



Lecture Series “Clinical Cases”

Blood

WS 2017/2018

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Medizinische Klinik und Poliklinik

Abt. für Onkologie, Hämatologie und Immunologie

What cells can be found in the blood? where do they originate?

(1) Blood Smear: which cells do exist?

- ➔ Differentiation, normal range
- ➔ Overview of physiological functions
- ➔ Consequences of lack or dysfunction

(2) Where and how are blood cells generated?

- ➔ Bone marrow from so-called hematopoietic stem cells

(3) Developmental hematopoiesis

Complete Blood Count

Medscape®		www.medscape.com
		Ref
WBC	13.8	4.0–11.0 K/Cumm
RBC	5.07	4.10–5.70 M/Cumm
Hemoglobin	16.2	13.0–17.0 g/dL
Hematocrit	46.6	37.0–49.0%
MCV	92	80–100 fL
MCH	31.9	27.0–34.0 UUG
MCHC	34.7	32.0–34.0 g/dL
RDW	14.2	11.0–15.0%
Neutrophils	73	40–74%
Lymphocytes	17	19–48%
Monocytes	7	3–11%
Eosinophils	2	0–7%
Basophils	1	0–2%
Platelets	333	130–400 K/Cumm

WBC, white blood cell; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width

Source: Lab Med © 2007 American Society for Clinical Pathology

Functions:

Erythrozytes:

Transport of oxygen

Leukocytes:

Immune defense

Platelets:

Blood clotting

Erythrocytes: Quantitative deviations

Anemia (deficiency of vit. B12 or folic acid, intrinsic defects of cellular membrane or enzymes, hemoglobinopathies)

Polycythemia (Polyglobulia) (e.g. EPO-dependant formation in polycythemia vera)

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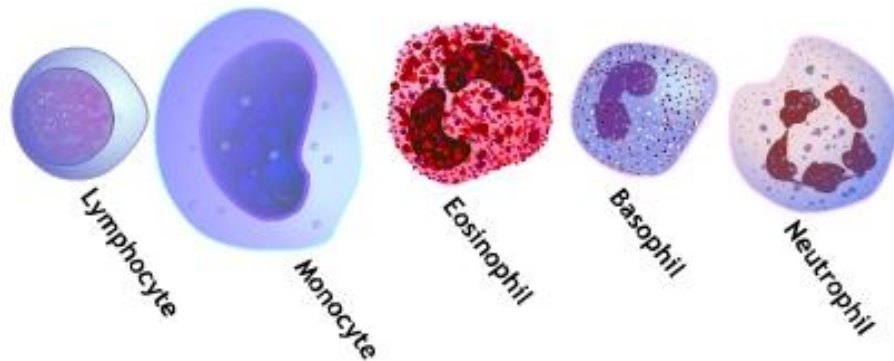


Platelets: Quantitative deviations

Thrombocytopenia: hemorrhages (z.B. ITP)

Thrombocytosis: vessel occlusion, thrombosis (z.B. essential thrombocytosis = ET)

WBC Differential



➤ **Innate Immunity:**

phagocytosis of foreign material/pathogens

- Neutrophils
- Monocytes /macrophages /dendritic cells

➤ **Adaptive Immunity:**

▪ **B Lymphocytes/plasma cells:**

can produce specific antibodies against pathogens after specific stimulation/activation

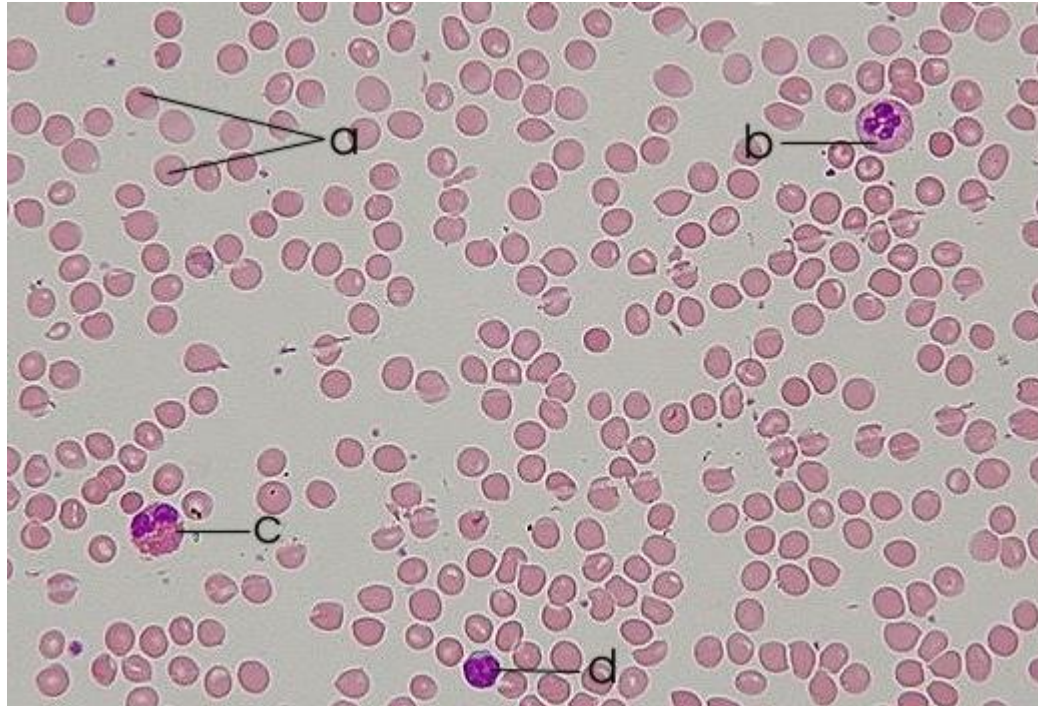
▪ **T Lymphocytes:**

coordinate the immune response
can directly attack bacteria/virus infected cells via cytotoxic mechanisms

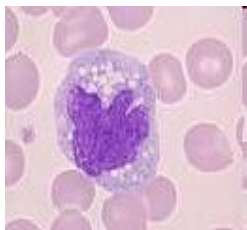
➤ Leukocytes produce different mediators (cytokines, chemokines) to coordinate the immune response.

WBC VALUES		
NORMAL RANGE, TOTAL WBCs: $4.5\text{--}11.0 \times 10^3/\mu\text{L}$ (ADULT POPULATION)*		
Normal ranges, differential leukocyte counts:		
3-part differential	Lymphocytes	$1.0\text{--}4.8 \times 10^3/\mu\text{L}$
	Mid-Cells	$0\text{--}0.9 \times 10^3/\mu\text{L}$
	Granulocytes	$1.8\text{--}8.1 \times 10^3/\mu\text{L}$
5-part differential	Lymphocytes	$1.0\text{--}4.8 \times 10^3/\mu\text{L}$
	Monocytes	$0\text{--}0.8 \times 10^3/\mu\text{L}$
	Neutrophils	$1.8\text{--}7.7 \times 10^3/\mu\text{L}$
	Eosinophils	$0\text{--}0.5 \times 10^3/\mu\text{L}$
	Basophils	$0\text{--}0.2 \times 10^3/\mu\text{L}$
Other normal WBC values:	Band neutrophils (%)	0-6%
	Segmented neutrophils (%)	40-70%

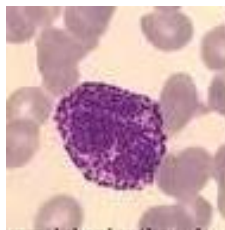
Cytology/examination of blood smear, peripheral blood of healthy adult):



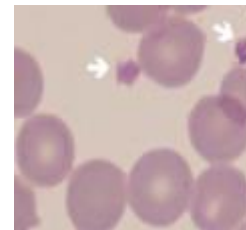
a = erythrocytes; b = granulocytes; c = eosinophilic granulocyte; d = lymphocyte



