

Blood

Erythrocytes

Thrombocytes

Blood groups

Leukocytes

- classification

- count

- granulocytes

Complete blood

count

Blood

- sanguis, *inis m.*, αἷμα
- 6-9 % of body mass (age, gender, hydratation)
(5,5 l ♂)
- suspended **formed blood elements**
- **hematocrite** = volume fraction of RBC within the blood
 - 40-50 % in man
 - 35-40 % in woman
 - centrifugation (citric acid, heparinized)
 - ↑ hematocrite → ↑ ↑ viscosity
- pH 7,36-7,44
- serum osmolality 275-295 mmol/kg H₂O

Blood

- O_2 , CO_2 , metabolites, hormones
 - O_2 bound to HB and dissolved in the plasma
 - $CO_2 \leftrightarrow HCO_3^-$ or bound to HB
- termoregulation
- homeostasis – constant internal conditions
- immunity

Blood plasma

- liquid part of blood, 55 %
- centrifugation of the blood
- water solution
 - 90 % water
 - 10 % dissolved
 - 7 % proteins
 - albumins
 - α - a β -globulins (transport, coagulation,...), γ -globulins (Ig)
 - complement – humoral immunity (inflammation, bacteriolysis...)
 - fibrinogen
 - 0,9 % anorganic substances
 - others: lipids, lipoproteins, aminoacids, hormones...
- **serum** = remains after the blood clotting (= plasma without fibrinogen and coagulant factors)

Formed elements of the blood

- erythrocytes (RBC = red blood cells)
- thrombocytes (platelets)
- leukocytes (WBC = white blood cells)
 - granulocytes: specific granules and segmented nuclei (polymorphonuclears)
 - neutrophilic
 - eosinophilic
 - basophilic
 - agranulocytes: without specific granules, spherical to kidney-shaped nucleus (mononuclears)
 - lymphocytes
 - monocytes

Erythrocytes

Ian Swammerdam 1638

- eosinophilic biconcave discs
 - 7,5 μm \times 2,5 μm (isotonic)
 - 7,2-7,4 \times 1,9 μm in blood smear
 - no nucleus (in mammals)
 - normocytes 82-98 fl
 - macrocytes > 9 μm
 - microcytes < 6 μm
 - anisocytosis = variable size
- count:
 - 4-5,8 $\times 10^6/\text{mm}^3$ peripheral blood in male
 - 3,5-4,2 $\times 10^6/\text{mm}^3$ in female
 - cca 6,7 $\times 10^6/\text{mm}^3$ in a newborn
 - polycythaemia = erythrocytosis
 - anaemia, cytopenia, oligocythaemia

Erythrocytes

- reticulocytes with remnant polyribosomes (< 1 % ery)
- elastic and deformable
 - membrane cytoskeleton
 - ankyrin, spektrin, aktin
 - mutation → hereditary sphaerocytosis (AD)
 - elasticity and osmotic resistance – age dependend
- life span 100-120 days
 - hemolysis, phagocytosis in red splenic pulp, bone marrow, liver
- glykocalyx with oligosaccharides
 - agglutinogens and blood groups

Hemoglobin

- 33 % of RBC volume
- 28-32 pg/erythrocyte
- 1 litre: 135-175 g Hb
 - **anaemia** = low Hb content
 - low RBC count
 - low Hb content (hypochromic anaemia)
- tetrameric metalloprotein with heme Fe²⁺
- types:
 - adult HbA (predominates) and HbA2
 - fetal HbF (higher affinity to O₂)
- transport functions
 - O₂ – oxyhemoglobin
 - CO₂ – carbaminohemoglobin
 - CO+Hb = 200× greater affinity than to O₂ → carboxyhemoglobin
- buffer

