ANEMIA AND THROMBOCYTOPENIA

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INTRODUCTION

- **Anemia is defined as a decrease in hemoglobin under lower level (for men 130 g/l, for women 120 g/l)**

  (Nutritional anaemias. Report of a WHO scientific group. World Health Organ Tech Rep Ser 1968)

- **Classification of anemia is based on:**

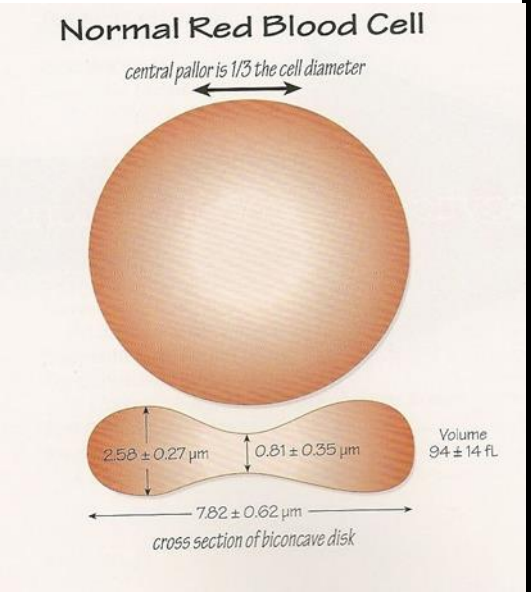
  - *red cell parameters*

  MCV (mean cell volume)

  MCH (mean cell hemoglobin)

  RDW (red cell distribution width)

  - *reticulocyte count (hyperproliferative and hypoproliferative anemia)*