monocyte

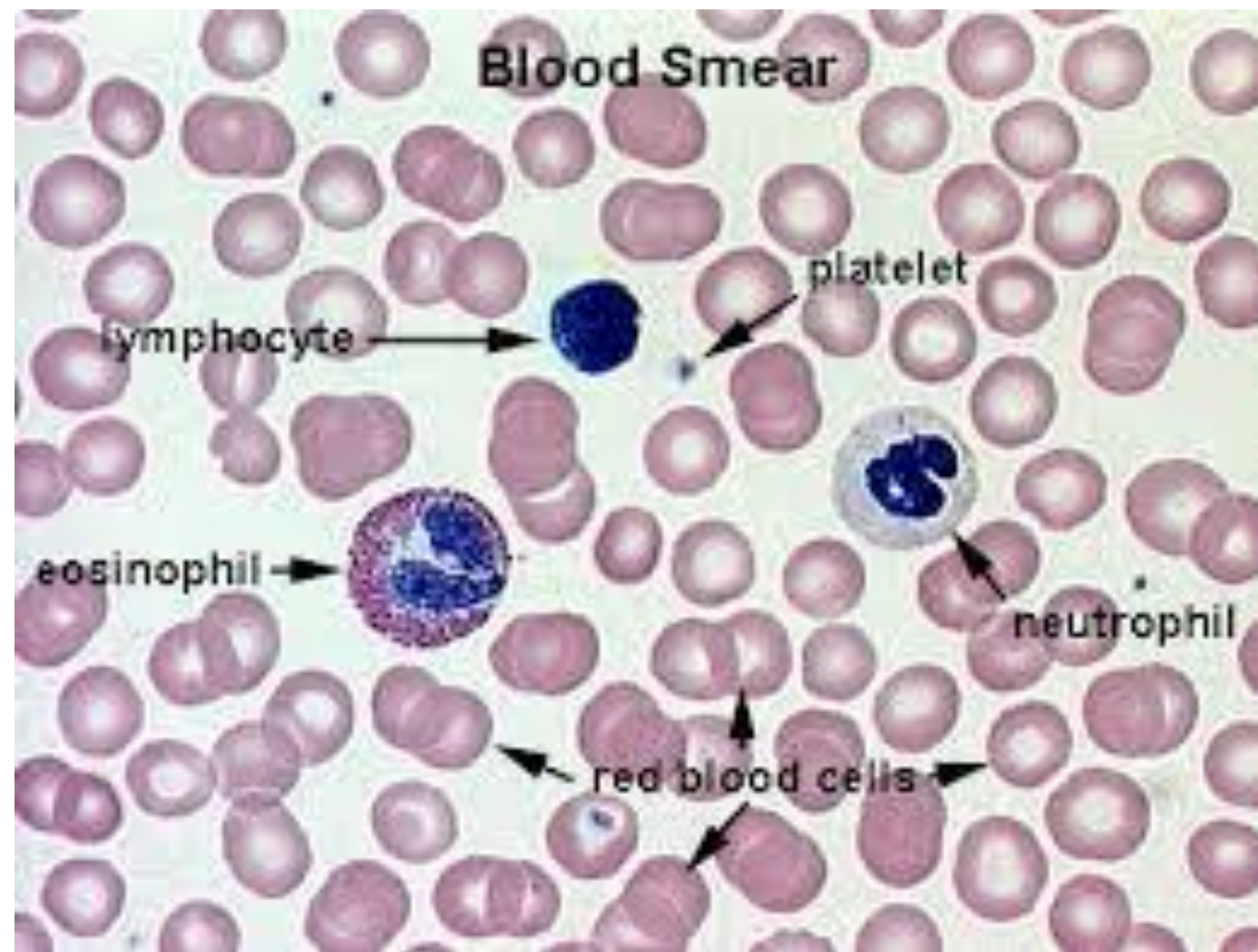


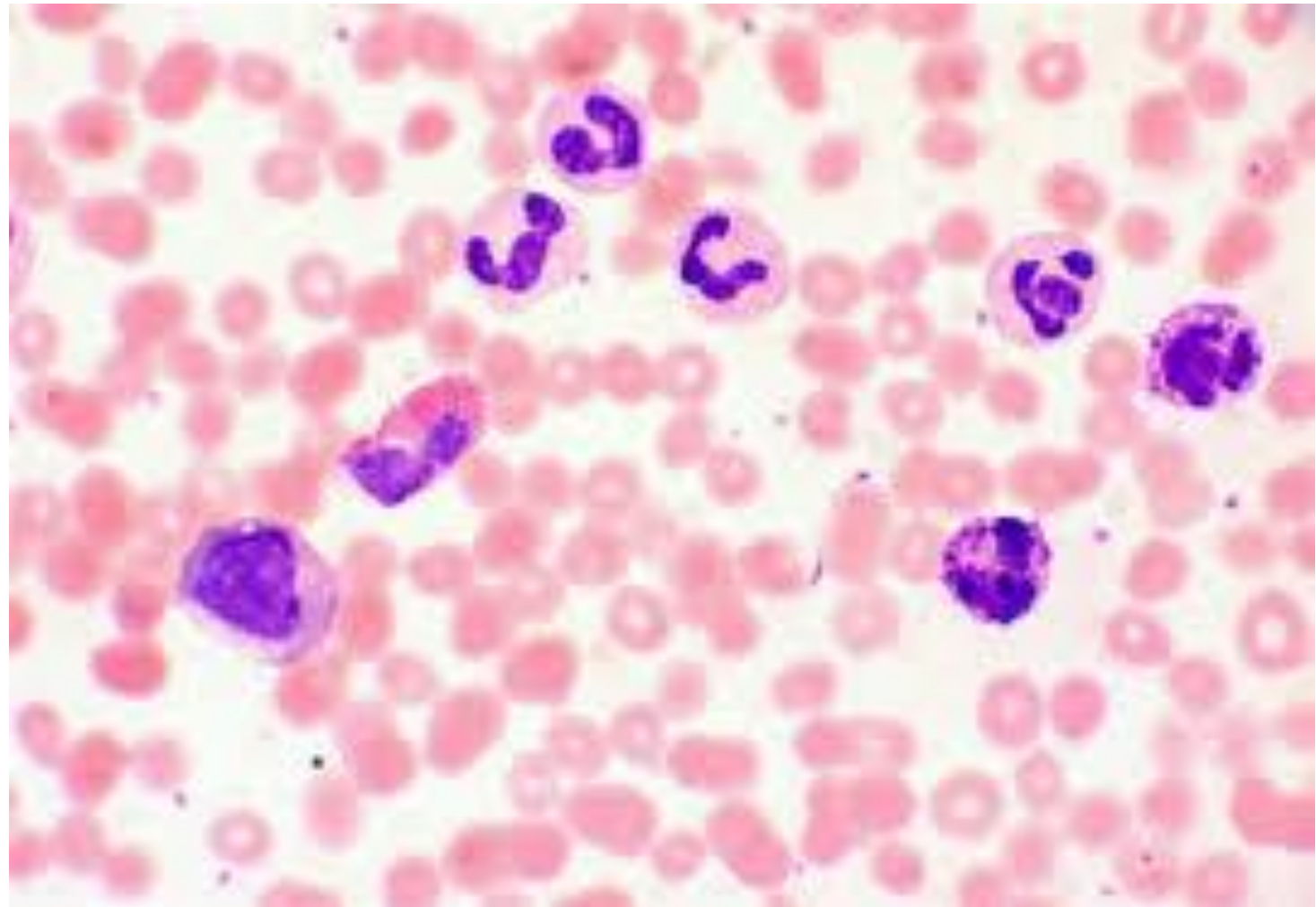
basophilic gr.



