Physiological and pathophysiological notes on pediatric hematology (pediatrics)

Blood

Total blood volume correlates with body weight. The **functions of the blood** mainly include the transport of a wide range of substances (O2, CO2, nutrients, metabolic products, vitamins, electrolytes etc.), heat transport, signaling functions (transport of hormones to target tissues), buffering functions and defense against foreign substances and microorganisms. **Erythrocytes** are involved in all these tasks (they distribute O2, they are involved in CO2 transport and pH maintenance; hemoglobin is an important intracellular buffer). From **leukocytes**, *granulocytes* are responsible for non-specific immunity, *monocytes* (macrophages and lymphocytes) are responsible for specific immune responses. **Platelets** have an essential function in stopping bleeding. The ratio between the volume of blood cells and the total volume of blood is called **the *hematocrit (hkt)***. Erythrocytes occupy more than 99% of the hematocrit.

The liquid component of the blood is **plasma**, in which electrolytes, nutrients, metabolic products, vitamins, gases and proteins are dissolved. The tasks of plasma proteins include humoral immunity, maintenance of colloidal-osmotic (oncotic) pressure, transport of water-insoluble substances and protection of certain substances against breakdown in the blood and excretion by the kidneys (eg *heme*). Such binding of smaller molecules to proteins reduces, on the one hand, their osmotic activity and, on the other hand, they can charge as haptens of antigenic effect. The combination of hormones, drugs and toxins with plasma proteins reduces their signaling, therapeutic or toxic effects, but at the same time prevents their rapid excretion. Many plasma proteins are involved in the coagulation process. If blood clots, plasma fibrinogen is consumed and **serum** is formed.

Bone marrow structure and function

During embryogenesis and fetal development, **hematopoiesis** passes from the *yolk sac* to the *liver* and from about the 28th week of gestation to the *bone marrow*. In childhood, hematopoiesis is concentrated in the bone marrow of *long bones*. Here it is gradually replaced by adipose tissue and at a later age the center of hematopoiesis is the *sternum, pelvis, ribs, cranium, pineal gland*. This knowledge is important when deciding where to aspirate bone marrow. The pelvis can be used for aspiration throughout the age, while the tibia can only be used in children under 2 years of age. An alternative source of hematopoietic stem cells may be peripheral blood after induction by growth factors or umbilical cord blood. In extreme cases, such as severe hemolytic anemia, **extramedullary hematopoiesis** can occur, especially in the *liver* and *spleen*.

Microscopically, the bone marrow consists of a network of vascular sinuses that separate fatty and hematopoietic islets, osteoblasts, and osteoclasts (important for bone remodeling).

Unlike peripheral veins, intramedullary vessels do not collapse during shock, so entry into the bone marrow is appropriate if the bloodstream at the periphery is collapsed or for any reason standard i.v. input.

Stem cells in the bone marrow are **pluripotent** (universal in terms of further differentiation). Stem cells can differentiate into muscle, heart, liver or nerve tissue.

Hematopoiesis

Cells within the hematopoietic islets in the **bone marrow** contain red blood cells, granulocytes (neutrophils, eosinophils, basophils), monocytes and macrophages, lymphocytes, platelets and their precursors. The first morphologically identifiable **precursors** are the *proerythroblast* (giving rise to the erythroblast), *myeloblast, monoblast, lymphoblast* and *megakaryoblast* (megakaryocyte precursor).

The lifespan of blood cells is as follows:

- **Erythrocytes** for 100 to 120 days (60 days in newborns),
- **Platelets** 7 to 10 days,
- **Granulocytes** 12 hours,
- **Lymphocytes** have a long lifespan

The shorter lifespan of erythrocytes in neonatal age is one of the factors involved in ***neonatal hyperbilirubinemia***.

