# Hematological examinations
## Seminar

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**(Petr Marsalek, 2 some overhead/ hand drawn slides)**

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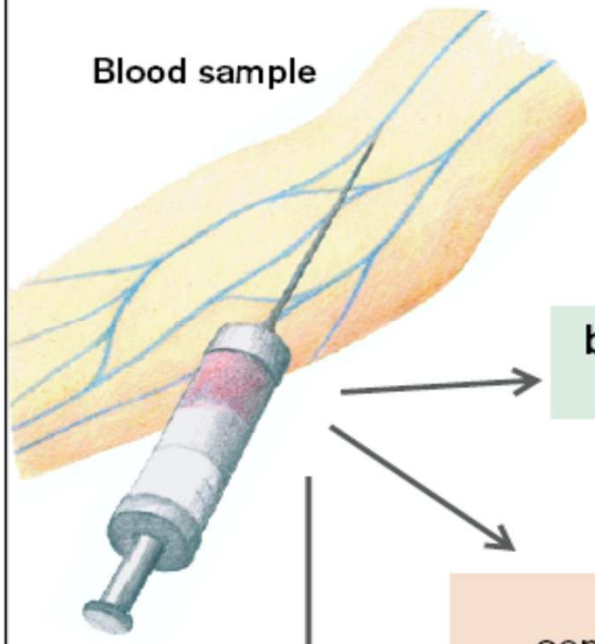
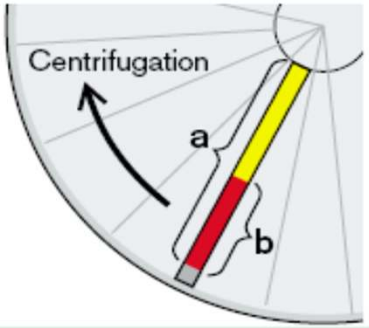
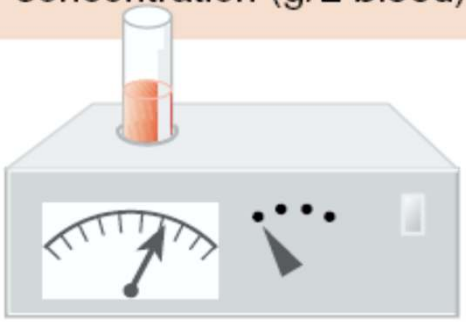
## A. The Erythrocyte Parameters MCH, MCV, and MCHC



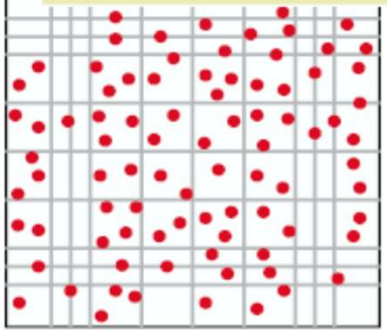




**b/a** = hematocrit (Hct) ($L_{RBC}/L_{blood}$)

Hemoglobin (Hb) concentration (g/L blood)



Erythrocytes (RBC) (number/L blood)



**MCH** (mean Hb mass/RBC)

$$= \frac{\text{Hb conc.}}{\text{No. RBC}} \text{ (g/RBC)}$$

Normal: ~30 pg

**MCV** (mean volume of one RBC)

$$= \frac{\text{Hct}}{\text{No. RBC}} \text{ (L/RBC)}$$

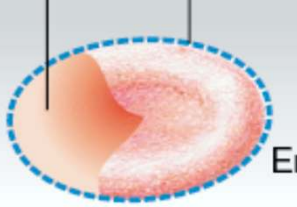
Normal: ~90 fl

**MCHC** (mean Hb conc. in RBCs)

$$= \frac{\text{Hb conc.}}{\text{Hct}} \text{ (g/L RBC)}$$

Normal: ~320 g/L

**MCH** / **MCV** = **MCHC**



# Hemoglobin (Hb)

Measured in a volume unit of the full blood,
i.e. in the serum with the red blood cells (RBC)