LABORATORY PARAMETERS ARE THE MOST USEFUL IN FORMULATING A PRACTICAL DIAGNOSTIC APPROACH

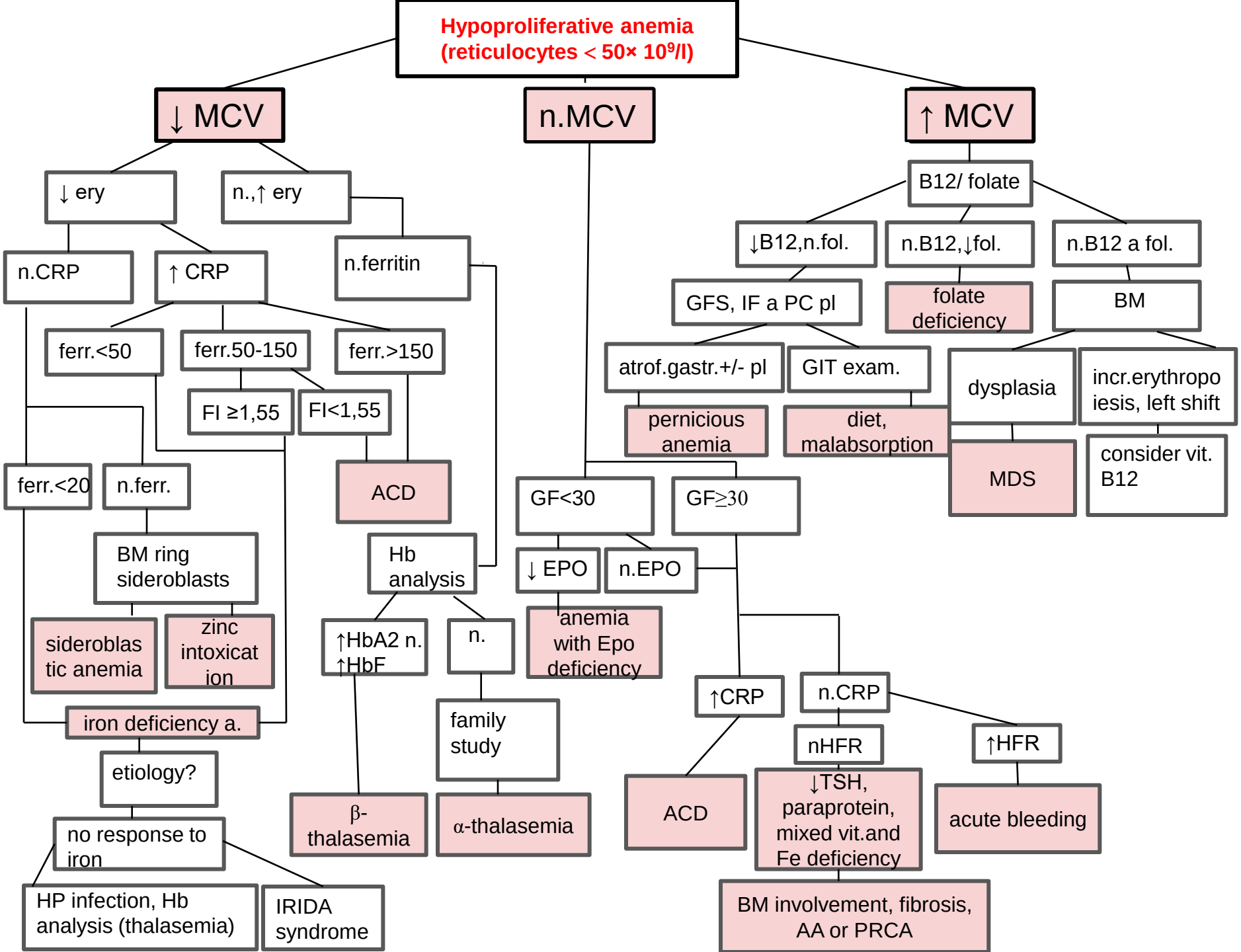
hemoglobin men 135-176g/l **erytrocytes (RBC)** men 4,0-5,9×10^{12}/l **hematocrit (HCT)** men 0,39-0,51	women 120-160g/l women 3,8-5,4 ×10^{12}/l women 0,35-0,46
MCV (mean cell volume) **MCH** (mean cell hemoglobin) **MCHC** (mean cell hemoglobin concentration) **RDW** (red cell distribution width)	84-96 fl 28-34 pg 320-370 g/l 10,0-15,2%
platelets (PLT) **MPV** (mean platelet volume) **leukocytes (WBC)**	150-400×10^{9}/l 7,8-11,0 fl 4,0-10,0 ×10^{9}/l