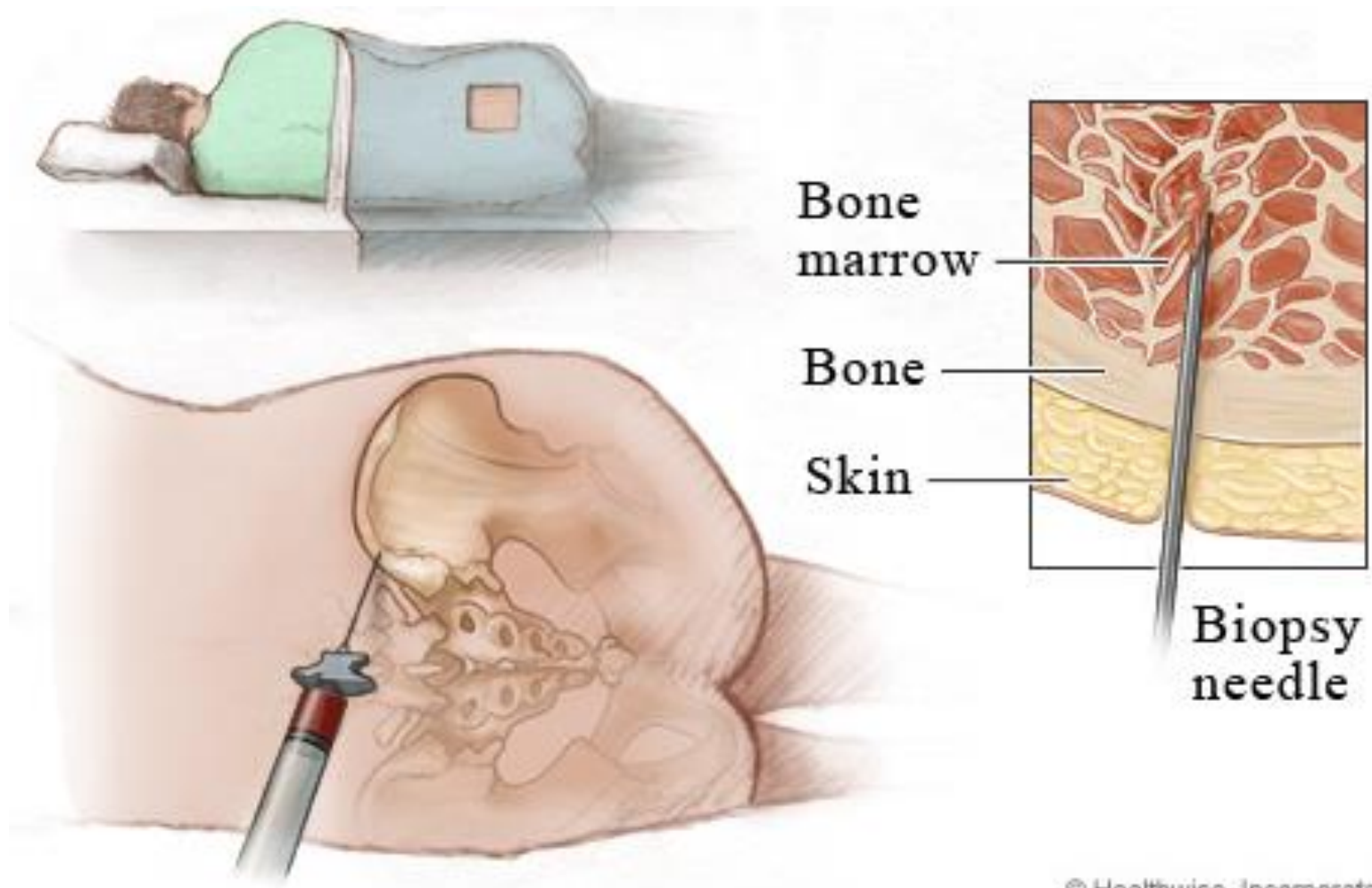
platelet

Blood Smear

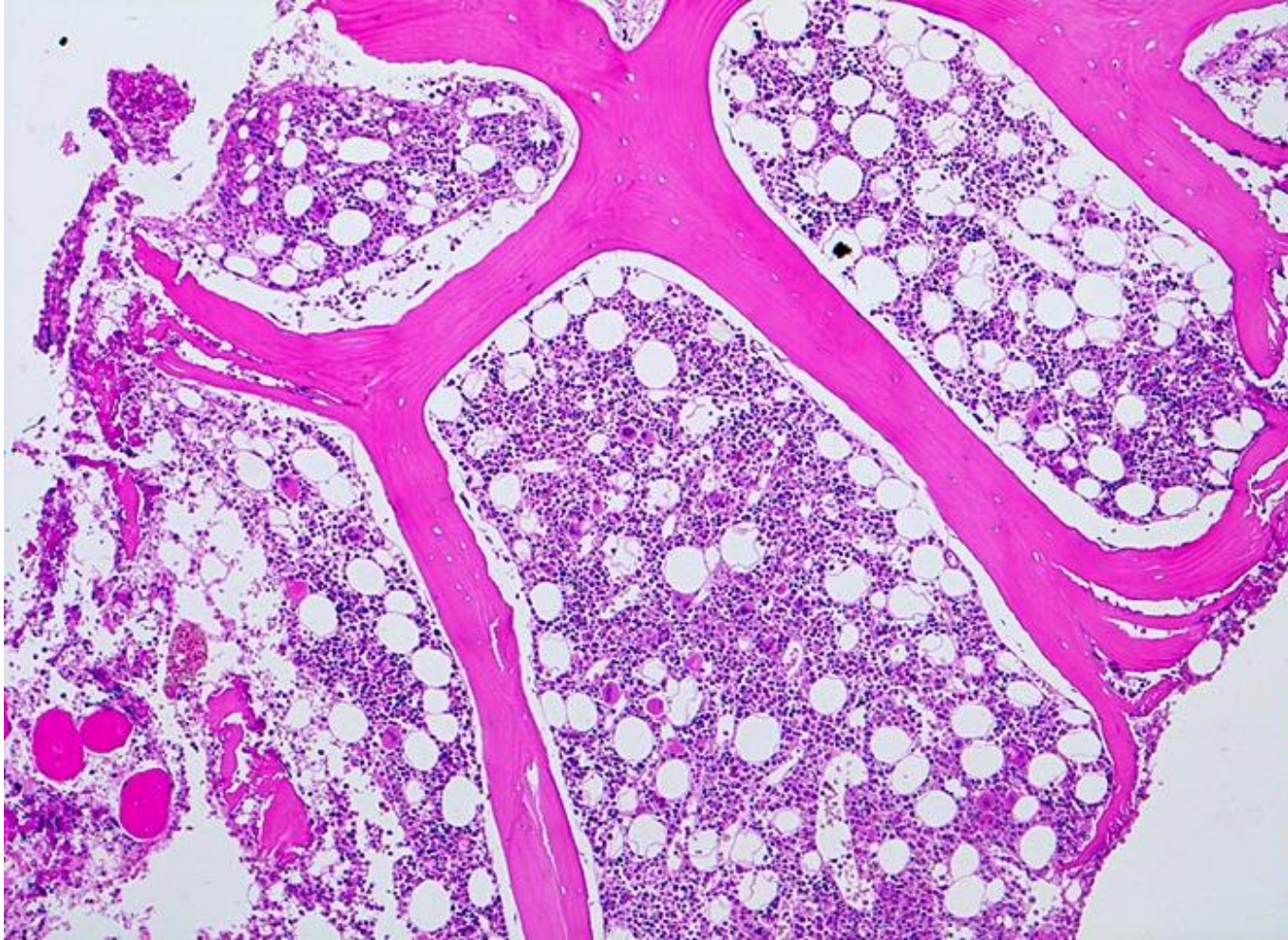




*Generation of blood cells
... in adults in the bone marrow:*

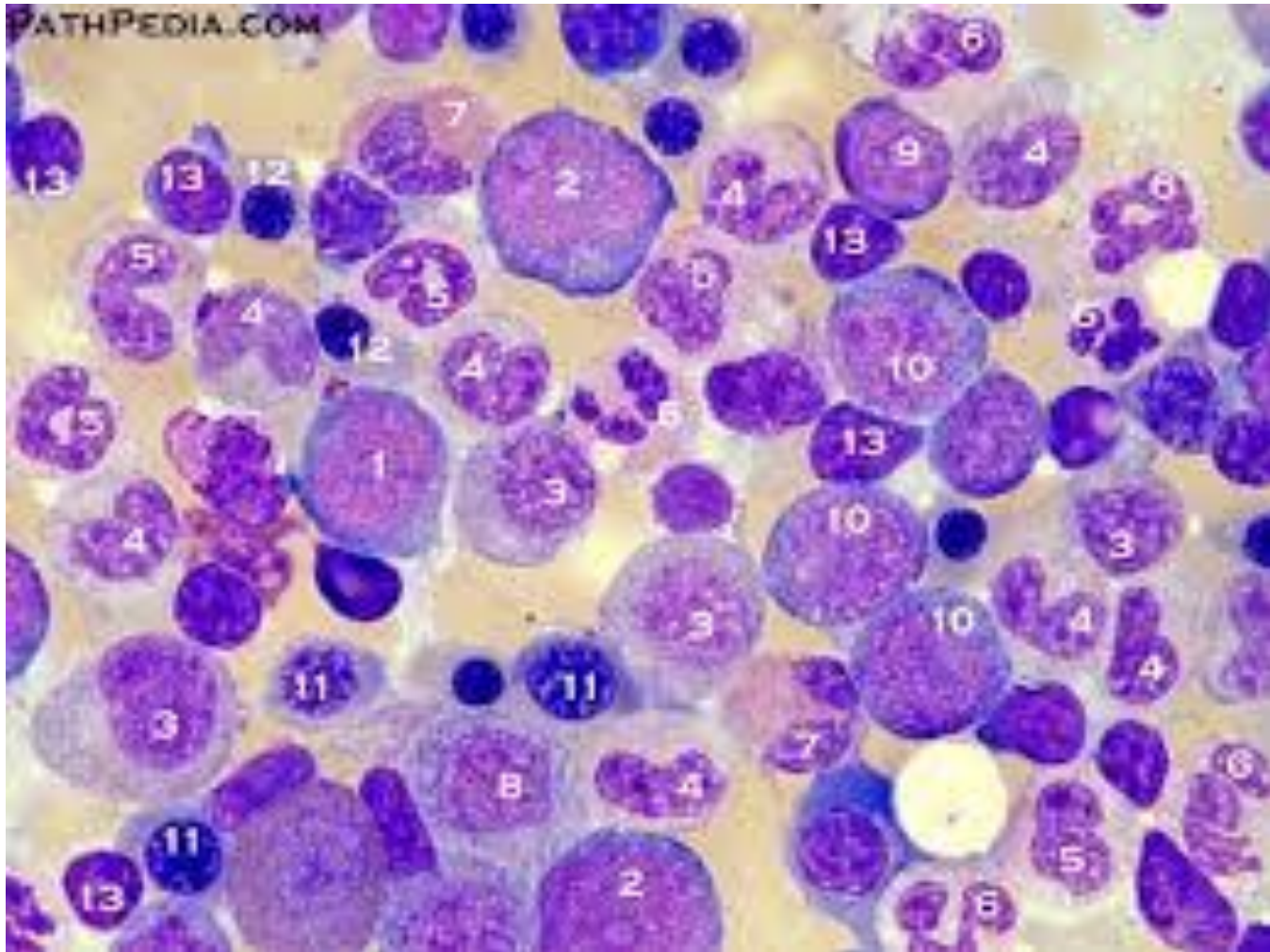


*Generation of blood cells
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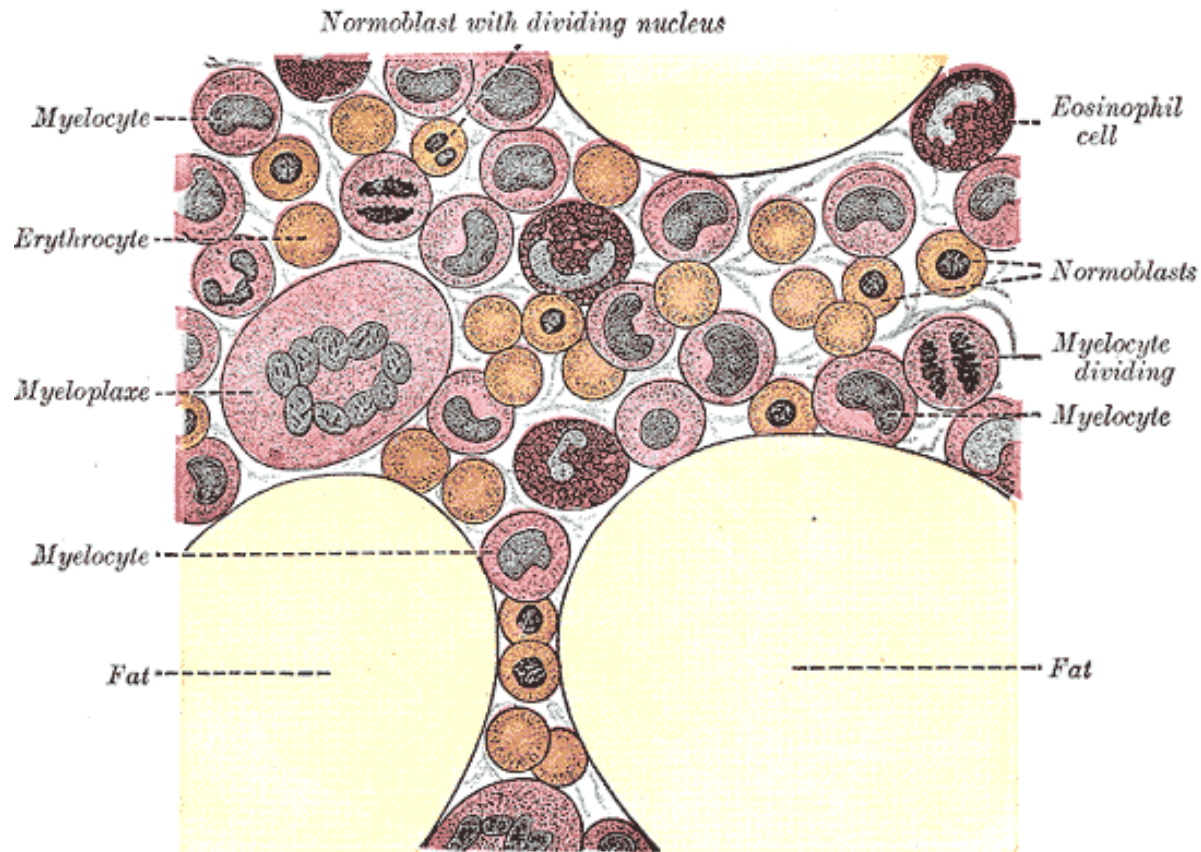
Histology/BM biopsy

*Generation of blood cells
... in adults in the bone marrow:*

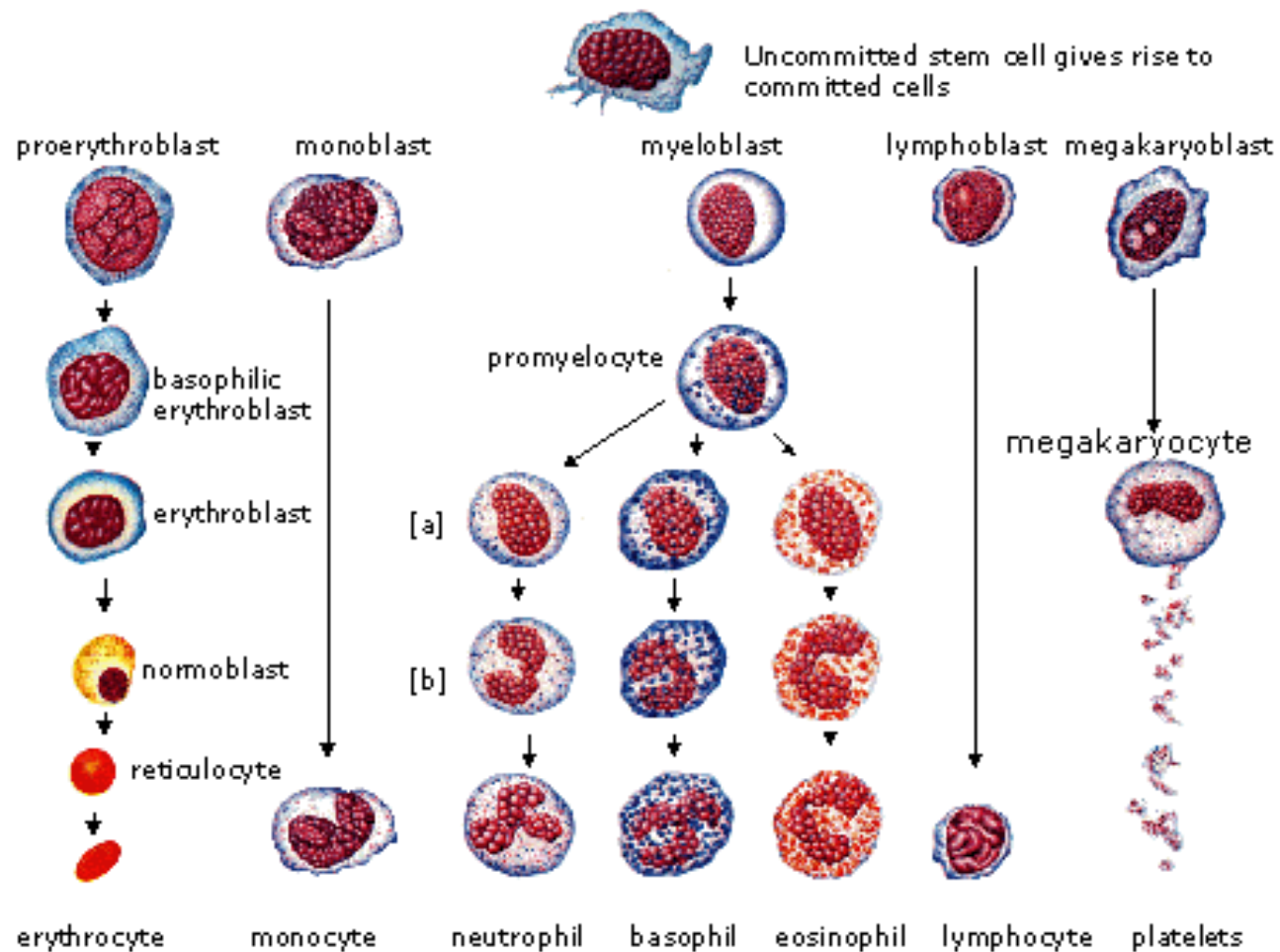


Cytology /BM smear

Generation of blood cells ... in adults in the bone marrow:



Generation of blood cells ... in adults in the bone marrow:

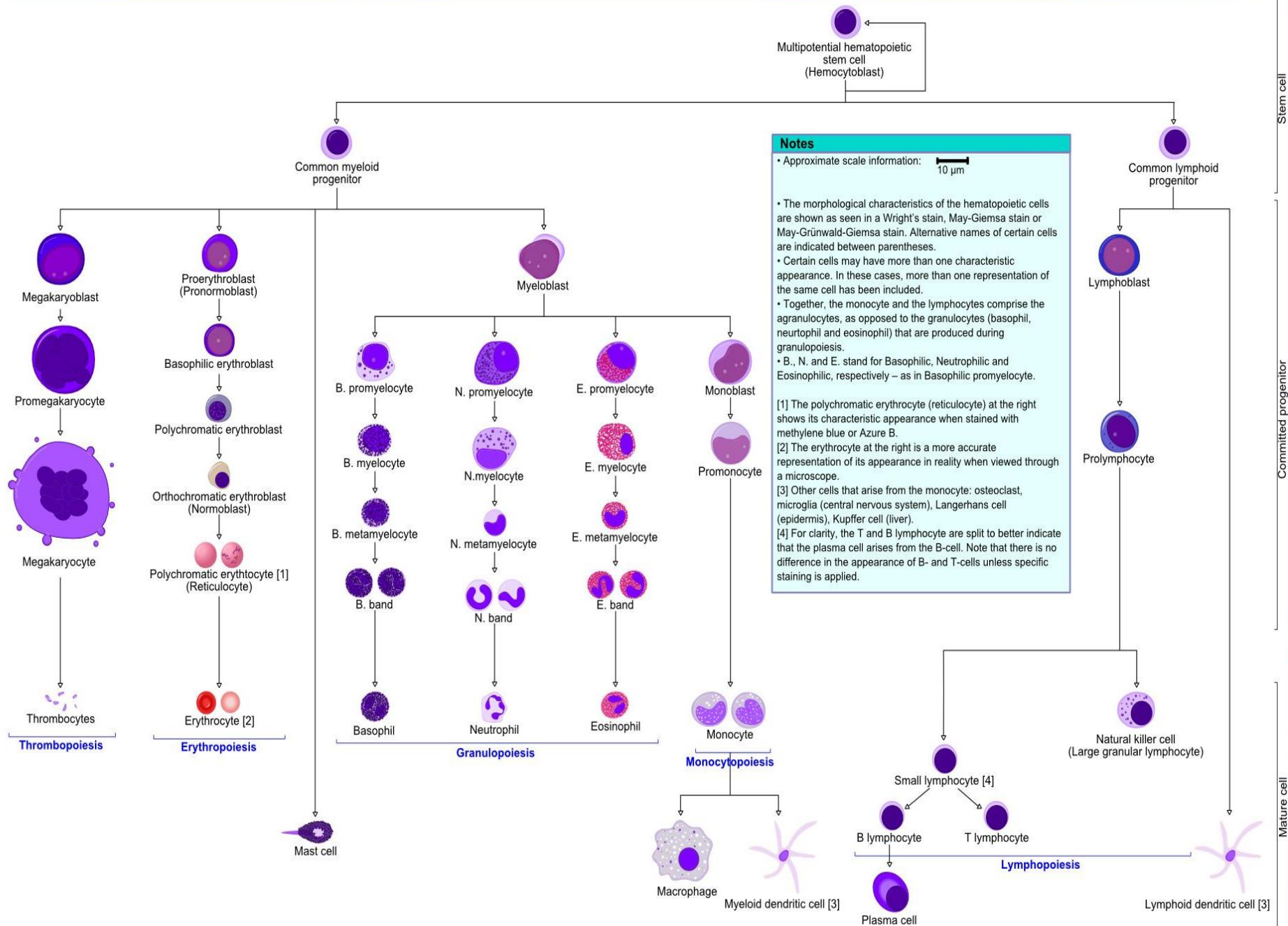


Hematopoiesis in humans

Bone marrow

Blood

Tissue



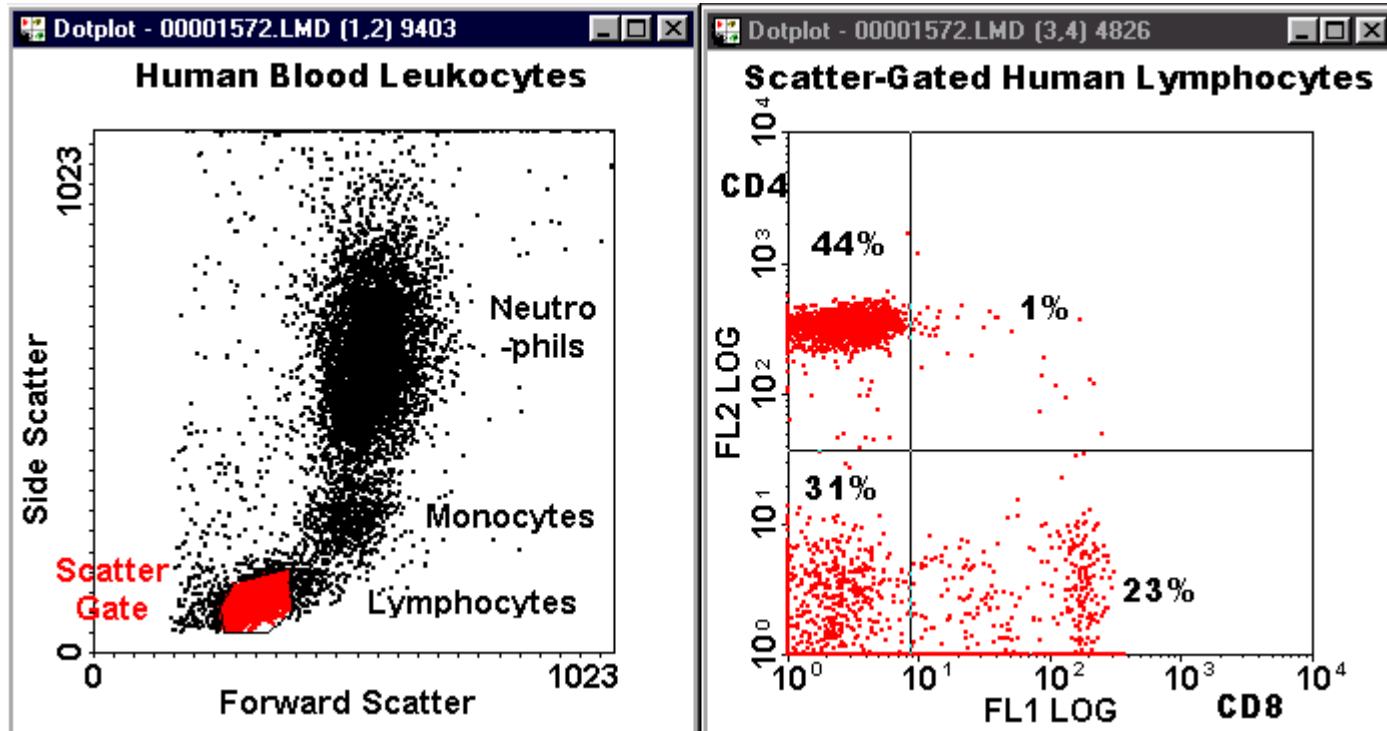
Stem cell

Committed progenitor

Mature cell

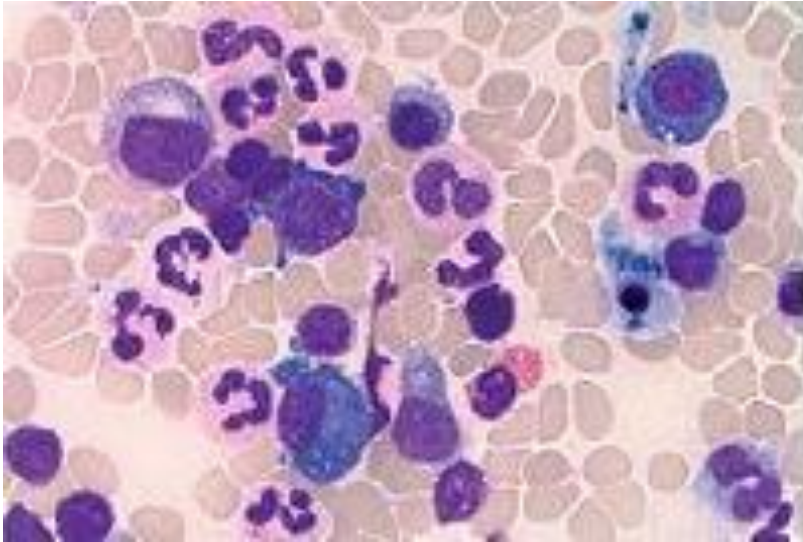
Measurement of blood cells based on physical properties (Flow cytometry)

Granularity

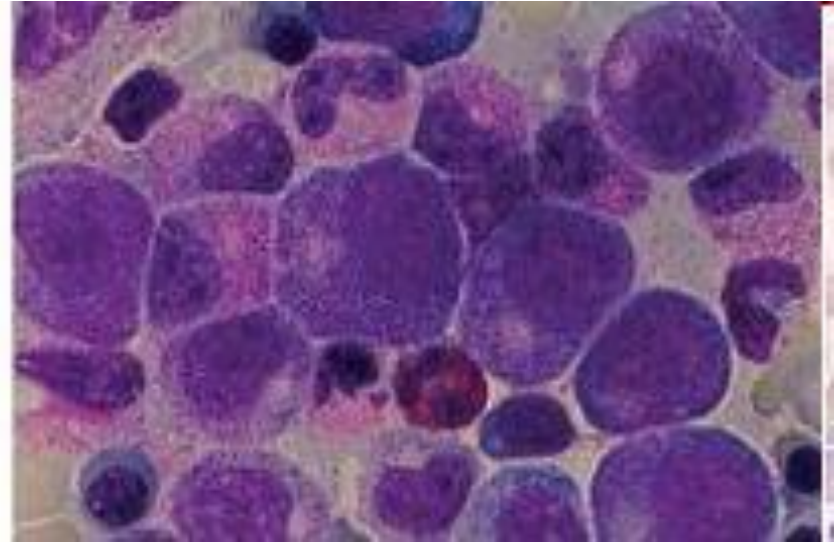


Cell Size

Bone marrow diseases

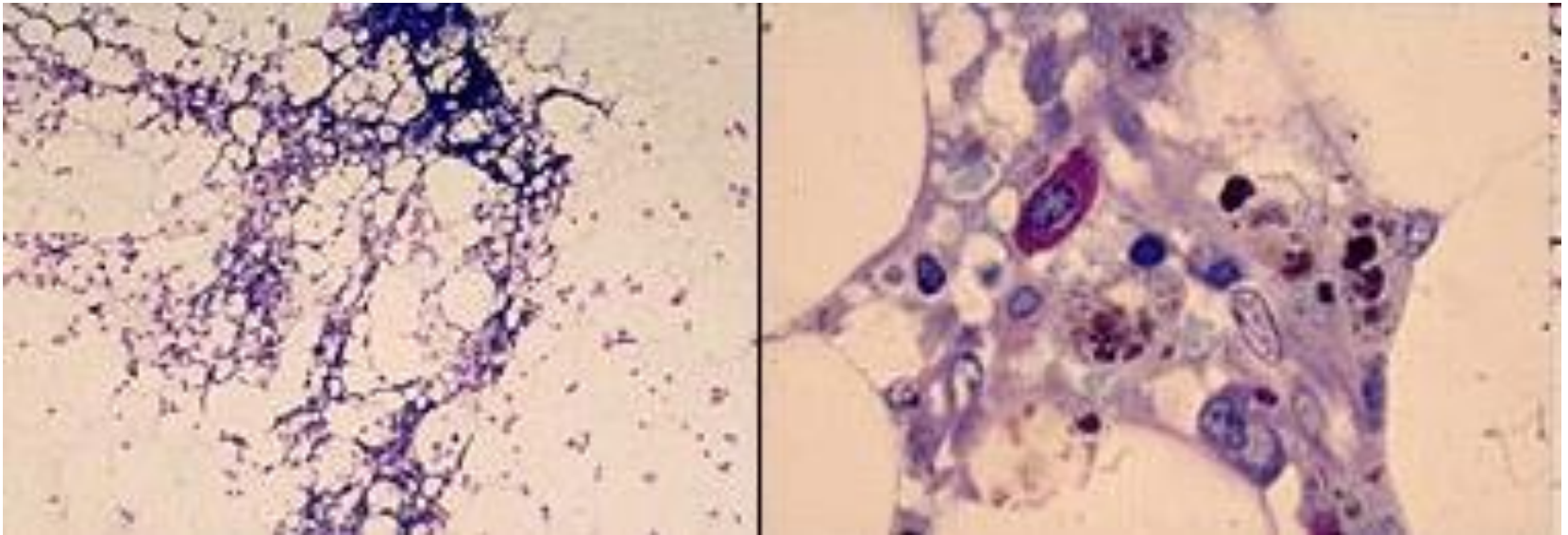


Healthy BM



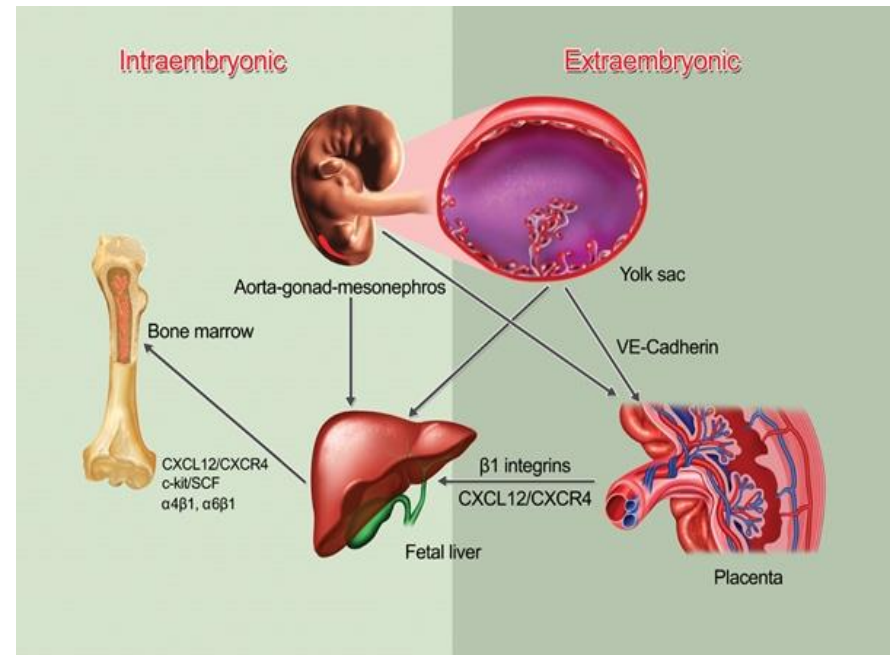
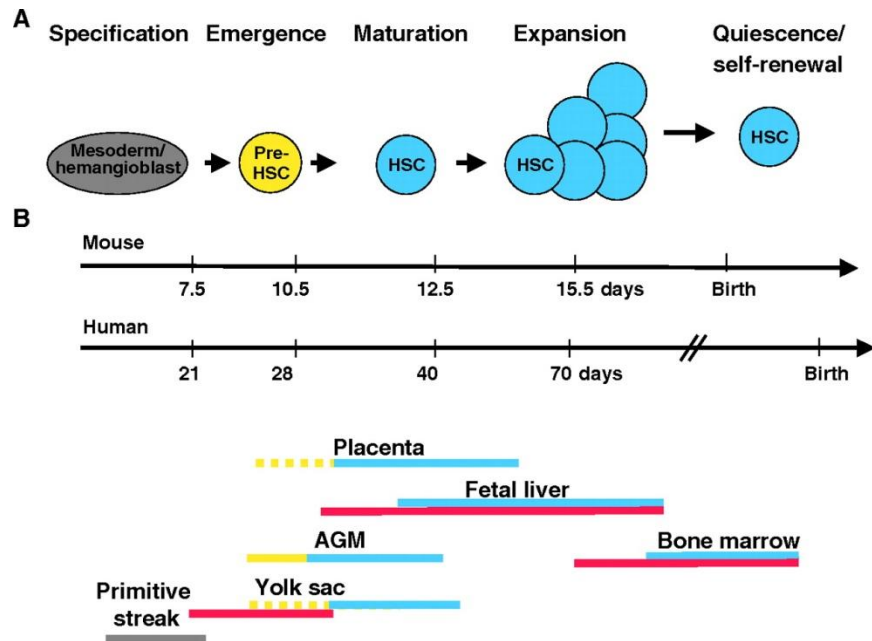
BM with CML