At the beginning of hematopoietic cell differentiation is a **pluripotent stem cell** that differentiates into a **lymphoid, erythroid** and **myeloid precursor cell**. Pre-T-lymphocytes (→ T-lymphocytes) and pre-B-lymphocytes (→ B-lymphocytes → plasma cells) differentiate from the lymphoid precursor cell. Erythroblasts (→ reticulocyte → erythrocyte) differentiate from the erythroid precursor cell. Megakaryoblast (→ megakaryocyte → platelet),

myeloblast (→ promyelocyte → myelocyte → metamyelocyte → rod → segment), monoblast (→ monocytes → macrophages), eosinophiloblast (→ eosinophil), grocytes and mast cells are differentiated from myeloid precursor cells .

Pluripotent stem cell

- **Lymphoid stem cell** (lymphoblast): 1-for T → T-lymphocyte;

2-for B → B-lymphocyte → plasma cell.

- **Erythroid precursor cell** (proerythroblast) → erythroblast → reticulocyte → erythrocyte.
- **Myeloid precursor cell**: 1-megakaryoblast → megakaryocyte → platelet;

2-myeloblast → promyelocyte → myelocyte → metamyelocyte → rod → segment;

3-monoblast → monocyte - macrophage

4-eosinophiloblast → eosinophil;

5-basophilic granulocyte;

6-mast cell.

Hematopoietic cells differentiate from progenitor cells under the influence of **hematopoietic growth factors** (**HGFs**). HGFs also affect extramedullary hematopoiesis, including T cells, macrophages, endothelial cells, and fibroblasts. HGFs include **erythropoietin**, **thrombopoietin**, G-CSF (granulocyte colony stimulating factor), GM-CSF (granulocyte monocyte colony stimulating factor). Erythropoietin, G-CSF and GM-CSF are used *therapeutically*. More recently, a new erythroid-stimulating protein (darbepoetin α) and a chemical modification of G-CSF (pegfilgastrim) have been synthesized. These latest preparations are still in the stage of research and clinical trials. There are also growth factors for the stem cells themselves, SCF (stem cell factor) and FL (flt 3-ligand).

Hemostasis

The hemostatic system protects a person from bleeding and blood loss. The following are involved in *hemostasis*:

- **Plasma factors**
- **Platelets**
- **Vascular wall**

Their interactions locally block the vascular wall, which pre-seals the **platelets** ("**white thrombus**") and subsequently creates a solid fibrin network ("**red thrombus**") in the **plasma coagulation system** and thus a **stable plug**. Excess thrombus formation resulting in occlusion of larger vessels and subsequent embolism must be ruled out. In order to maintain this balance, the hemostatic system is activated very quickly locally if necessary, but its undesired spread is prevented (partly by feedback) by *inhibitory factors*. The **fibrinolytic system** ensures the dissolution of excess thrombi. *Platelets* are nuclear-free disks formed by fragmentation of the cytoplasm of bone marrow megakaryocytes. In endothelial injury, ***von Willebrand factor (vWF)***, produced by endothelial cells, causes the platelets to adhere immediately to exposed collagen. Platelets are activated by their own **adhesion**, ie they capture each other (**aggregation**), which is stimulated by ***thrombin***. Platelets change their shape and secrete mmj. **vasoconstrictor** (*serotonin, plateled derived growth factor = PDGF, thromboxane A2*) and **aggregation stimulant** (*fibronectin, vFW, fibrinogen*). In addition, thromboxane A2, together with the simultaneously released ADP and the inflammatory mediator PAF (platelet activating factor), enhances platelet activation. During aggregation, platelets contract and change shape significantly. They attach to the fibronectin subendothelial matrix and also to the fibrinogen that binds the platelets together.