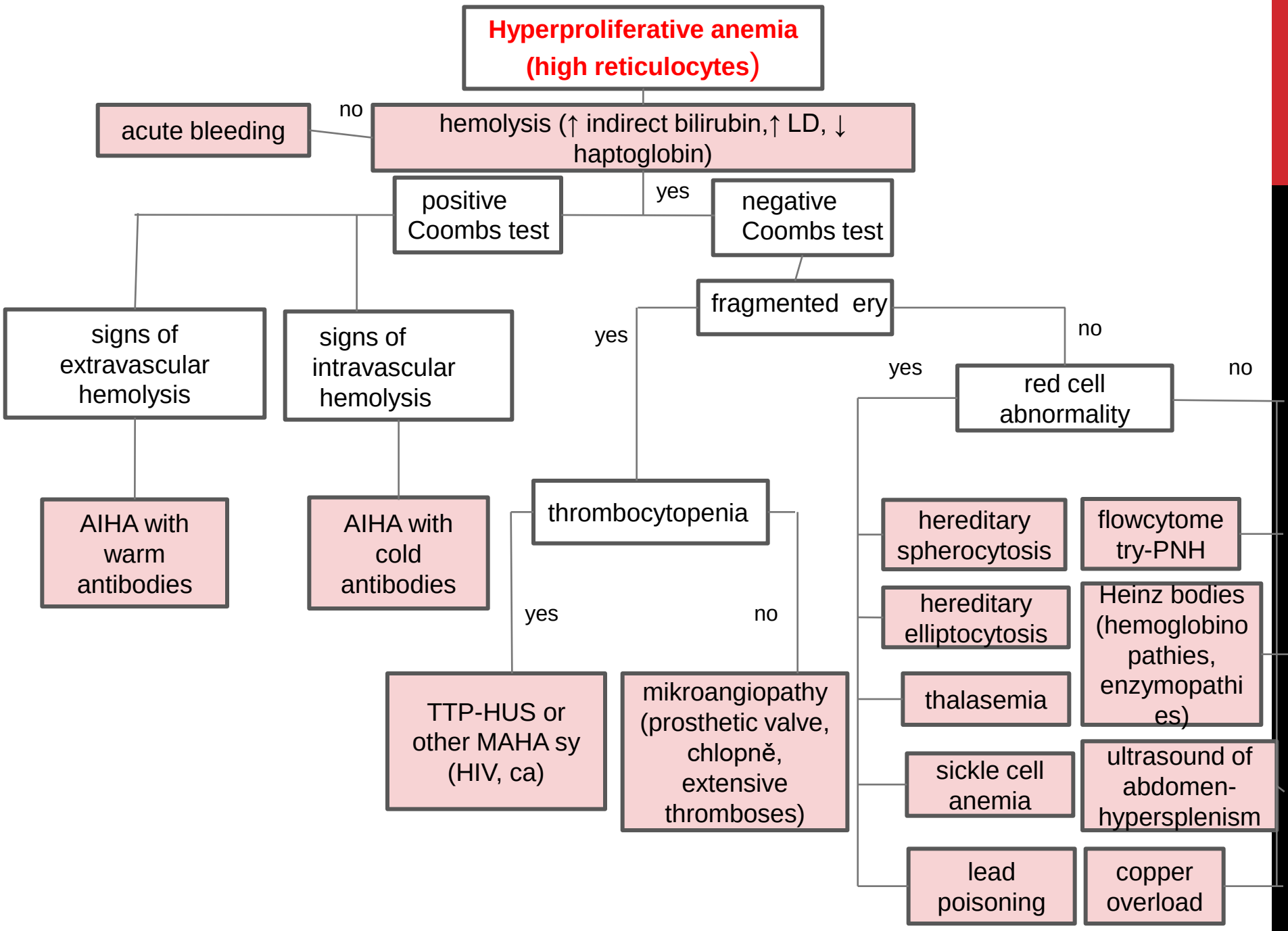
CASE HISTORY

Positive anamnestic data	Possible intepretation
dyspnoe, weakness, palpitation, dizziness	nonspecific symptoms without clear significance
bleeding symptoms	sideropenia, bleeding tendency, locus minoris resistentiae (tumor, inflammation)
infection, tumors, chronic inflammation, fever, renal disease, thyropathy	suspicion on anemia of chronic disease, eventually bone marrow involvement of tumor
toxic effects	alcohol, drugs, lead…
jaundice, dark urine	suspicion on hemolytic anemia
nail fragility, hair breaking, swallowing disorder	sideropenia
nutrition, anorexia, weight loss	sideropenia, vitamine B12 deficiency in vegetarians/vegans, suspicion on tumor, anemia of chronic disease
crawling, neurologic symptoms	vitamin B12 or folate deficiency
drugs (antitrombotic, antireumatic therapy, therapy and other)	sideropenia, myelosuppression
family history of anemia	hereditary hemolytic anemia, congenital hematopoietic disorders

PHYSICAL EXAMINATION

positive clinical examination	possible interpretation
skin/mucosal symptoms: • jaundice • hemorrghage (haematoma, suffusion, petechia) • pallor/“straw skin coloring“ • mucosal changes in the tongue	• hemolytic anemia • I • ron deficiency anemia • without siginificancy/pernicious anemia • vitamin B 12/iron deficiency
abnormality of secondary hemopoetic organs (lymph nodes, liver, spleen)	suspicion on malignancy, in case of isolated splenomegaly on hemolysis
melaena, enterrorhagia, haemorhoids	iron deficiency, anemia of chronic disease
neurological symptomatology	vitamin B 12 or folate deficiency, rarely iron deficiency
cor/vascular circulation	murmurs, tachycardia, heart failure; usually without significancy for diferential diagnosis of anemia
palpable tumor	anemia of chronic disease, iron deficiency, bone marrow involvement