Bone marrow diseases



„Empty marrow“ in aplastic anemia

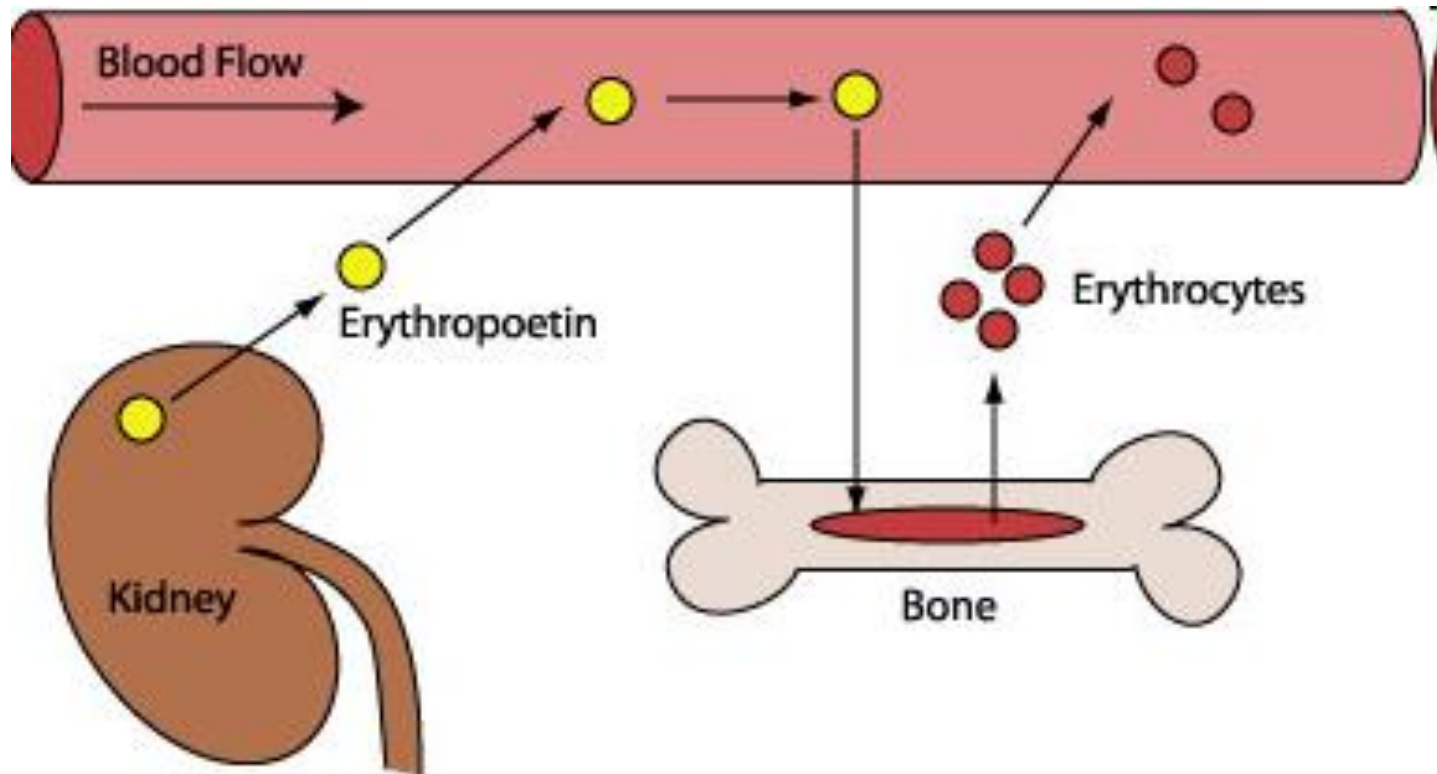
Generation of blood cells ... during embryonic development(developmental hematopoiesis):



Summary

- Naming of different blood cell types
- Main functions of blood cells
- Normal CBC
- Examples for pathological deviations in the CBC
- BM versus peripheral blood

Erythropoiesis in the bone marrow



Definition of anemia

Reduction of

- Hemoglobin Hb
- Hematocrit HCT/Packed Cell Volume PCV
- Erythrocytes

Hb < 13.0 g/dL [m]

HCT < 42% [m]

Hb < 12.0 g/dL [w]

HCT < 38% [w]

Clinical features of anemia



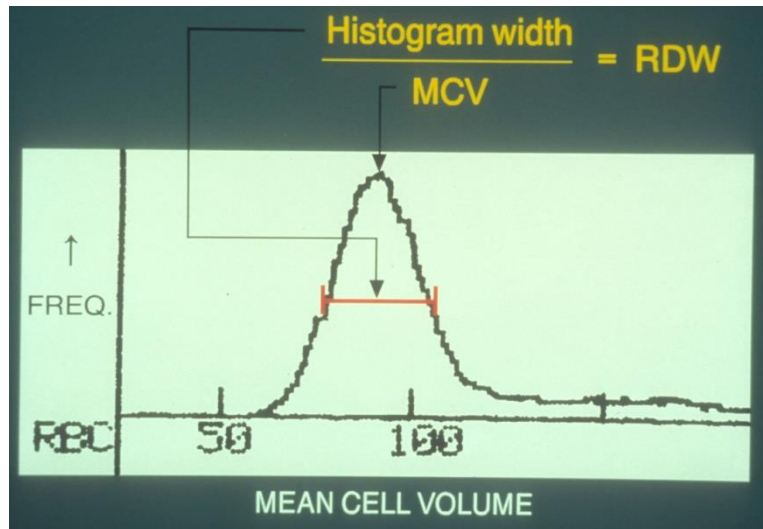
Determination of erythrocyte indices

MCV: mean corpuscular volume [80-95 fL]

MCH: mean corpuscular hemoglobin [28-34 pg]

MCHC: mean corpuscular hemoglobin concentration [31-37 g/dL]

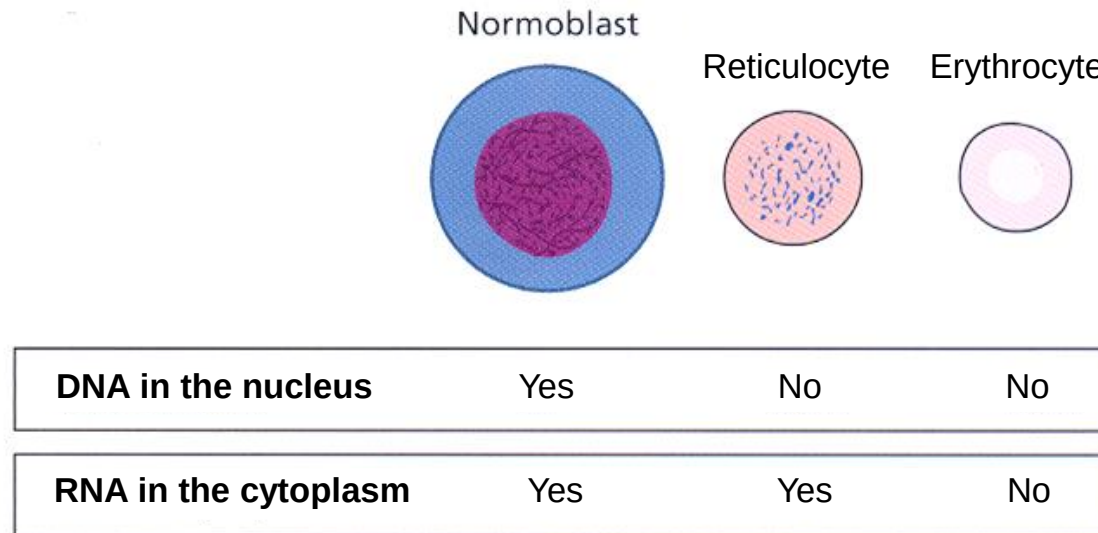
Physiological size distribution



MCV 80-95 fL normocytic
MCV < 80 fL microcytic
MCV > 95 fL makrocytic

MCH 28-34 pg normochromic
MCH < 28 pg hypochromic
MCH > 34 pg hyperchromic

Hypo- vs. Hyperregenerative Anemia



Hyporegenerative Anemia (Production Issue):

Retis



Hyperregenerative Anemia (Destruction Issue):

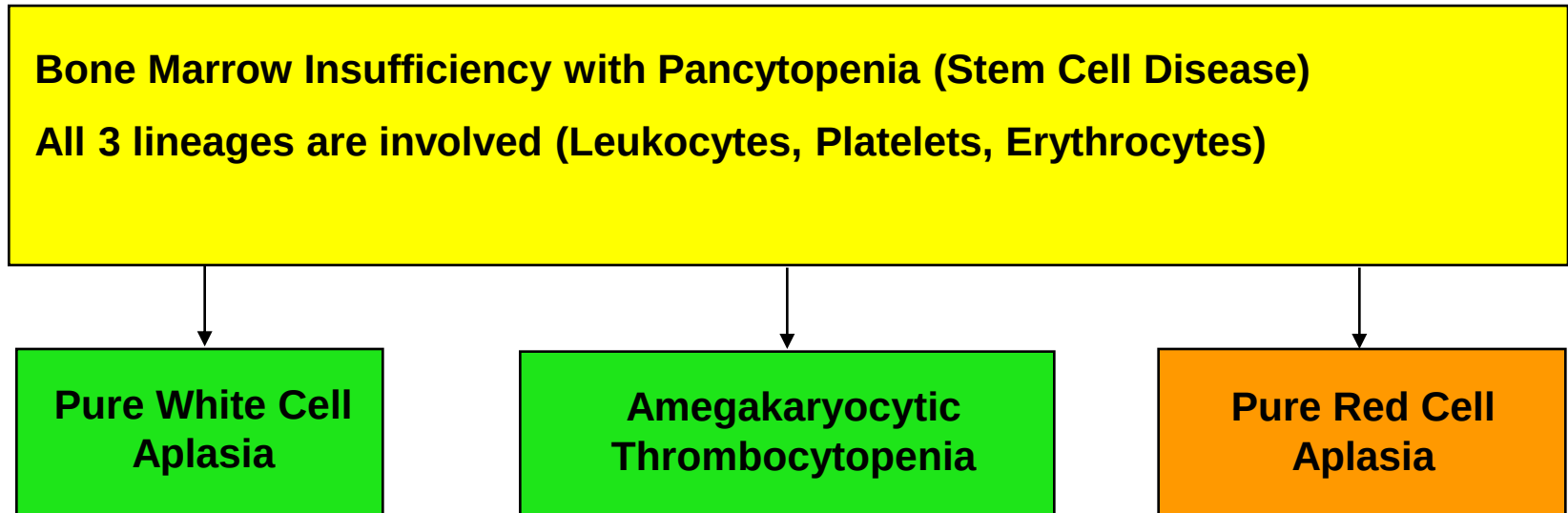
Retis



Causes of Anemia

Principle	Etiology	Form of Anemia
<u>Problems with RBC Generation</u>	1. Defect of the erythropoietic stem cell 2. Defects of DNA synthesis 3. Defects Hb synthesis 4. Lack of erythropoietin 5. Multiple factors	Aplastic anemia AA Myelodysplastic Syndrome Lack of Vit. B12 and Folic Acid Iron Insufficiency Renal Anemia Anemia of chronic disease (cancer, rheumatoid arthritis, infection)
<u>Increased Destruction</u>	1. Defects of the erythrocyte 2. Factors independent of the erythrocyte	<u>Corpuscular hemolytic anemia:</u> -Membrane defects -Enzymopathies -Hemoglobinopathies <u>Extracorpusc. hemolyt. anemia:</u> -Antibodies -Pharmacologic agents -Infectious diseases -Physical/chemical injury -Metabolic causes
<u>Loss of RBC</u>	Bleeding/acute blood loss	Bleeding anemia
<u>Distribution Problem</u>	Pooling of blood cells in an enlarged spleen	Hypersplenism