The coagulation system consists of a number of **factors**:

Procoagulant factors

- *factor I* = **fibrinogen** (its synthesis takes place in the liver)
- *factor II* = **prothrombin** (its synthesis takes place in the liver)
- *factor III* = **tissue thromboplastin** (tissue factor, TF; is in endothelial cell membranes)
- *factor IV* = **calcium**
- *factor V* = proaccelerin, so-called labile factor
- *factor VI* = none
- *factor VII* = proconvertin, so-called stable factor
- *factor VIII* = antihemophilic factor A; represents a macromolecule with 2 components
    - antihemophilic factor = procoagulant factor VIII C, is a smaller protein
    - f. VIII R: Ag (related antigen) = vWF (large molecule with antigenic properties)
- *factor IX* = Christmas factor, antihemophilic factor B
- *faktor X* = Stuart–Prowerové faktor
- *factor XI* = PTA (plasma thromboplastin antecedent), plasma thromboplastin precursor
- *factor XII* = Hageman's factor
- *factor XIII* = **fibrin stabilizing factor**
- factors involved in the **initiation of endogenous activation** (after contact with collagen):

- **precalikrein** (Fletcher factor), a precursor of kallikrein
- kininogen (Fitzerald factor), a kinin precursor

Anticoagulant factors

- **antithrombin III**
- **α 2-macroglobulin**
- **α 1-antitrypsin**
- **protein C**
- **protein S**

With the exception of calcium, these are proteins that are mostly synthesized in the **liver** (f. I, II, V, VII, IX, X, XIII, kininogen). **Vitamin K-dependent factors** include f. II, VII, IX, X, protein C and S.

Anemia

Markers of red blood count:

- Hemoglobin Hb, unit g / l
- hematocrit HTK, given in%,
- number of PE erythrocytes, expressed in millions / mm3,
- reticulocytes = early forms of erythrocytes, normal up to 2%, in children <1 week up to 5%,
- **MCV** = mean erythrocyte volume = HTK / PE, unit fl,
- **MCH** = mean Hb content in erythrocytes = Hb / PE, unit pg,
- **MCHC** = mean Hb concentration in erythrocytes = MCH / MCV = Hb (g / dl) / HTK,
- **RDW** = erythrocyte distribution width, predicts anisocytosis, norm 13 to 15,
- **sTfR** = soluble transferrin receptors (the most reliable marker in sideropenic anemia, not affected by inflammation in the body).

Pathophysiological classification of anemias

Anemia from erythrocyte loss

- *extravascular*: posthemorrhagic anemia;
- *intravascular*: hemolytic anemia.

Anemia from erythrocyte dysfunction or Hb

- in the absence of substances (Fe, vitamin B12, folic acid);
- from bone marrow depression;

Red blood cell parameters predicting anemia

age group	Hb g/l	HTK	MCV (fl)
newborns	< 140	< 0,44	<100
infants	< 100	< 0,32	<70
toddlers and preschoolers	<105 to 110	< 0,32	<73
younger schoolchildren	< 115	<0.33	<75
older schoolchildren	< 120	<0.34	<77

Differential diagnosis of sideropenic anemia and anemia of chronic diseases

Sideropenic anemia	***microcytes***	***increased RDW***	***reduced Fe***	***increased CVK***	***increased sTfR***	***reduced ferritin***	***initially microcytosis***
Anemia of chronic diseases	***normocytes***	***normal RDW***	***reduced Fe***	***reduced CVK***	***normal sTfR***	***increased ferritin***	***initially hypochromia***

Hemolytic anemia

Hemolytic anemias are mostly **normocytic**, *thalassemia* is **microcytic**. In the **laboratory** we find anemia, reticulocytosis, increased unconjugated bilirubin, increased urobilinogen in the urine, hyperplasia of the erythrocyte lineage in the bone marrow, increased Fe, increased LDH (isoenzymes LDH 1 and LDH 2), decreased haptoglobin (intravascular hemolysis leads to increased free H which forms complexes with haptoglobin). After depletion of the haptoglobin capacity, the remaining free Hb in the form of dimers passes through the glomerular membrane. **Hemoglobinuria** is found in *intravascular hemolysis*. In hemoglobinuria / myoglobinuria, there is no exception to the discrepancy between a markedly positive blood test on urine chemistry and a test in urinary sediment, which may be completely normal. **The Coombs test** predicts the presence of autoantibodies (**Coombs direct test** = detection of incomplete erythrocyte-bound Ig, **Coombs indirect test** = detection of incomplete free Ig in plasma).