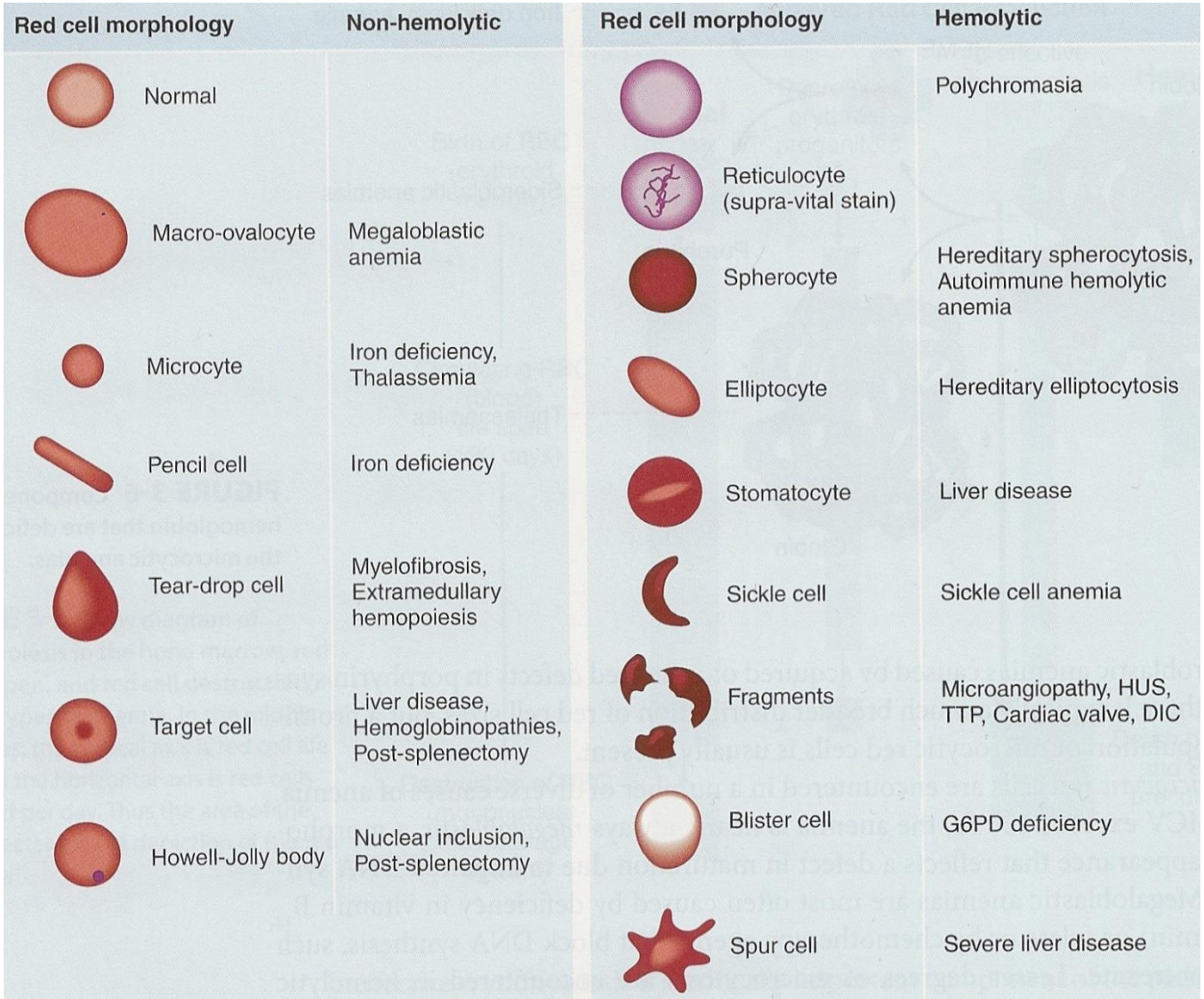

Hypoproliferative anemia (reticulocytes < 50× 10⁹/l)
↓ MCV
n.MCV
↑ MCV
↓ ery
n.,↑ ery
n.CRP
↑ CRP
n.ferritin
ferr.<50
ferr.50-150
ferr.>150
FI ≥1,55
FI<1,55
ferr.<20
n.ferr.
ACD
BM ring sideroblasts
Hb analysis
sideroblastic anemia
zinc intoxication
↑HbA2 n. ↑HbF
n.
iron deficiency a.
family study
etiology?
β-thalasemia
α-thalasemia
no response to iron
HP infection, Hb analysis (thalasemia)
IRIDA syndrome
GF<30
GF≥30
↓ EPO
n.EPO
anemia with Epo deficiency
B12/ folate
↓B12,n.fol.
n.B12,↓fol.
n.B12 a fol.
GFS, IF a PC pl
folate deficiency
BM
atrof.gastr.+/- pl
GIT exam.
dysplasia
incr.erythropoiesis, left shift
pernicious anemia
diet, malabsorption
MDS
consider vit. B12
↑CRP
n.CRP
nHFR
↑HFR
ACD
↓TSH, paraprotein, mixed vit.and Fe deficiency
acute bleeding
BM involvement, fibrosis, AA or PRCA


Hyperproliferative anemia (high reticulocytes)
acute bleeding
no
hemolysis (↑ indirect bilirubin,↑ LD, ↓ haptoglobin)
positive Coombs test
yes
negative Coombs test
signs of extravascular hemolysis
signs of intravascular hemolysis
fragmented ery
yes
no
AIHA with warm antibodies
AIHA with cold antibodies
thrombocytopenia
yes
no
TTP-HUS or other MAHA sy (HIV, ca)
mikroangiopathy (prosthetic valve, chlopně, extensive thromboses)
yes
red cell abnormality
no
hereditary spherocytosis
flowcytometry-PNH
hereditary elliptocytosis
Heinz bodies (hemoglobinopathies, enzymopathies)
thalasemia
sickle cell anemia
ultrasound of abdomen-hypersplenism
lead poisoning
copper overload