Aplastic Anemia



Ursachen: 1. Idiopathic AA (70%), cause unknown

2. Secondary AA (25%)

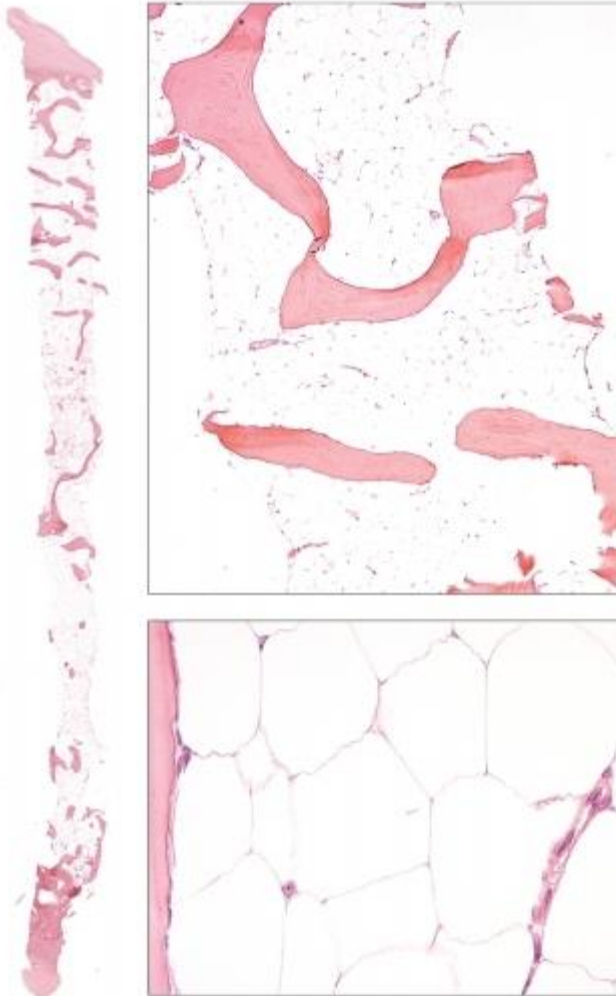
Pharmacologic agents (chloramphenicol, sulfonamide, gold etc.),
Chemicals (benzene), virus infections (parvovirus B19, EBV),
Ionising radiation.

3. Congenital AA (<5%)

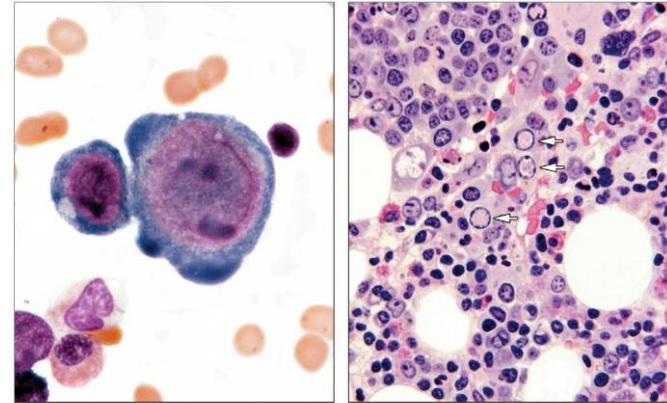
Fanconi anemia, Blackfan Diamond syndrome, Dyskeratosis congenita

Aplastic Anemia

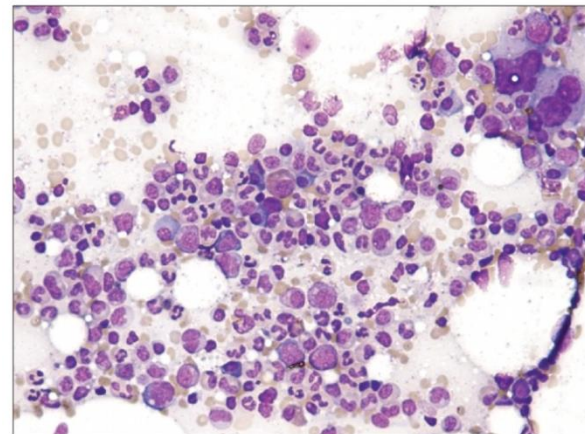
Aplastic Anemia



**Pure Red Cell Aplasia
caused by Parvovirus B19**



**Pure Red Cell Aplasia
(idiopathic)**



Therapy der Aplastic Anemia

1. Supportive Therapy:

- Transfusions
- Antibiotics

2. Causal Treatment:

- Allogeneic Stem Cell Transplantation
- Immunosuppressive Therapy (CsA, ALG, ATG)
- With doc. viral genesis: Therapy with 7S-Immunglobulins

Iron Metabolism

Loss of iron per day:	Male	1 mg
	Menstruating Female	2 mg
	Pregnant Female	3 mg

Body iron:

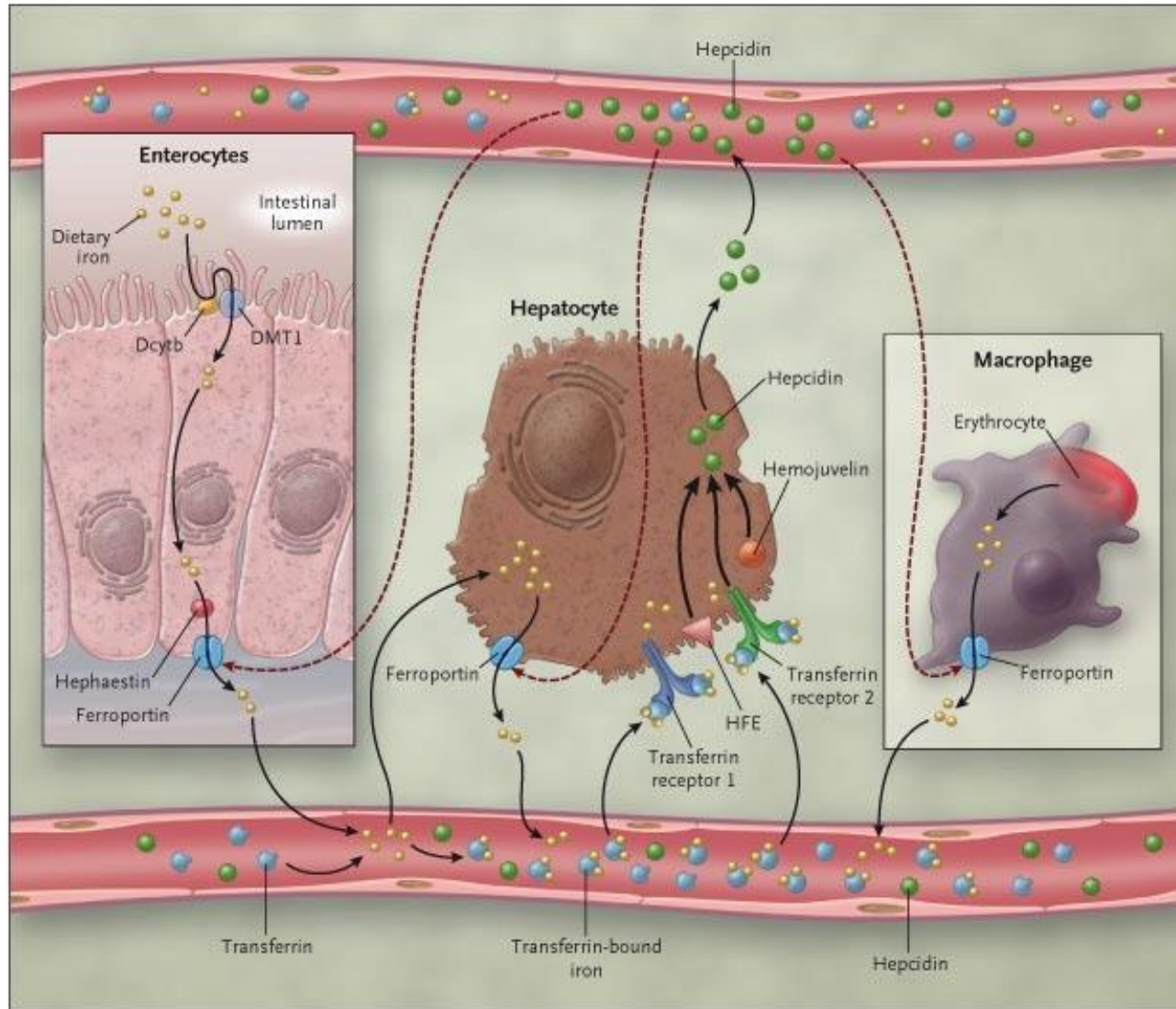
70 kg Male: 3.5 g Iron (50 mg/kg body weight)

60 kg Female: 2.1 g Iron (35 mg/kg body weight)

Distribution of iron in the human organism:

1. Heme Iron (70%) [Iron in the heme of hemeoglobin](#)
2. Storage Iron (18%) [Intracellular storage as ferritin and hemosiderin](#)
3. Functional Iron (12%) [Myoglobin und other iron-containing enzymes](#)
4. Transport Iron (0.1%) [Iron bound to transferrin](#)

Mechanism of Iron Homeostasis



Causes of Iron Insufficiency

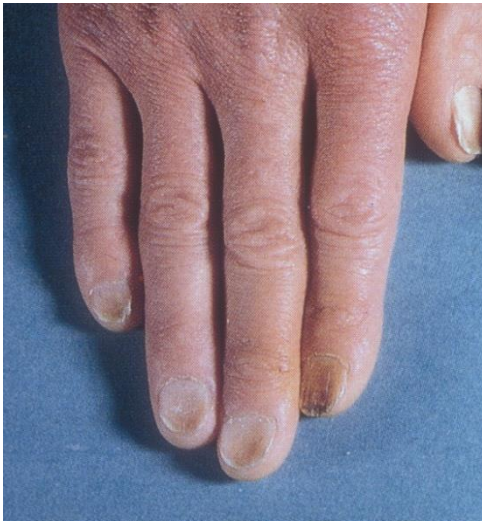
1. **Lack of intake** (babies, children, vegetarians)
2. **Issues with iron resorption** (gastrectomy, celiac disease, CIBD)
3. **Increased demand** (growth, pregnancy, breast feeding period)
4. **Iron loss (> 80 % of cases!!!)**
 - Genitourinary bleedings in females (menorrhagia)
 - Gastrointestinal bleedings
 - Other types of blood loss (e.g. ENT)
 - HP-positive gastritis, gastric lymphoma
 - Münchhausen-Syndrome, borderline personality disorder

Clinical Symptoms of Iron Insufficiency Anemia

General anemia symptoms: Pallor, fatigue etc.

Unspecific psychiatric/neurologic Symptoms: Cephalgia, issues with concentrating, irritability, pikacism.

Koilonychia



Oral rhagades



Plummer-Vinson-Syndrome



Diagnostic work-up and therapy

Diagnostic work-up: PMH, clinical symptoms, labs

Hb, Fe, ferritin, MCV, transferrin-saturation, retis ↓
transferrin, sTfR, platelets, RDW ↑

Therapy:

1. Treatment of bleeding cause (hemorrhage)
2. Iron substitution (oral Fe^{2+} , parenteral Fe^{3+})

Important differential diagnoses

Cave: Hypochromic, microcytic anemia mit normal or elevated ferritin is not an iron insufficiency anemia!!!!