**Reference values**

| | | |
|---|---|---|
| **women:** | 125 – 155 g / L (litre) | 12.5 – 15.5 g / 100 mL |
| **men:** | 135 – 175 g / L | 13.5 – 17.5 g / 100 mL |

**Higher:** polycytaemia, dehydration
**Lower:** anaemia, hyperhydration

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# RBC count

measured in quantities (times) x $10^{12}$/ L (litre)
or x $10^{9}$/ 1 mL or x $10^{6}$ / 1 μL

**Reference values**

**women :** 3.8 – 5.2 x $10^{12}$ / L (litre)
**men:** 4.2 – 5.8 x $10^{12}$ / L

**Lower:** anaemia, hyperhydration
**Higher:** polycytaemia, dehydration

# Hematocrit (HCT)

HCT gives **blood cell volume** to **total cell volume** ratio

## Reference values

**women :** 0.35 – 0.46 35 - 46 %
**men:** 0.38 – 0.49 38 – 49 %
in newborns: up to 0.60, then lower, at the beginning of puberty 0.39, end of puberty returns to normal values

**lower:** anaemia, extra-cellular fluid (ECF) expansion
**higher:** polycytaemia, dehydration



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# MCV – mean cell volume

**Reference value = 87.5 fL (80-96fL)**

MCV differs in RBC anomalies (sickle cell anaemia, poikilocytosis, anisocytosis etc.) According to its value we distinguish normo-, micro- and macrocytic anaemias

**MCV=** hematocrit x $10^3$ / RBC count ( x $10^{12}$/ L litre)

Note: most of the time is the MCV automated



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# MCH – mean Hb conc. in 1 RBC

**Reference value:** 29 pg (28-33pg), 18 fmol

**higher:** macrocytic anaemia,
**lower:** microcytic anaemia

**Formula:**
MCH = Hb conc. (in g / 100 mL of blood) /
RBC count (x $10^{12}$ / L)

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# MCHC = mean Hb conc. in RBCs