LABORATORY EVALUATION IN THE DIAGNOSIS OF ANEMIA

- **blood count, cell differential of white blood cell count, reticulocyte count**
- **red cell morphology**
- **serum iron, transferrin saturation, serum ferritin, transferrin (or total iron binding capacity)**
- **folate, cobalamin, serum bilirubin, lactate dehydrogenase, erythropoietin level, iron resorption testing, occult blood test …..**
- **bone marrow examination with iron staining**

Red cell morphology



Red cell morphology	Non-hemolytic
Normal	
Macro-ovalocyte	Megaloblastic anemia
Microcyte	Iron deficiency, Thalassemia
Pencil cell	Iron deficiency
Tear-drop cell	Myelofibrosis, Extramedullary hemopoiesis
Target cell	Liver disease, Hemoglobinopathies, Post-splenectomy
Howell-Jolly body	Nuclear inclusion, Post-splenectomy

Red cell morphology	Hemolytic
	Polychromasia
Reticulocyte (supra-vital stain)	
Spherocyte	Hereditary spherocytosis, Autoimmune hemolytic anemia
Elliptocyte	Hereditary elliptocytosis
Stomatocyte	Liver disease
Sickle cell	Sickle cell anemia
Fragments	Microangiopathy, HUS, TTP, Cardiac valve, DIC
Blister cell	G6PD deficiency
Spur cell	Severe liver disease

From Bunn HF, Aster JC: Pathophysiology of blood disorders, 2011

ANEMIA CLASSIFICATION ACCORDING TO RED CELL PARAMETERS

MCV (mean cell volume):

<84 fl - microcytic a.

84-95 fl - normocytic a.

>96 fl - macrocytic a.

RDW (red cell distribution width)

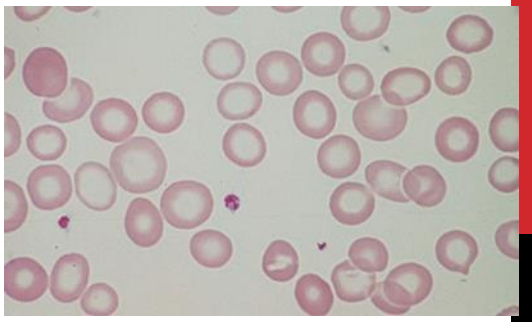
> 15,2 % – a. with anisocytosis

< 15,2 % – a. with homogennous red cell population

MCH (mean corpuscular hemoglobin):

28 - 34 pg	**normochromic a.**
< 28 pg	**hypochromic a.**

MICROCYTIC HYPOCHROMIC ANEMIA



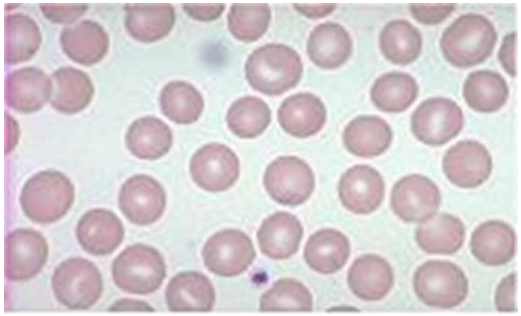
- iron deficiency anemia
- thalassemia

RDW>15,2

- anemia of chronic disease
- thalassemia
- sideroblastic anemia

RDW<15,2

NORMOCYTIC NORMOCHROMIC ANEMIA



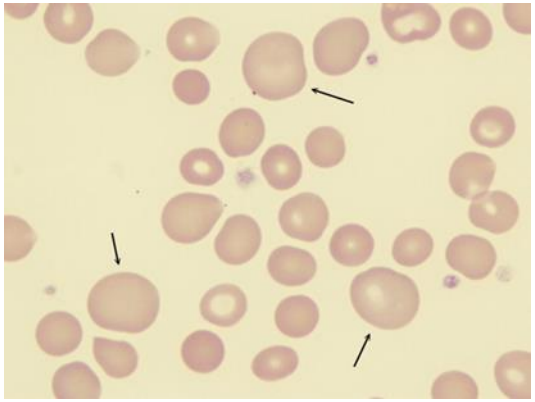
- iron deficiency anemia (initial stage)
- myelofibrosis

RDW>15,2

- primary bone marrow disorder (aplastic anemia, myelodysplastic syndrome…)
- anemia of chronic disease
- acute posthemoragic anemia
- sideroblastic anemia
- hemolytic anemias (hereditary spherocytosis, hemoglobinopathy)