Important differential diagnoses:

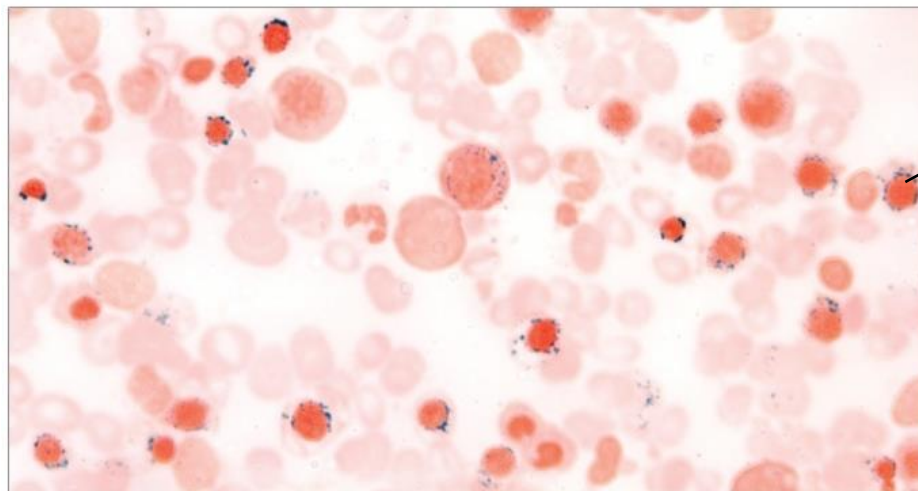
- Anemia of chronic disease (Tumor, chron. Infekt etc.)
- Sideroachrestic anemia
- Thalassemia

Sideroachrestic Anemia

Sideroachrestic Anemia/Sideroblastischic Anemia

- Acquired, inherited or idiopathic disorder of iron usage.
- Deposition of iron in erythroblasts (sideroblasts), monocytes, macrophages parenchymatous organs (e.g. liver)

**BM smear with
numerous sideroblasts**



Sideroblast

Thalassemia

- Quantitative disorder of Hb synthesis
- Common in the mediterranean area (Thalassa Θάλασσα „Meer“)
- Genetically fixed dysregulation of the synthesis of the globin chains (α -thalassemia, β -thalassemia)
- Diagnosis by Hb-electrophoresis, HPLC

β -Thalassämie

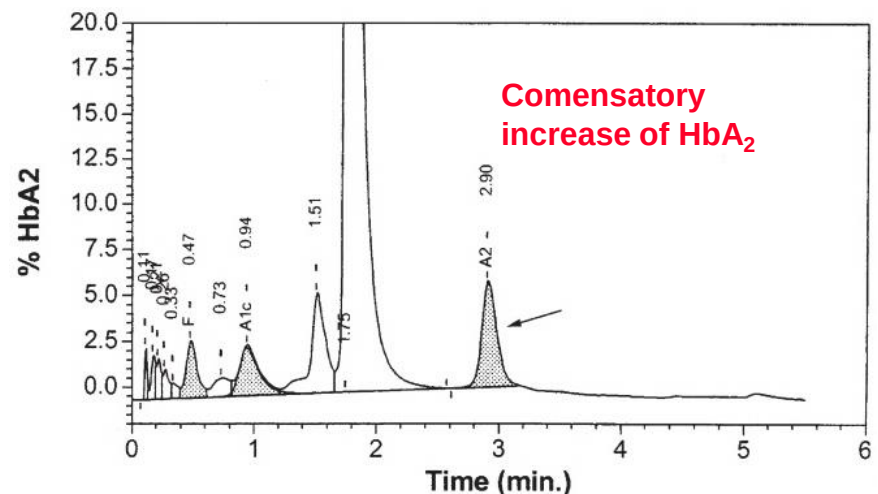


HbA ($\alpha\alpha/\beta\beta$)

HbA₂ ($\alpha\alpha/\delta\delta$)

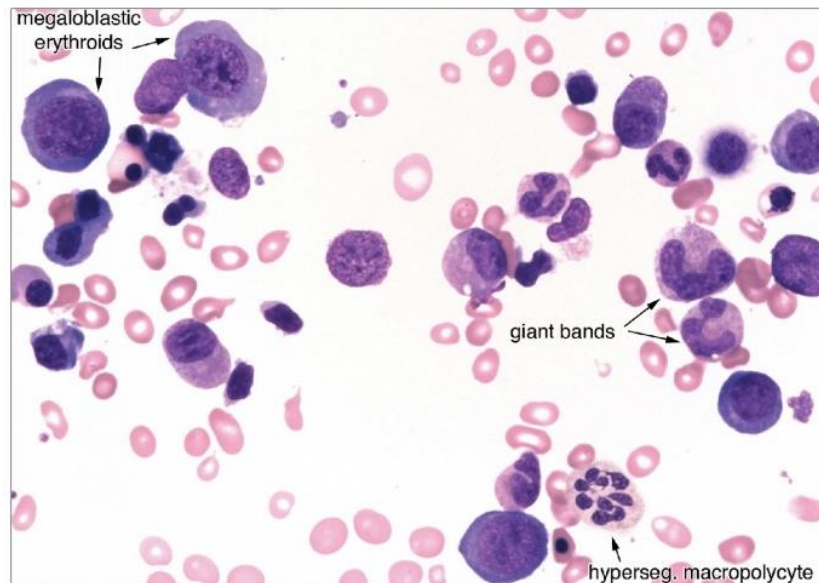
HbF ($\alpha\alpha/\gamma\gamma$)

HPLC



Megaloblastic Anemia

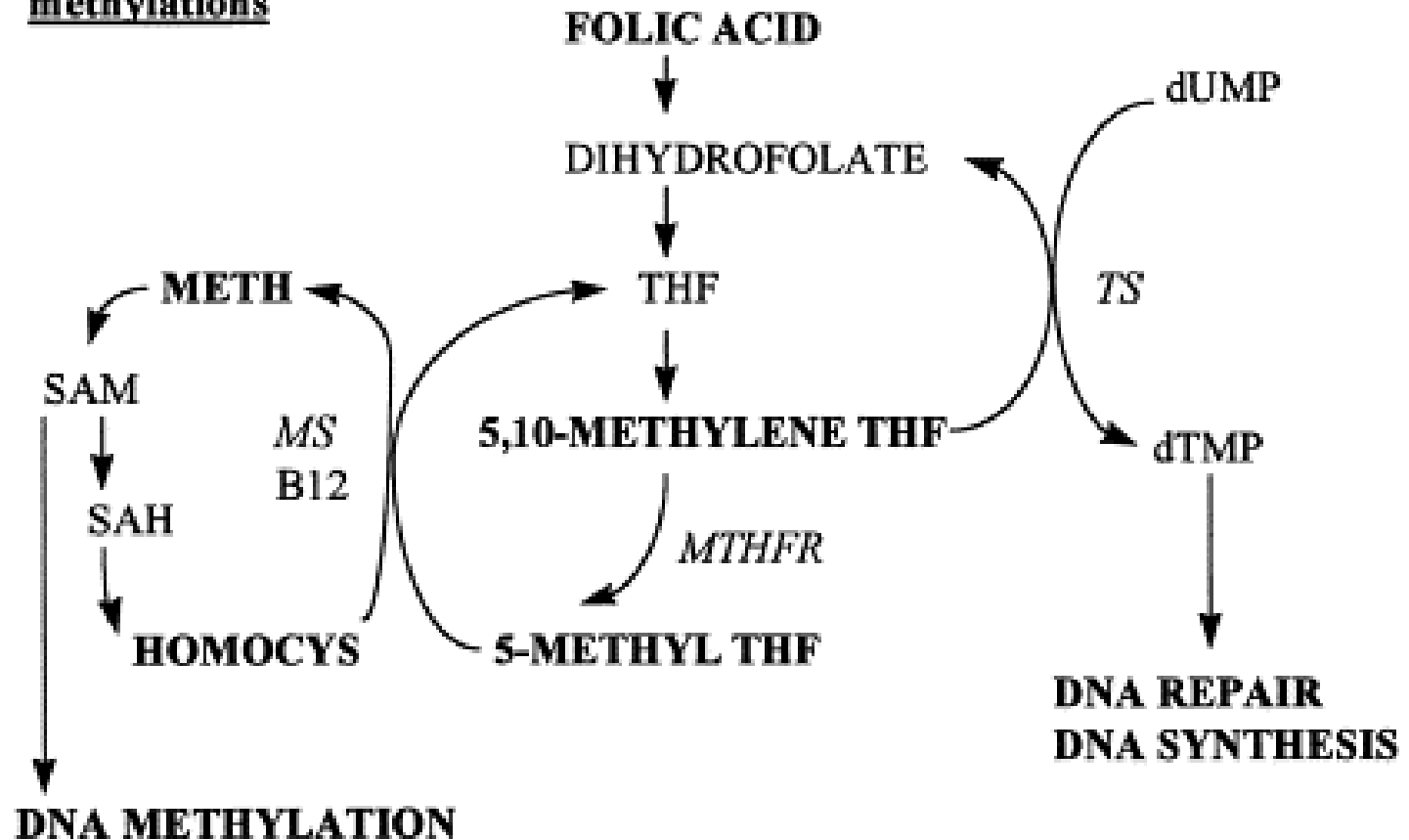
- Lack of Vitamin B₁₂ (Cobalamin) a/o Folic Acid with consecutive dysregulation of DNA synthesis and nuclear maturation.
- Appearance of megaloblasts
- **Leading symptoms:** Makrocytic anemia, in the case of Vit. B₁₂-insufficiency additional neurological und gastrointestinal symptoms.



Role of Folic Acid and Vitamin B₁₂

Methionine synthesis and SAM-mediated methylations

Thymidylate Synthesis



Dietary Vitamin B₁₂ und Folsäure

	Vitamin B ₁₂	Folic Acid
Main diets	Only in animal products (meat; milk)	In most foods including liver, vegetables and yeast.
Cooking	Little effect	Easy destruction
Minimal intake	1-2 µg	100-150 µg
Storage	Sufficient for 2-3 years	Sufficient for 4 months
Resorption	Ileum (Intrinsic Factor)	Duodenum and Jejunum

Causes for Vit. B₁₂ or Folic Acid Insufficiency

Vitamin B₁₂-Insufficiency

- Vegetarian/vegan diet
- Lack of intrinsic factor (gastrectomy, pernicious anemia)
- Gastrointestinal diseases with malabsorption syndrome
- Increased need (fish tapeworm)
- Intestinal bacterial overgrowth (e.g. „blind loop syndrome“)

Folic Acid-Insufficiency

- Insufficient diet, unbalanced diet (e.g. alcoholics)
- Increased need (pregnancy, hemolysis)
- Gastrointestinal diseases with malabsorption syndrome
- Impairment of dekonjugation by certain medications
- Treatment with folic acid antagonists (e.g. methotrexate, trimethoprim)

Symptoms of Megaloblastic Anemia

Trias in severe Vitamin B₁₂-Insufficiency:

- **Hematologic Syndrome:** anemia, jaundice
- **Gastrointestinale Syndrome:** Trophic mucosa alterations, atrophische Glossitis Hunter
- **Neurologic-psychiatric Syndrome:** Funicular myelosis with ataxia and loss of vibration sensitivity.