**Reference value** = 34 +- 2 %

**higher:** hereditary spherocytosis
**normal to lower:** macrocytic anaemia
**lower:** microcytic anaemia

**Formula:**
MCHC = Hb conc (in g /100 mL blood) x 100 /
hematocrit

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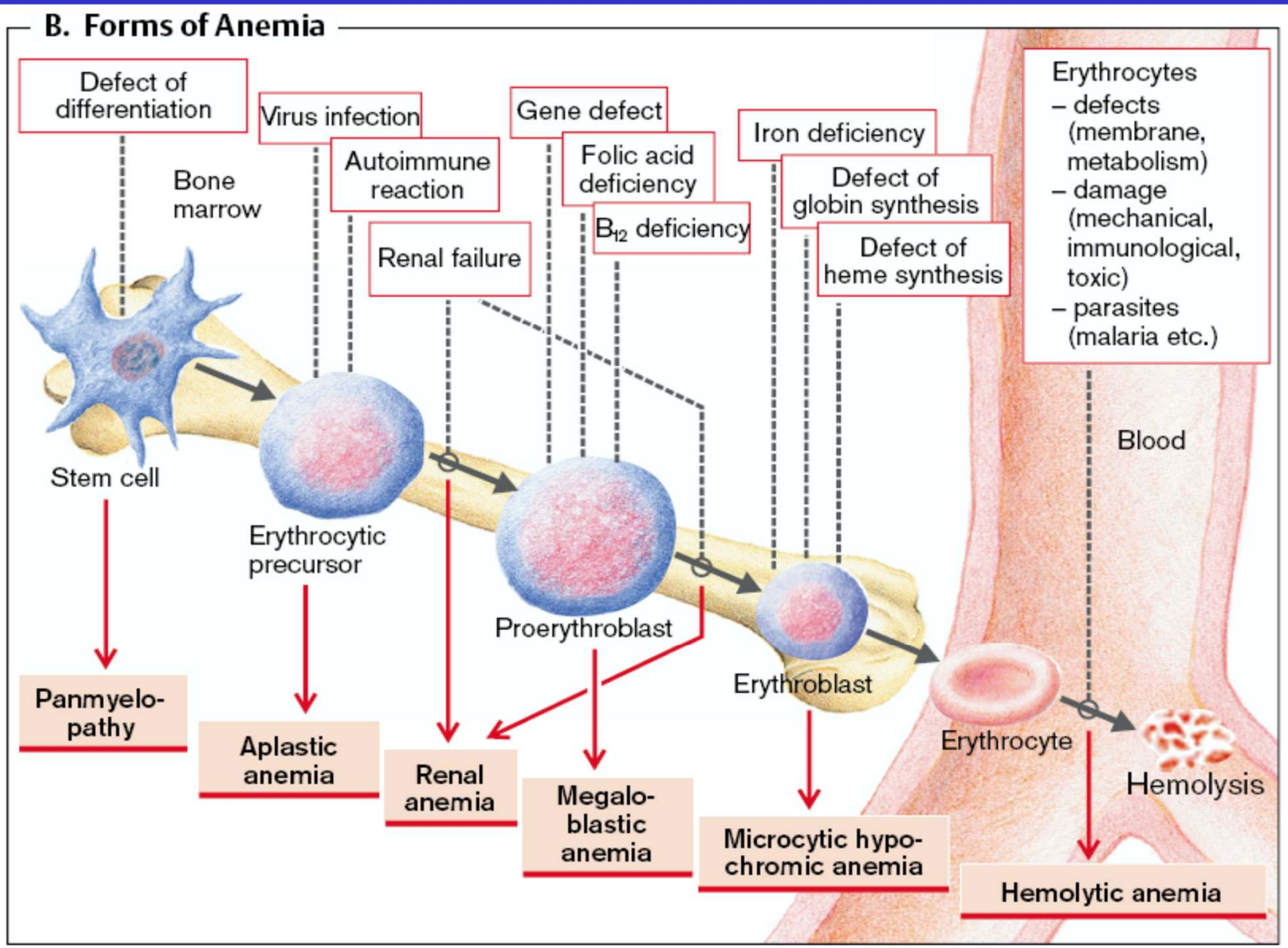
B. Forms of Anemia
Defect of differentiation
Virus infection
Autoimmune reaction
Gene defect
Folic acid deficiency
$B_{12}$ deficiency
Iron deficiency
Defect of globin synthesis
Defect of heme synthesis
Renal failure
Erythrocytes
– defects (membrane, metabolism)
– damage (mechanical, immunological, toxic)
– parasites (malaria etc.)
Bone marrow
Blood
Stem cell
Erythrocytic precursor
Proerythroblast
Erythroblast
Erythrocyte
Hemolysis
Panmyelo-pathy
Aplastic anemia
Renal anemia
Megalo-blastic anemia
Microcytic hypo-chromic anemia
Hemolytic anemia

# Reticulocytes (RTC) count

In per cent % of all cells in the red line.

**Reference value:** both women and men 0.5 – 1.5 %
a) In intra-vital staining (brilliant cresyl blue) is the percentage of RTC counted manually
b) In flow cytometry sometimes absolute values are given

**higher:** bleeding, haemolytic (compensatory erythropoietic activity)

**lower to none detected:** erythropoesis suppression, Bone marrow suppression

## Bone marrow cytology

**Sternal punction:** from manubrium sterni.
Only cytologic analysis of bone marrow blood.
(eg. cytogenetics, FACS (fluorescent- activated
cell sorting, and other methods of molecular biology)

**Trepanobiopsy:** from spina iliaca superior posterior.
The sample contains also bone marrow tissue.
This is necessary for the histological investigation
of bone tissue.