Diagnosis according to erythrocyte morphology

-*schistocytes*: hemoglobinopathies, bacterial toxins, parasites, circulatory disturbances, HUS;

-*spherocytes + positive direct Coombs*: autoimmune hemolytic anemias (cold Ig = viral infections, thermal Ig = autoimmune diseases);

-*spherocytes + negative direct Coombs*: hereditary spherocytosis;

-*eumorphic erythrocytes*: hepatic / renal insufficiency, Wilson m .;

-*Heinz bodies in erythrocytes*: G-6-P dehydrogenase deficiency;

-*morphologically bizarre erythrocytes*: pyruvate kinase deficiency;

-*target cells + basophilic erythrocyte dotting*: β-thalassemia minor.

Pancytopenia

At least 2 of the 3 criteria must be met:

- platelets <20,000,

- granulocytes <500,

- reticulocytes <0.02%.

Etiology

-*idiopathic*;

-*drugs*: cytostatics, antiepileptics, antithyroid drugs, chloramphenicol;

-*toxins*: benzene, insecticides, heavy metals;

-*infections*: viral hepatitis, EBV, Parvovirus B19.

Pathology of the white blood line

White blood cell parameters

- leukocytosis:> 10,000,
- leukopenia: <4,000,
- neutropenia: <1,000 (in children 2 weeks to 1 year), <1,500 (in children over 1 year),
- agranulocytosis: <500,
- lymphocytosis:> 5,000,
- lymphopenia: <1,500,
- monocytosis:> 800 or> 10% in diff.
- eosinophilia:> 600 or> 10% in diff.,
- basophilia:> 100 or> 1% in diff.

Differential diagnosis of leukocytosis and neutrophilia

-Pyogenic infections,

-connective tissue disease,

-glomerulonephritis,

-acidosis,

-uremia,

-acute bleeding,

-hemolysis

-burns,

-surgery,

-liver necrosis,

-dehydration,

-corticoids,

-catecholamines,

-lead and mercury poisoning,

-bone marrow metastases

Physiological causes of leukocytosis and neutrophilia

-Stress,

-physical exertion,

-intense crying in the newborn,

-smoking.

Leukemoid reactions

The leukemoid reaction represents a situation where we find tens of thousands of leu / mm3 + a significant shift to immature forms. The determination of ALP in neutrophils can help in differential diagnosis (ALP is reduced in leukemia, ALP is normal or increased in leukemoid response in infection or systemic disease). When in doubt, it provides certainty of bone marrow aspiration.

Differential diagnosis of lymphocytosis

Absolute lymphocytosis

- infectious lymphocytosis,
- infectious mononucleosis ,
- pertussis ,
- lights ,
- TBC ,
- hyperthyroidism

Relative lymphocytosis

- morbilli ,
- exanthema subitum .

Differential diagnosis of neutropenia

Congenital

- reticular dysgenesis ,
- congenital pluripotent stem cell disorder
- Kostmann ,
- cyclic neutropenia,
- Schwachmann sy.,
- neutropenia in DMP: glycogenosis , Gaucher.

Gained

- isoimmune neonatal neutropenia,
- vitamin B12 deficiency ,
- folic acid deficiency ,
- autoimmune etiology,
    - SLE ,
    - JIA ,
    - SCID ,
    - Hyper IgM syndrome,
    - Evans syndrome,
    - lymphoma ,
    - drugs: chloramphenicol , antiepileptics , thyrostatics ,
    - infections: EBV , CMV , HHV 6, HIV , Parvovirus, hepatitis , varicella , parotitis ,
- idiopathic neutropenia.

Differential diagnosis of eosinophilia

- Allergic diseases,
- skin diseases: eczema atopicum,
- parasitic infections: toxocariasis, oxyuriasis,
- m.Hodgkin,
- scarlatina.