Symptoms in severe Folic Acid Insufficiency:

- Megaloblastic anemia w/o funicular myelosis
- Folic Acid Insufficiency in pregnant women increases the risk for neural tube defects (e.g. spina bifida)

Symptoms of Megaloblastic Anemias

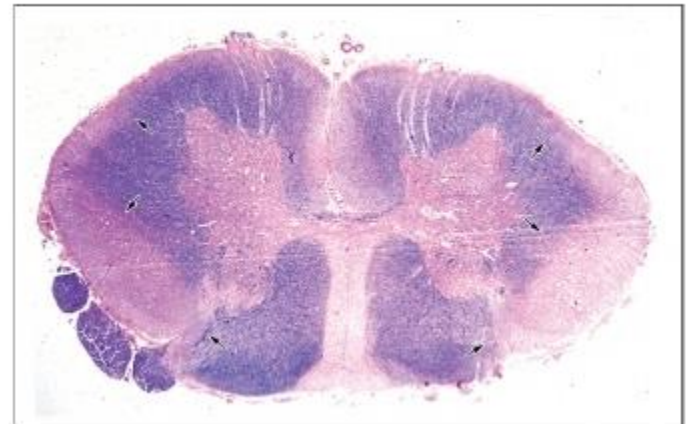
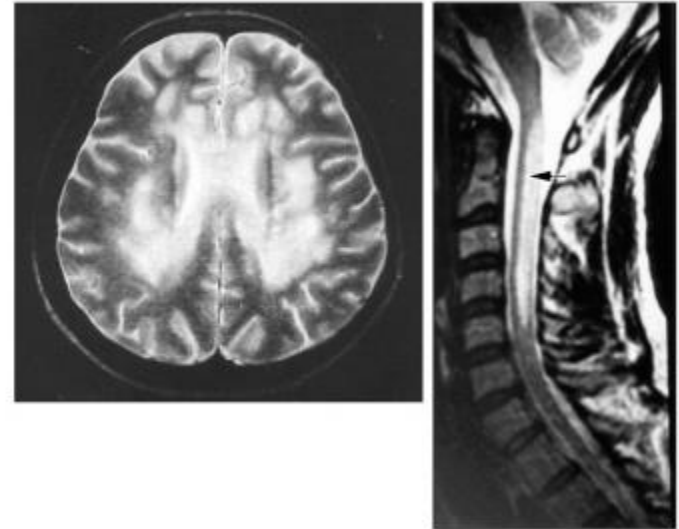
Atrophic Glossitis Hunter



Autoimmungastritis with achlorhydrie



Funikular Myelosis



Therapy of Megaloblastic Anemia

Therapy of Vitamin B₁₂-Insufficiency:

- Treatment of the underlying disease (e.g. treatment of fish tapeworm infestation).
- Parenteral substitution of hydroxycobalamin.
- In chronic-atrophic Type A-Gastritis: regular follow-up gastroscopies (increased risk for gastric cancer).

Therapy of Folic Acid Insufficiency:

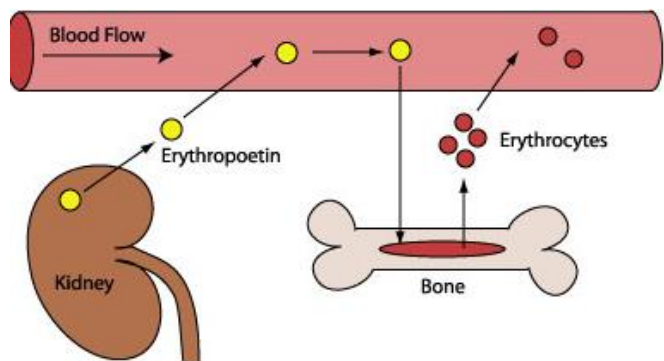
- Causal therapy (e.g. alcohol abstinence, compensation of unbalanced diet).
- Substitution of folic acid (5 mg/d oral).
- Special scenario: Prophylactic application of folic acid during pregnancy for the prevention of neural tube defects.

Renal Anemia

Normochromic, normocytic hyporegenerative anemia, which develops during the course of a renal insufficiency (creatinine > 3.5 mg/dL, creatinine clearance < 30 mL/min).

Main Cause: Lack of Erythropoietin

Other factors: Decreased erythrocyte survival time, iron deficiency, inappropriate dialysis



Therapeutic options:

1. Substitution of erythropoietin
2. Kidney transplantation

Hemolytic Anemias

I. Corpuscular hemolytic anemias

1. Inherited membrane defects(spherocytosis, elliptocytosis)
2. Enzymopathies (G6P-DH-Insufficiency, PK-Insufficiency)
3. Hämoglobinopathies (Sickle Cell Anemia, Thalassemia)
4. Acquired membrane defects (PNH)

II. Extracorpuscular hemolytic anemia

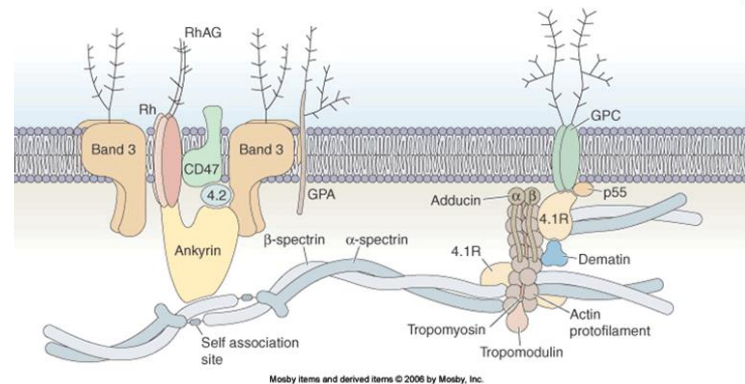
1. Hemolysis by allo-antibodies (Transfusion, Rh-Inkompatibilität)
2. Autoimmune hemolytic anemia
3. Hemolysis during infectious diseases (Malaria, Babesia, Bartonella)
4. Hemolysis durch physical and chemical effects (Runner's anemia, snake venom etc.)
5. Hemolysis in metabolic disorders (e.g. Zieve Syndrome)
6. Mikroangiopathic hemolytic anemia (HUS, TTP)

Spherocytosis/Elliptocytosis

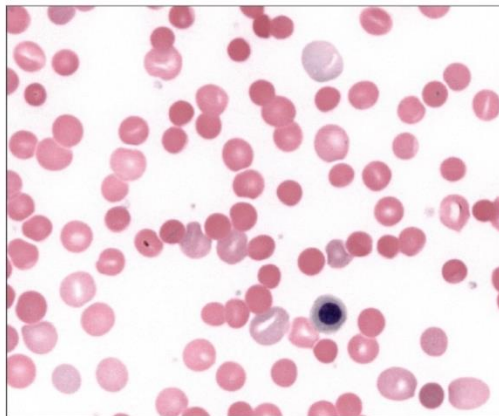
Most common inherited hemolytic anemia in Northern Europe (Prävalenz 1 :5000)

Cause:

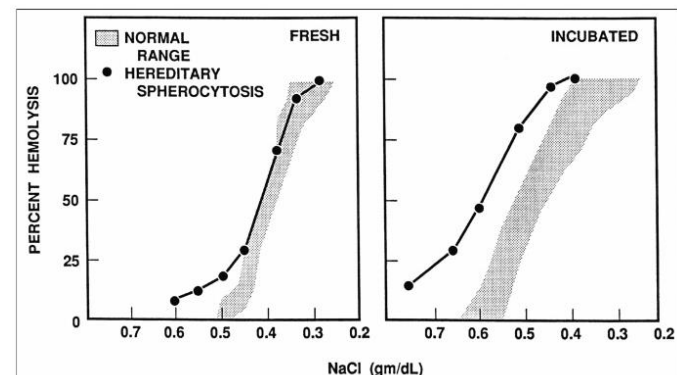
- Defect of Ankyrin
- Defect of α/β -Spectrin
- Defect of Band3
- Defect of Protein4.2



Blood Smear



Decreased osmotic resistance



Inherited Enzymopathies

Glucose-6-Phosphat-Dehydrogenase-Insufficiency

- Except for Diabetes most common genetic disorder
- Most common in African and Asian populations
- X-chromosomal-recessive inheritance
- Decreased synthesis of reduced Glutathion (Protection from Oxidation)
- Triggering of hemolytic crises by infections, fava beans and ceratin parmacologic agents (Chinin, Primaquin, Sulfonamide).

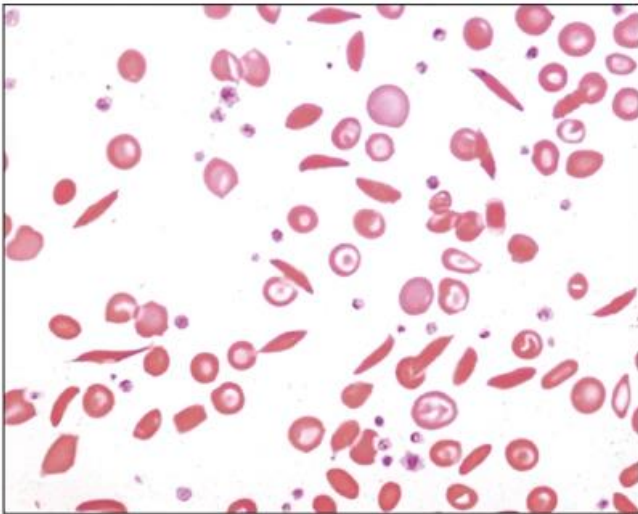
Pyruvatkinase-Insufficiency

- Most common inherited defect of glycolysis
- Autosomal-recessive inheritance
- Mature erythrocytes do not have mitochondria and are therefore dependent on glycolysis as their sole source of energy.

Sickle Cell Anemia

- Most common hemoglobinopathy: 20-40% of the population in subsaharan Africa as well 5-10% of the African-American population in North America are heterocygous conductors.
- A point mutation in the β gene locus on chromosome 11 leads to the production of an abnormal hemoglobin termed HbS.
- In HbS glutamate in position 6 of the β chain is replaced by valine.
- Symptoms: Hemolytic anemia, painful vaso-occlusive crises.

Peripheral Blood Smear:

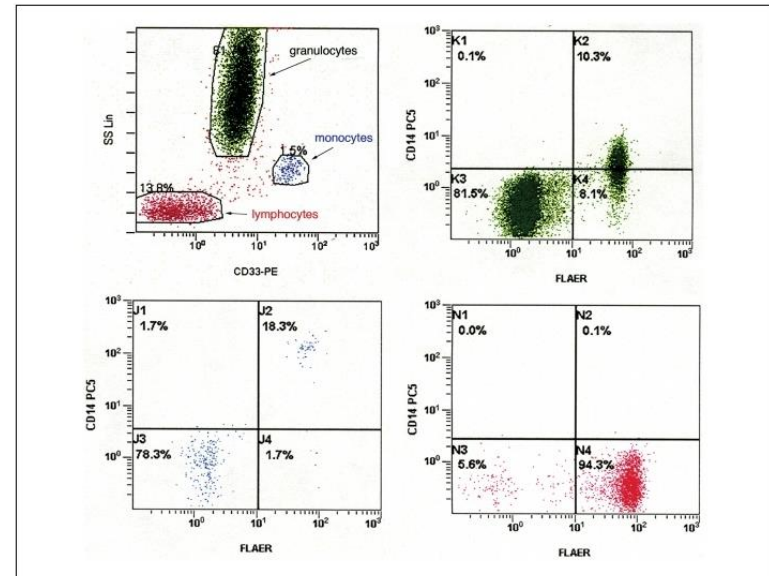
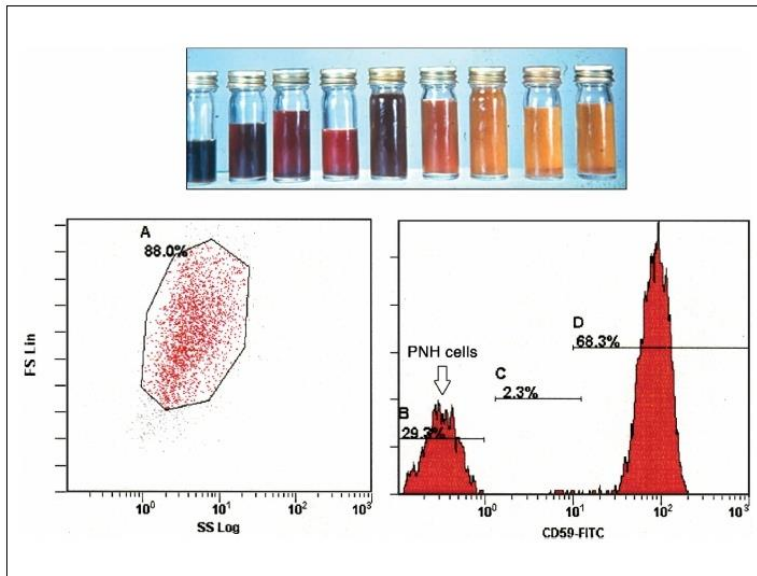


Typical skin ulcerations:



Paroxysmal nocturnal hemoglobinuria (PNH)

- Acquired clonal disorder of the pluripotent HSC with GPI-anchor deficiency.
- Only acquired corpuscular hemolytic anemia.
- Deficiency of GPI.anchored proteins on the cells of all three lineages (complement regulating proteins CD55 and CD59)



Thank you very much for your attention!!!!