# Readout of the bone marrow cytology

- normo-, hypo- and hyper-celularity, dysplastic bone marrow

- presence of blasts, normal and pathologic sideroblasts

- differentiation of normal and leukemic blasts (enzymatic activity staining, aberant chromosomes, cell determinants, oncogenes and others)

# Erythropoetin

**Method**: ELISA (enzyme linked immuno essay) or RIA (radio-immuno essay) in serum or plasma

**Lower level (low production):** kidney disorder, anemia with lo erythropoeietin of unknown origin, protein catabolism, higher $p_aO_2$, polycythaemia vera (raised cell count).

**Higher level (hi production):** secondary polycythaemia, lower $p_aO_2$, sideropenic anaemia and other haemolytic anaemias

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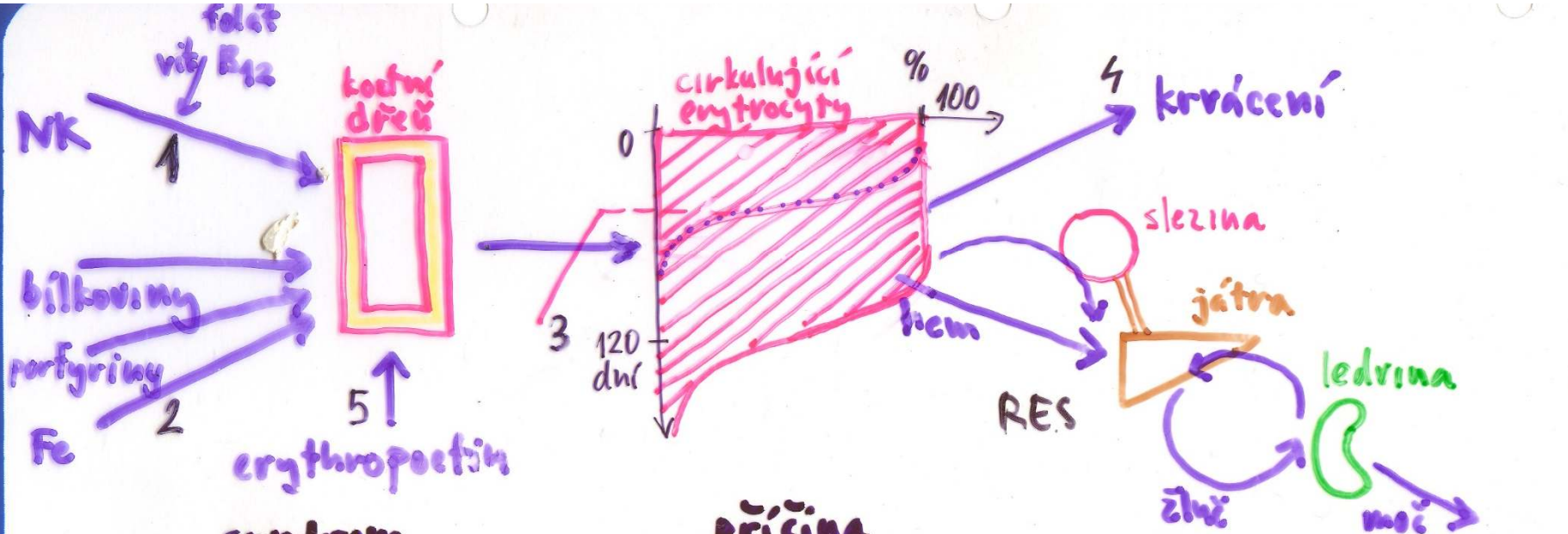


| | syndrom | příčina |
|---|---|---|
| 1 | makrocytární anémie | ↓ vit. B12, folát |
| 2 | hypochromní anémie | ↓ Fe |
| 3 | hyperbilirubinémie | hemolýza |
| 4 | **posthemoragická anémie** | exsanguinace (**meléna, menstruace, atd.**) |
| 5 | hyper-kreatininemie | chronická renální insuficience |

# Iron (Fe, ferrum) – basic facts.

**Free iron is toxic**. Free iron generates **free oxygen radicals**. When free oxygen radicals get out of control, the toxic effects are called "**oxidative stress**".