RDW<15,2

MACROCYTIC ANEMIA



- pernicious anemia
- megaloblastic anemia in pregnancy
- sideroblastic a.
- autoimunne hemolytic anemia

RDW>15,2

- aplastic anemia
- myelodysplastic syndrome
- liver disease, hypothyroidism

RDW<15,2

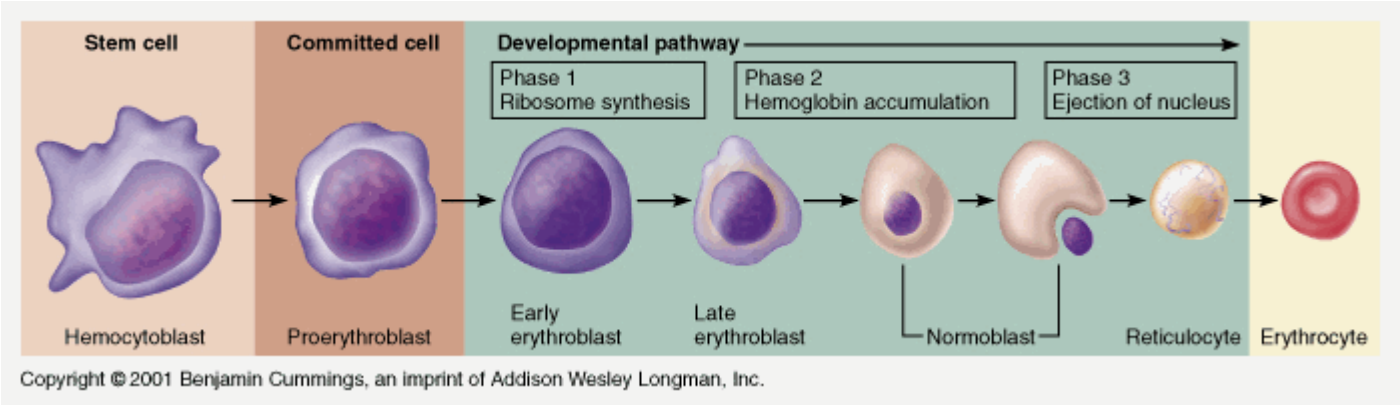
RETICULOCYTES

Low count

1. **Iron deficiency anemia**
2. **Megaloblastic a.**
3. **Sideroblastic a.**
4. **Congenital dyserytropoetic a.**
5. **MDS**

High count

1. **Hemolytic anemias**
2. **Chronic blood loss**



PATHOPHYSIOLOGICAL CLASSIFICATION OF ANEMIA

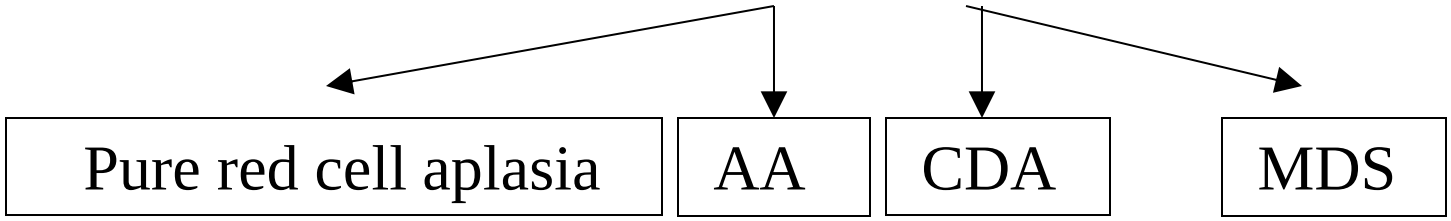
1. Anemia due to decreased red cell production