Hemorrhagic diathesis

Differential diagnosis of clinical manifestations of hemorrhagic diathesis

Coagulopathy

- Deep localization → bleeding into muscles, joints
- Bleeding is delayed / prolonged
- It returns even after a few hours, local compression does not stop well
- The scope is more of isolated manifestations

Trombocytopenia/trombocytopathy

- Surface localization → petechiae (they are in the level), suffusion, bleeding from the mucous membranes
- The bleeding is immediate
- Compression can be well stopped
- The extent is usually multiple manifestations

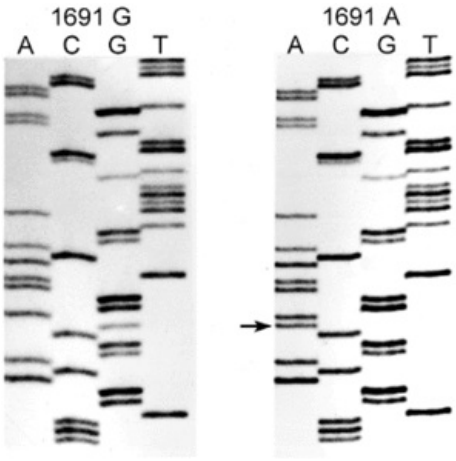
Vaskulopathy

- Bleeding with a thrombocytopenia / -pathy pattern
- Often symmetrical distribution
- May come in waves
- On the skin at the same time rashes, edema , ev. necrosis
- Petechiae are palpable = above niveau skin

Differential diagnosis of trombophilic conditions

Congenital trombophilic conditions

- Deficiency of **AT III**
- **Protein C** deficiency
- **Protein S** deficiency
- **Resistance to activated protein C** (= impaired binding of protein C to f. V, the so-called **Leiden mutation** , which occurs in up to 5% of the population)



Leiden mutation

Acquired trombophilic conditions

- vascular catheters
- vasculitis
- diabetes mellitus
- hyperhomocysteinemia
- dehydration
- hyperviscosity: polycythemia , extreme leukocytosis
- thrombocytosis
- hyperlipidemia
- malignancy
- after contraception
- nephrotic syndrome
- HUS
- antiphospholipid syndrome

Basic tests in pediatric hematology

Blood count

Platelet counts are especially important for assessing coagulation disorder . There is a risk of serious bleeding if their number falls below 20,000. If DIC syndrome or microangiopathic hemolytic syndrome (HUS , TTP) is suspected , we also require examination for the presence of fragmented erythrocytes (*schistocytes*).

Activated partial thromboplastin time (aPTT)

Tests the internal coagulation system . It indicates the function of factors VIII., X to XIII. It is a control test for heparin dose titration (the antidote to heparin is protamine sulphate). The shortening of the aPTT value is accompanied by the activation of the coagulation system, but in children also incorrectly performed sampling.

The extension of aPTT leads to:

- **Lack of coagulation factors** of the internal system when their production is impaired or their consumption is increased (massive bleeding, extensive thrombosis , DIC),
- **High concentration of fibrin cleavage products** ,
- **Presence of coagulation factor inhibitors**: heparin (attention to artificial admixture when taken from CVK), a specific inhibitor directed against the coagulation activity of one of the factors, in the presence of a non-

specific inhibitor (antiphospholipid antibody = lupus anticoagulant).

Even a small prolongation of aPTT, ie by 5 to 8 seconds, may already indicate a mild form of haemophilia (**CAVE**: in this case, preoperative screening is necessary). APTT cannot be used to monitor efficacy in low molecular weight heparin (LMWH) therapy . The efficacy of LMWH must be verified by determining antiXa (aXa) activity. The recommended range is 0.5 to 1.0 aXa U / ml. A blood sample for control should be taken 3 to 5 hours after administration.

