular discs to small globules with numerous pseudopodia and begin to connect with one another-forming the thrombocyte aggregation. The **thrombocyte aggregation** runs simultaneously with the arachidonic acid metabolism activation, with increase in free cytoplasmatic calcium level and with the release of a lysolecithinic substance. The aggregation of thrombocytes ceases to be reversible. After the above mentioned reaction is triggered, begins the release of platelet granule content. ATP, ADP, serotonin, calcium are released from dense granules. Platelet factor 4, the platelet growth factor, beta-thromboglobulin, vWF and a lot of other factors are released from alpha granules. During release of these factors the thrombocyte membrane is rebuilt. The negative charge of phospholipids is "unveiled" and the platelet factor 3 is generated.

During the thrombus formation the surrounding intact endothelium inhibits its excessive growth. The control is performed by inactivation of ADP (a potent agonist) by ADP-ase. Adrenaline and serotonin inactivate the endothelial cells. Endothelium enables the thrombin binding with antithrombin III; endothelium synthesizes and releases the antiaggregation PGI₂. PGI₂ binds with specific receptors localized on the membranes of thrombocytes activating so cyclic AMP bound with the membrane. In such a way the arachidonic acid metabolism, the calcium flux and the aggregation of thrombocytes are inhibited.

At site of impairment the coagulation cascade is activated via extrinsic or intrinsic pathways. All damaged cells in organism produce thromboplastin or the tissue activating factor, which further activates the factor VII (extrinsic pathway). In the presence of calcium and tissue activating factor activated factor VIIa may convert factor X into factor Xa. Factor Xa with negative charge of phospholipids, cal-

cium and factor V converts prothrombin into thrombin.

The intrinsic pathway is triggered via contact system. The proenzyme-factor XII is activated by damaged cells or by subendothelial structures. In combination with kallikrein and kininogen it activates the factor XI. Factor XIa activates the factor IX which, in combination with negative charge of phospholipids, calcium and factor VIII forms the complex activating the factor X. Further course of coagulation cascade is identical (as above described in extrinsic pathway) regard less of the beginning.

The **thrombocytes** accelerate the haemocoagulation:

1. by increasing the contact activity through the receptors for factor XI,
2. by tissue thromboplastin production,
3. by supply of membrane phospholipids which facilitate the factor X and prothrombin activation.

Thrombin modifies the factor V and VIII to induce rapidly the coagulation cascade into its most important phase, – the thrombin formation and the fibrinogen conversion into the fibrin.

Fibrinogen is a large six-chained molecule. Thrombin cleaves first the two A peptides and later two B peptides from this molecule. The fibrin monomer formed in this way is able to non enzymatic polymerization and it changes into a gel. Fibrin formed in this way remains relatively easily soluble and can be destructed by fibrinolytic system. The non soluble fibrin polymer is produced after its interaction with factor XIII. Factor XIII can stabilize fibrin only when it is converted by thrombin.

The action of thrombin and of other coagulation enzymes **is limited by three mechanism:**

1. The activated coagulation enzyme are drifted by blood flow from the site of impairment. The drifted enzymes are rapidly inactivated during the passage through the liver. This clearance mechanism includes the action of inhibitors and the interaction of macrophages.
2. The proteolytic enzymes produced during coagulation not only activate the coagulation factors but they destroy them simultaneously. The degradation is directly performed by degradation of factor Xa by thrombin, or it can be the

result of activation of inhibitory system through protein C (a vitamin K – dependent plas-matic protein activated by serine proteinases). Thrombin forms a complex with thrombomodulin (a protein localized on the surface of endothelial cells having a strong affinity for thrombin). The thrombin-thrombomodulin complex activates the C protein. In the presence of phospholipids and of S protein (a further cofactor) the activated C protein inactivates the factor Va and VIIIa. C protein is involved also in fibrinolysis initiation.

3. The third control mechanism are the original inhibitors: especially the alpha 2 macroglobulin (a component of serum proteins), α -1-antitrypsin (a glycoprotein with inhibitory action in serum), antithrombin III (the main regulator of blood coagulation; it neutralizes the thrombin serine proteinase), α -2-antiplasmin (one single-chained glycoprotein forming a complex with plasmin) and other factors. In coagulation cascade regulation antithrombin III plays the key role. It inactivates the serine proteases. Heparin and the contact with damaged endothelium accelerates this effect.

The fibrin deposition and its removal from circulation is regulated by fibrinolytic system – a multicomponent system composed of circulating plasminogen proenzyme, of activators, cofactors and inhibitors. The human plasminogen is a single-chained globulin easily adaptable by proteolysis. Plasminogen (lys-plasminogen) has higher affinity for fibrin and alpha 2-antiplasmin. It is easily activated also by urokinase. The plasminogen molecule contains two parts – one of them has an active site and the other has a binding site for the binding with fibrin and alpha-2-antiplasmin. The plasminogen activation occurs in the case when the activator cleaves the peptide binding and two chains of plasmin enzyme arise. The plasmin activation may occur in three ways:

1. Intrinsic mode represents the proactivator activation through the contact system
2. In the extrinsic system are the activators released into the blood flow from damaged tissue of blood vessel wall or of thrombocytes
3. During the treatment the streptokinase or uroki-

nase (fibrinolytic substances) are applied into the blood flow.

Plasmin hydrolysis many fibrin bindings causing thus fibrinolysis. The fibrin rests are named fibrin degradation products (FDP). Besides fibrin, plasmin involved in the factor V and VIII degradation. Massive fibrinolysis is simultaneously inhibited by a potent inhibitor – the alpha-2-antiplasmin and by a less strong alpha-2-macroglobulin.

2.4 Disorders of primary haemostasis

The spontaneous stopping of bleeding from a smaller damaged vessel is performed with essentially important life saving mechanisms. In the process of bleeding stopping from a vessel a complex of reactions between three participating systems is involved:

1. the vessel wall,
2. the thrombocytes,
3. the plasmatic coagulation factors.

If all occurs in framework of physiological usefulness the result is the normal haemostasis. In case that certain limits are exceeded, the reactions may lead to pathologic bleeding or to undesired thrombosis. The haemostasis initiation occurs during few seconds following vessel injury. Its termination may last even an hour. In the temporary consequence three successive phases can be distinguished:

The first phase – the primary haemostasis includes:

- the constriction of the injured vessel
- the subendothelial collagen exposure
- the adhesion and agregation of thrombocytes on the damaged surface

During these processes the primary haemostatic (thrombocytic) plug is formed in about 3 to 7 minutes. In this process the von Willebrand's factor