2. Anemia due to increased red cell destruction

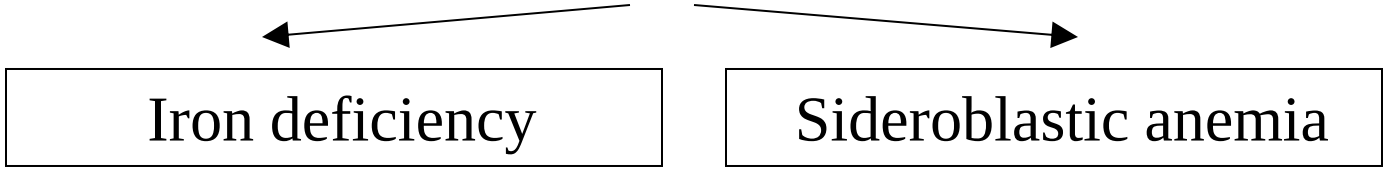
3. Acute posthemoragic anemia

ANEMIA DUE TO DECREASED RED CELL PRODUCTION

1. Decreased erythroid progenitors, ineffective erythropoiesis

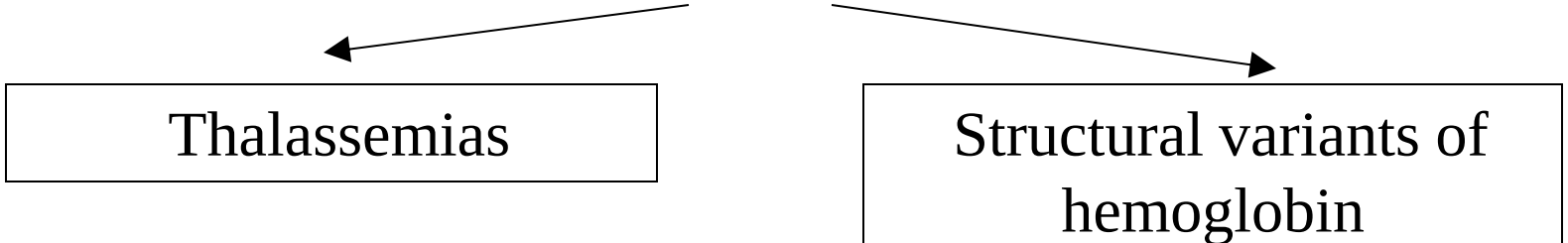


2. Disorders of heme synthesis

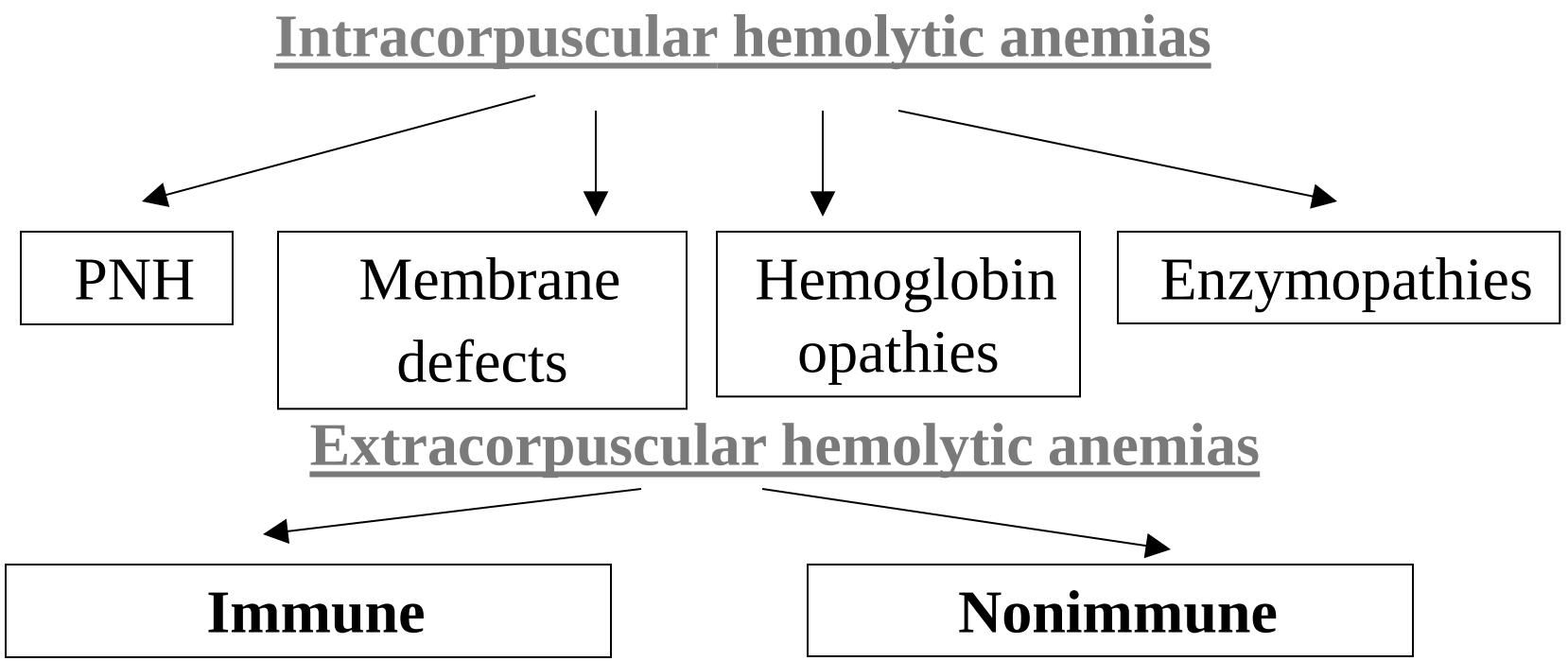


3. Disorders of DNA synthesis: megaloblastic