Sickle cell anaemia

- AR – manifested in HbS homozygotes
- milder malaria in homo/heterozygotes

Blood groups

- circulation W. Harvey 1682 → blood transusion experiments since XVII.
 - unsuccessfull
 - techniques developed i.v. nutrition in dogs
 - light red blood in the pulmonary circulation
- 1901 Karl Landsteiner 3 aglutination groups A, B, 0
- 1902 AB
- MN-system, P-system, Rh-system
- 1930 NP
- 1907 Jan Janský: Hematological studies in psychotics: 4 groups I, II, III, IV
- AB0:
 - H antigen (rare: Mumbai phenotype hh)
 - modified by two types of glycosyltransferases → A or B
 - codominant aleles of the AB0 locus

Blood groups

Group = agglutinogens in RBC	Gene	Central Europe	Hemagglutinins in plasma
A	AA, A0	43	Anti-B
B	BB, B0	12	Anti-A
AB	AB	5	-
0	00	40	Anti-A + anti-B

Rh+	85 %
Rh-	15 %

- Rh-incompatibility
- fetal erythroblastosis
- anti-D gamma-globulin

cross match

Thrombocytes

- cell fragments of megakaryocytes, no nuclei
 - 2-4 μm
 - hyalomere = peripheral zone
 - granulomere = central zone
 - alpha, delta, lambda granules
 - Ca^{2+} , ADP, ATP, PDGF, vWF, serotonin, fibrinogen, enzymes
 - invaginating membrane \rightarrow canalicular system \rightarrow degranulation upon activation
 - thrombocytopenia \rightarrow bleeding
- $150\text{-}400 \times 10^3/\text{mm}^3$
- function
 - endothelial damage \rightarrow primary hemostasis \rightarrow platelet thrombus (unstable)
 - degranulation
 - \rightarrow serotonin \rightarrow vasoconstriction
 - platelet factors \rightarrow secondary hemostasis \rightarrow coagulation cascade \rightarrow fibrin
 - thromboplastin \rightarrow prothrombin \rightarrow thrombin \rightarrow fibrinogen polymerized into fibrin
 - contracting thrombocytes (actin, myosin) \rightarrow retracted thrombus
 - remodelling of thrombus, thrombolysis (plasmin)



Hemostasis

- primary: platelet trombus
- secondary: clotting
→ fibrin network

Leukocytes

- count
 - $4-10 \times 10^3/\text{mm}^3$
 - higher count in children
 - \uparrow = leukocytosis, \downarrow = leukopenia
- migration
 - rolling, marginal pool
 - adhesion
 - diapedesis into the interstitial connective tissue
 - lymphocytes – recirculation, homing
- differential count
 - neutrophils 45-70 (55-70) %
 - lymphocytes 20-45 (18-40) %
 - monocytes 2-12 (3-8) %
 - eosinophils 0-5 (2-5) %
 - basophils 0-2 (0-1) %
- granules and nuclei
 - granulocytes
 - agranulocytes

Neutrophilic granulocytes

- 10-12 μm
 - specific granules (baktericidal enzymes)
 - azurophilic granules (lysosomes, elastase, myeloperoxidase)
 - 2-5 nuclear segments
- hours in blood, 1-2 days in tissues, chemotactic
 - circulating neutrophils
 - reserve: inflammation \rightarrow activation
 - adhering marginal pool
 - bone marrow reserve
- active microphages
- pus
- \uparrow = neutrophilia; \downarrow = neutropenia, agranulocytosis

Maturing neutrophils

- Arneth's count
 - 1 segment (stabs) = 5 %
 - 2 segments (35 %)
 - 3 segments (41 %)
 - 4 segments (17 %)
 - 5 segments (2 %)
- Hynek's nuclear number = 2,7
- shift to the left = younger forms prevail
- shift to the right = elderly forms

- *1904 J. Arneth: Die neutrophilen weißen Blutkörperchen bei Infektionskrankheiten*
- *Dr. Kristian Hynek: sedimentation of RBC used for diagnostic purposes, Westergren-Fahrhaeus*