Prothrombin time (Quick, PT, prothrombin time)

Tests the external coagulation system. Indicates the function ff. II., V., VII., X (the test is less sensitive to the decrease of f. II and fibrinogen). The test is closely related to the activity of vitamin K- dependent factors (vitamin K- dependent factors: ff. II., VII., IX., X). The INR (International Normalized Ratio, which excludes the effect of the reagent used on absolute PT values) is used to assess the efficacy of drugs that reduce the activity of vitamin K-dependent coagulation factors (warfarin). The reasons for the shortening of the PT value are the same as for aPTT. PT prolongation occurs in ff deficiency. II, VII, IX, X.

Thrombin time (TT)

It measures **the thrombin-fibrinogen response**. The prolongation of TT is due to a reduction in the level of fibrinogen or its functional insufficiency (dysfibrinogenemia), as well as the presence of heparin, fibrin or fibrinogen fission products, or other pathological inhibitors. It is also extended at DIC.

Fibrinogen

Fibrinogen (FBG) is an acute phase protein . Its plasma concentrations increase not only in inflammation, in cancer, in the first days after major operations, but also in pregnancy. Decreased fibrinogen levels lead to a decrease, blood loss (major injuries, bleeding) or hemodilution. A marked decrease in fibrinogen may be a sign of increased fibrinogen consumption (DIC) or thrombolytic therapy.

Antithrombin III (AT III)

AT values are given either in absolute values or as a percentage of functional activity. Low AT activity is accompanied by a decrease in its production (liver lesions), increased consumption (DIC, major surgeries, extensive thrombosis) or kidney loss (nephrotic syndrome). There is also a slight decrease in AT values during pregnancy and when using hormonal contraception . Congenital AT deficiency is rare.

D-dimers

It is **a non-specific fibrin cleavage product**. Its increased value indicates the activation of fibrinolytic mechanisms, in a situation where a fibrin clot has already formed.

Ethanol-gelation test, fibrin split test and protamine test

The ethanol-gelation test (EGT), **the fibrin split test** and **the protamine test** are used to determine soluble fibrin monomer complexes and to assess the degree of activation of the coagulation system. Tests are *positive* for DIC, thromboembolic disease , sepsis and sometimes in the acute stages of systemic autoimmune diseases . They can be *false positive* when traumatic, incorrectly taken.

Tests to determine primary hemostasis

- Thrombocytopenia: blood count,
- Thrombocytopathy: bleeding test (Duke, Simplate),
- Vasculopathy: capillary fragility test.

Physiological values of coagulation parameters

Parameter	Physiological values
Antithrombin III	> 60 to 70%
aPTT	< 40 s
Quick	< 13 s (> 70%)
INR	0.8 to 1.2
D-dimers	< 150 (slight elevation of D-dimers values has low specificity)
Fibrinogen	< 2 to 4g/l
Thrombin time	+/- 20%

Physiological values of some coagulation parameters, which are dependent on the age of the child

TEST	newborns	1 month	6 months	Big children
F II = prothrombin (%)	50 (25-70)	70 (35-100)	90 (60-120)	100 (70-150)
AT III (%)	60 (30-90)	80 (50-100)	100 (80-120)	100 (60-125)
Protein C (%)	35 (20-50)	45 (20-65)	60 (40-80)	100 (65-130)
Protein S (%)	35 (15-60)	60 (35-90)	90 (55-120)	100 (50-120)

- *Note:* For time-expressed tests (aPTT, PT), it depends on the reagent used. Therefore, each laboratory has its own range of normal values.
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References

Sources

- HAVRÁNEK, Jiří: *Hematology - general introduction*. (edited)

Related articles

- White blood cell pathology (pediatrics) • Neutropenia in children
- Diseases of the white blood component:
  - Leukemiea: Acute myeloid leukemia • Acute lymphocytic leukemia • Chronic myeloid leukemia • Chronic lymphocytic leukemia
  - Malignant lymfoma: Hodgkin's lymphoma • Non-Hodgkin's malignant lymphoma
  - Histiocytosis
- Diseases of the red blood component: Anemiea • Polyglobulia