Total body iron content = 50 – 70 mmol, ie. 3 – 4 g.

Hemoglobin is packed with Fe, contains 65 – 75 % Fe, myoglobin: 3 – 5 % Fe, enzymes with the hem group 0.2 %. In plasma is 0.1 % bound to transferin, ferritin and haemosiderin contain 15 – 20 % Fe.

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# Iron - continued

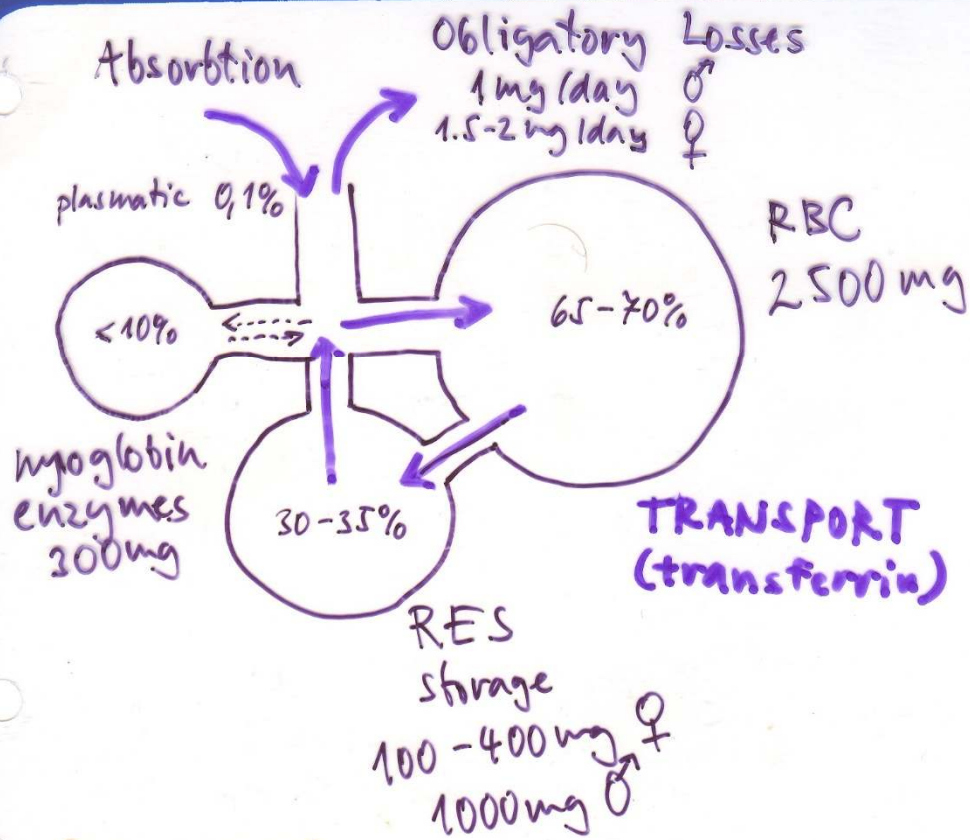
**Resorption:** duodenum, upper part of small intestine. **Daily intake**: circa 18 μmol Fe (1 mg), balanced **daily losses**.

**Output:** is not controlled.

**Resorption** depend on 1) erythropoietic activity of bone marrow, 2) Amount of iron stored
**Losses** depend on ferritin content in desquamated cells

Once iron is resorbed, it enters a "closed system" of
**plasm- bone marrow – RBCs -storage**

Iron stores are in form of **ferritin** and **haemosiderin**




## Iron pools

Iron content in RBC = tot. body Hb $\cdot \frac{\text{mol. weight of iron}}{\text{mol. weight of Hb}}$

Feritin (plasma): 35 µg/l ♀ 95 µg/l ♂ (RIA)

(sideropenia: < 12 µg/l)

# Iron content in serum

All transported iron is bound to transferin, it is called **serum iron** pool, and the amount of binding sites is called **TIBC** (total iron binding capacity).