Eosinophilic granulocytes

- 12-15 μm
 - bilobed nucleus
 - eosinophilic granules
 - internum: large granule with crystalline centre = MBP major basic protein, ECP – eosinophil cationic protein
 - externum: surrounding matrix, lytic enzymes, peroxidase
- migrate into lamina propria and skin
- anti-microbial and anti-parasitic effects
 - binding to antigen-IgE complex \rightarrow degranulation
 - *Ascaris* (roundworm), *Fasciola hepatica* (common liver fluke)...
 - IgE-mediated (type I) allergy: atopic eczema, asthma, allergic rhinitis (eicosanoids = derivatives of arachidonic acid)
- \uparrow = eosinophilia

Basophilic granulocytes

- 12-15 μm
 - irregular shaped nucleus
 - large specific basophilic granules
 - heparin: sulphated GAG
 - histamin: bronchoconstriction, vasodilation
- producing eicosanoids and cytokines
 - inflammatory mediators
 - chemotactic and differentiation factors
 - supporting the differentiation of Th2-lymphocytes → allergy

Life span

- erythrocytes
 - 120 days
- thrombocytes
 - 10 days
- neutrophilic granulocytes
 - circulating in blood < 1 day
 - interstitial tissue 1-2 days
- monocytes
 - circulating 1-3 days
 - → macrophages (months)

Blood smear and blood count

- Pappenheim stain
 - Giemsa-Romanowski
 - May-Grünwald
- manual counting chambers (Bürker)
 - calibrated grids
 - 0.1 below the cover slip
- automated hematological analyzers
 - selective hemolysis
 - electronic impedance measurement of RBC and thrombocytes
 - cells = low conductivity: a cell between electrodes → changing the impedance
 - flow cytometry
 - capillary, laser beam (size, granules, nuclei)

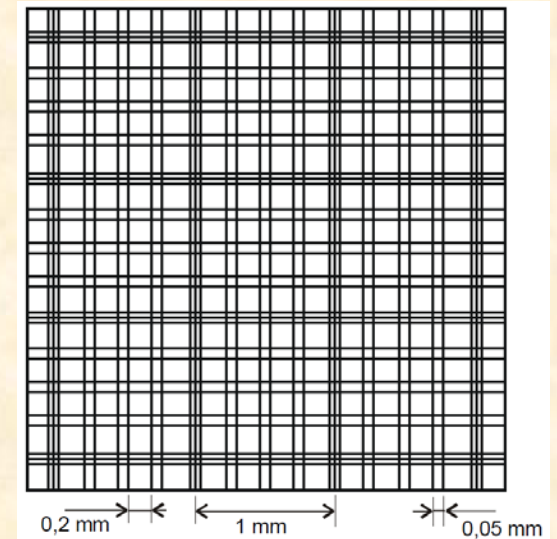


Table for assessing the differential leukocyte count in 100 leukocytes.
 Count 10 leukocytes per column, i.e. 100 leukocytes in total.

Types of leukocytes:	1	2	3	4	5	6	7	8	9	10	<i>Sum of the values in a row:</i>
neutrophils											<i>a =</i>
eosinophils											<i>b =</i>
basophils											<i>c =</i>
lymphocytes											<i>d =</i>
monocytes											<i>e =</i>

The blood smear contains: % of neutrophils
 % of eosinophils
 % of basophils
 % of lymphocytes
 % of monocytes

Table for assessing the nuclear number (Hynek's figure), i.e. the mean number of lobes counted in 100 neutrophils.
 Count 10 neutrophils per column, i.e. 100 neutrophils in total.

Number of lobes in a neutrophil:	1	2	3	4	5	6	7	8	9	10	Sum of the values in a row:
1											a =
2											b =
3											c =
4											d =
5											e =

$$\text{Nuclear number} = (a*1 + b*2 + c*3 + d*4 + e*5)/100 = \dots$$

Voluntary blood donation

Recipients

- injury
- major surgery
- **hematological disorders:** hemophilia, thalassemia, leukemias, anemias
- shock, drug reactions, burns, poisoning

Recipient safety

Screening of donors for HIV, viral hepatitis etc.

Donor safety

Registration, questionnaire, complete blood count, interview, donation (8 - 12 minutes)

Volume: 450 ml +/- 10%; recovery; women max. 3x per year, men 4-5x per year