**Reference values:**

| | |
|---|---|
| newborns | 17.90 – 44.75 µmol / L (litre) |
| babies | 8.95 – 21.48 µmol / L |
| women | 7.16 – 26.85 µmol / L |
| men | 8.95 – 28.64 µmol / L |

# TIBC (total iron binding capacity)

**Reference values:**

44.75 – 71.60 μmol / 1 litr

Higher values of TIBC are correlated with lower iron in serum (more binding sites in transferrin).

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# % trasferrin saturation
## (sometimes listed as % iron saturation)

% transferrin saturation is ratio
**serum iron (to) TIBC**

**Reference values:**
20 – 55 %

Lower values in **iron deficiency**.

**Both low transferin saturation**

together with low values of **TIBC**:
hemochromatosis, hemosiderosis, liver diseases



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# Serum ferritin

Correlates with the level of iron storage in organism.

**Reference values: in** μg / 1 Litre, or ng / mL

| | |
|---|---|
| newborn | 25 – 200 |
| 1. mo. | 200 – 600 |
| 6. mo. – 15 years | 7 – 140 |
| women | 12 – 150 |
| men | 15 – 150 |

**lower:** detectable already in **first stages of sideropenic anemia**

**higher:** in anemias of chronic diseases or in tumours

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# Soluble transferrin receptor (sTfR)

Its level is measured by the ELISA method.

**higher:** iron deficiency, intensive erythropoesis (hemolytic anaemias, β-thalasemia, polycytemia) = higher TfR Expression in cellular surfaces

**lower:** bone marrow suppresion, chronic renal failure

Note: sTfR is generated by proteolysis of TfR in specific extra-celular domain, sTfR is a monomer detectable in plasma or serum.
TfR and sTfR are correlated, sTfR is an indirect indicator of TfR expression in organism.



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# WBC (white blood cells) count

Given in **quantities** x $10^9$ / 1 litr; x $10^6$ / ml; x $10^3$ / µl

**Leukocytosis**

**„Physiological“**: higher physical activity, pregnancy,
in newborns

**Other causes**:

acute infections, tissue necroses, bleeding, higher ACTH production,
(ACTH = adreno- cortico- tropic hormone)
also in higher level of gluco-corticoids (even in treatment),
leukemoid reaction, stress, intoxication

**Leukopenia**

Migration of especially granulocytes into the „marginal pool,“
ineficient granulopoesis,
hypersplenism, antibodies against leukocytes,
Bone marrow suppression

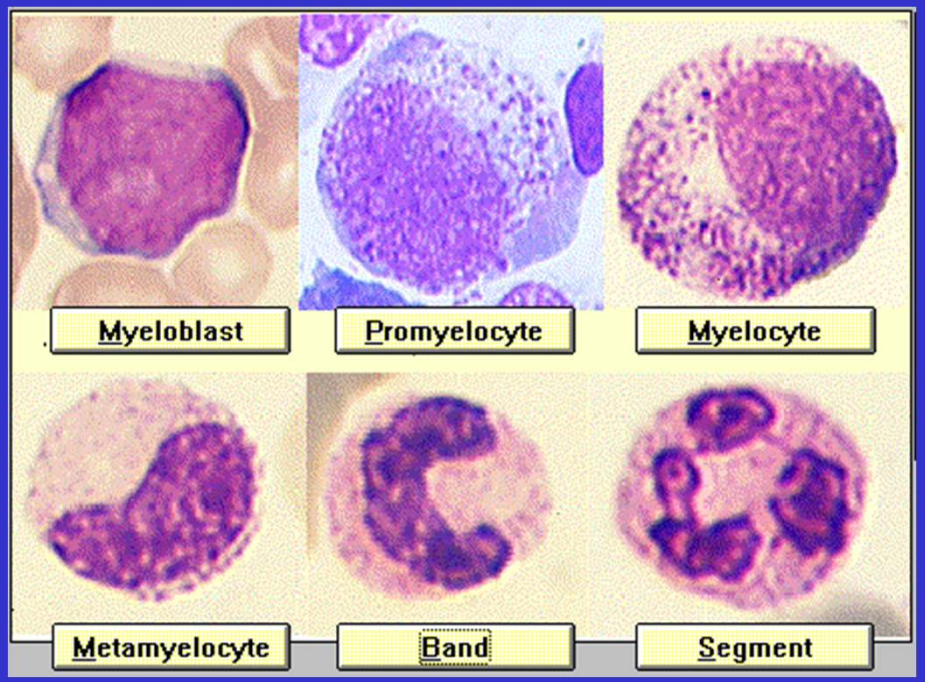
# Differential WBC count

Different WBC elements (PMN and MNC, poly- morpho- nuclear cells and mono- nuclear cells) in spread.
**Granulocytosis – neutrophil** granulocytes

**Eosinophilia –** especially in alergic states, in connective tissue disorders („collagenoses“), in skin disorders (pemphigus vulgaris, dermatitis exfoliativa), in parasitic diseases,
**Eosinopenia –** higher production of ACTH and gluco-corticoids, beginning of serious infections

**Basophilia –** in CML ( = chronic myeloid leucemia), Hodgkin lymphoma, polycytemia vera, diabetes, hypertyreosis
**Basopenia** – higher production of ACTH and gluco-corticoids

# Developmental stages of granulocytes



# Alterations of lymphocyte count

**Lymphocytosis**

especially viral infections,
infectious mononucleosis,
group of cytomegalovirus infection,
ALL (= acute lymphocytic leukemia),
CML ( = chronic myeloid leucemia)

**Lymphopenia**

higher ACTH,
irradiation,
effect of cytostatics,
Lymfangiektasias in GIT,
ductus thoracicus drainage,
(in tumours)

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# Some lymphocyte enzymatic activities I.

## Alcalic phosphatasis (ALP)

**normal (low)** activity - in normal blasts and granulocytes

**higher** activity – stress, higher gluco-corticoids, gravidity, polycythaemia vera

**lower to zero** activity – CML, PNH (paroxysmal nocturnal hemoglobinuria), MDS (myelo-dysplastic syndrome)

# Some lymphocyte enzymatic activities II.

## Acid phosphatase

**zero activity** – normal blasts
**lower** activity – AML, CLL
**higher** activity – CML

## Peroxidase activity

**zero values** – normal blasts
**higher to high values** – MDS, AML

# Some lymphocyte enzymatic activities III.

## Activity of non-specific esterases

**characteristic** in monocytes
(used in diagnostics of leukemias with mono-cytic components)

**zero activity** = mono-cyto-blasts

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# Flow cytometry and FACS
# (Fluorescence-activated-cell-sorting)

**Flow cytometry** – cells in suspension can be counted and analyzed. Cells can be separated based on physical (eg. volume), optical (number of granules) chemical (e.g. DNA content, pH of the ICT) and immunological differences (after tagging of surface receptors by mono-clonal antibodies).

**FACS** (Fluorescence-activated-cell-sorting) is flow-cytometry based cell sorting.

**Use in hematology:**
Flow cytometry and FACS can analyse cells according to cell differentiation Determinants, leucocyte marker expressions, DNA analysis, imuno-pheno-typing in myelo-proliferation etc.



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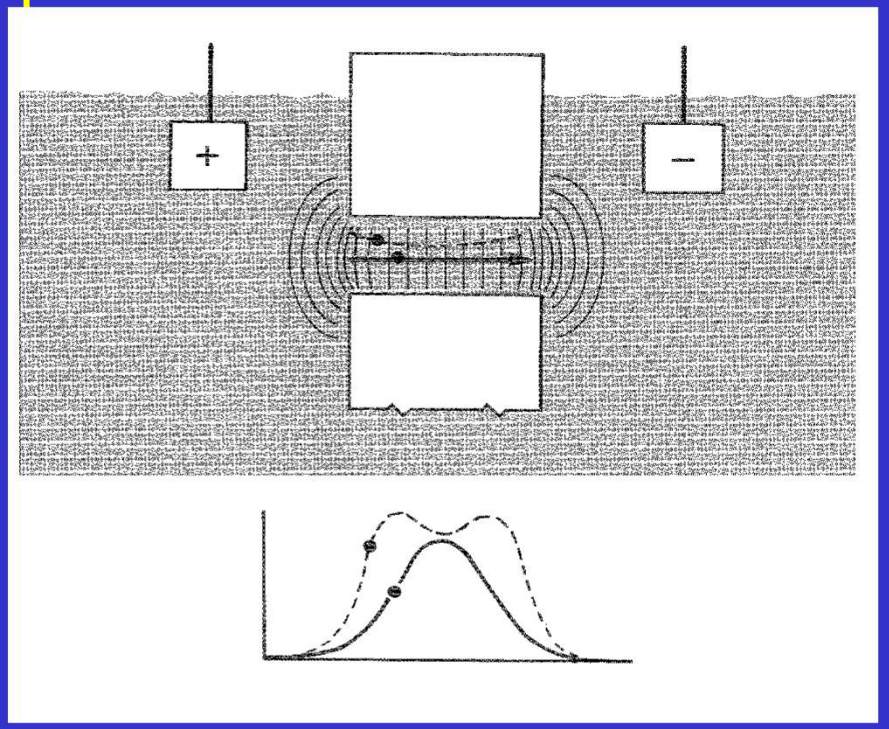
– Automatic cell sorting by hematologic analyzers

# How does the analyzer work ?

- impedance detection – when particle moves between electrodes, impedance is higher

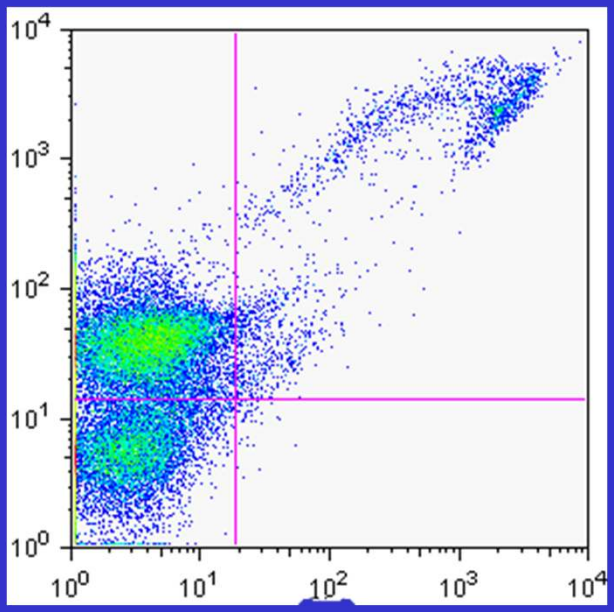
- impulse count = particle count

- impulse amplitude = particle volume




- optical detection – when particle moves in focused light beam, light is scattered

# **Flow cytometry and FACS**
(Fluorescence-activated-cell-sorting)




Sorting of cell populations using FACS

one stream in line with the light beam (Forward Scatter or FSC) and several perpendicular to it (Side Scatter (SSC)

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# Some cell differentiation determinants

- **CD3 –** T-lymphocytes, TCR (T-cell receptor)
- **CD4** – helper T-lymphocytes
- **CD7 –** T-lymphocyty, NK (natural killer) cells
- **CD8 –** cytotoxic/ supressor T-lymphocytes, NK cells, thymocytes
- **CD21, CD22 –** precursors and mature B-lymphocytes
- **CD34** – precursors of haematopoietic cells
- **CD50 –** granulocytes
- **CD52 –** eosinophilic granulocytes
- **CD61 –** thrombocytes, megakaryocytes
- **CD70 –** Reed-Sternberg cells, activated B- a T-lymphocytes
- **CD77 – cells of** Burkitt lymphoma, activated B-lymphocytes