4. Disorders of globin synthesis



ANEMIA DUE TO INCREASED RED CELL DESTRUCTION

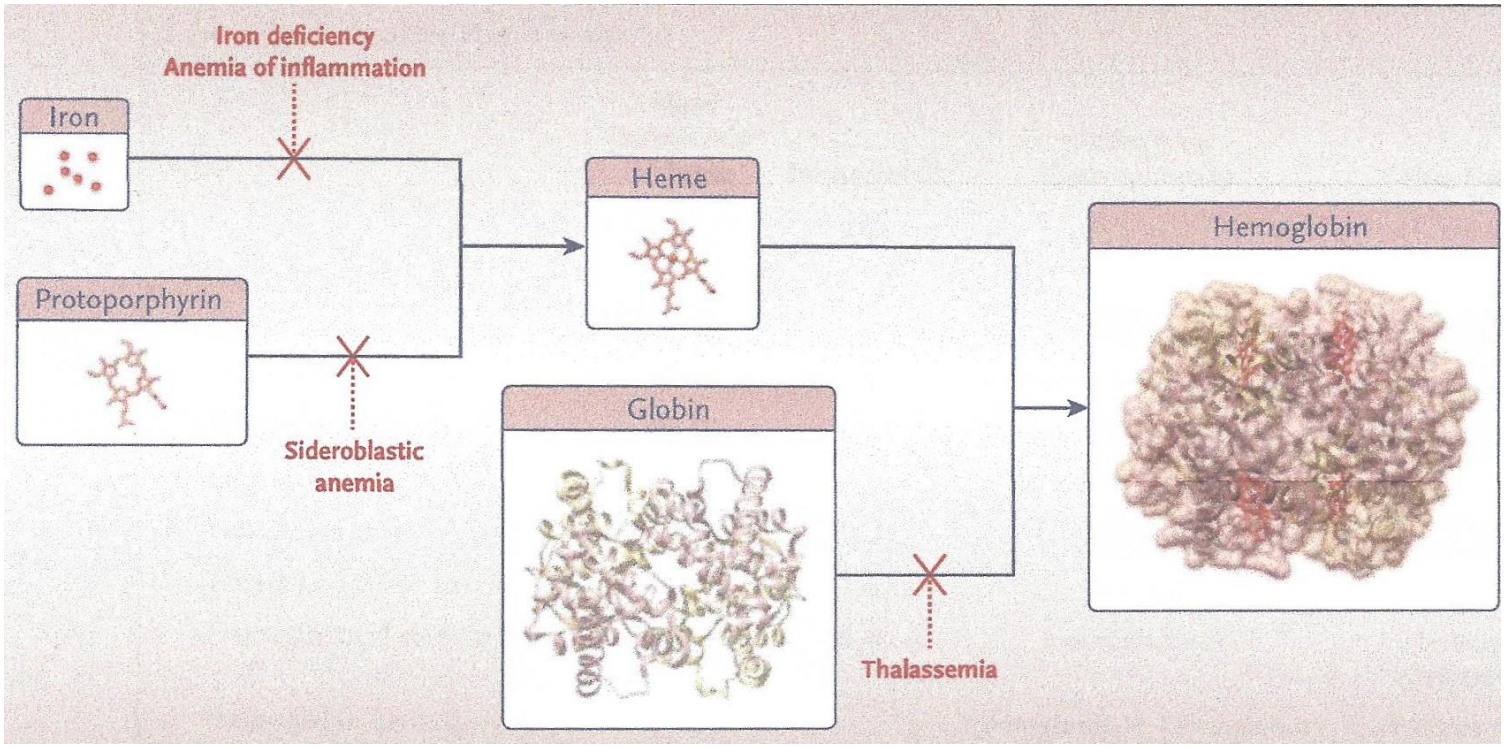


ANEMIA

THE PREVALENCE OF DIFFERENT TYPES

Type of anemia	Prevalence
Iron deficiency	25 %
Anemia of chronic disorders	25 %
Acute bleeding (posthemorrhagic)	25 %
Megaloblastic anemia	10 %
Hemolytic anemia	< 10 %
Bone marrow failure	< 10 %

MICROCYTIC ANEMIA

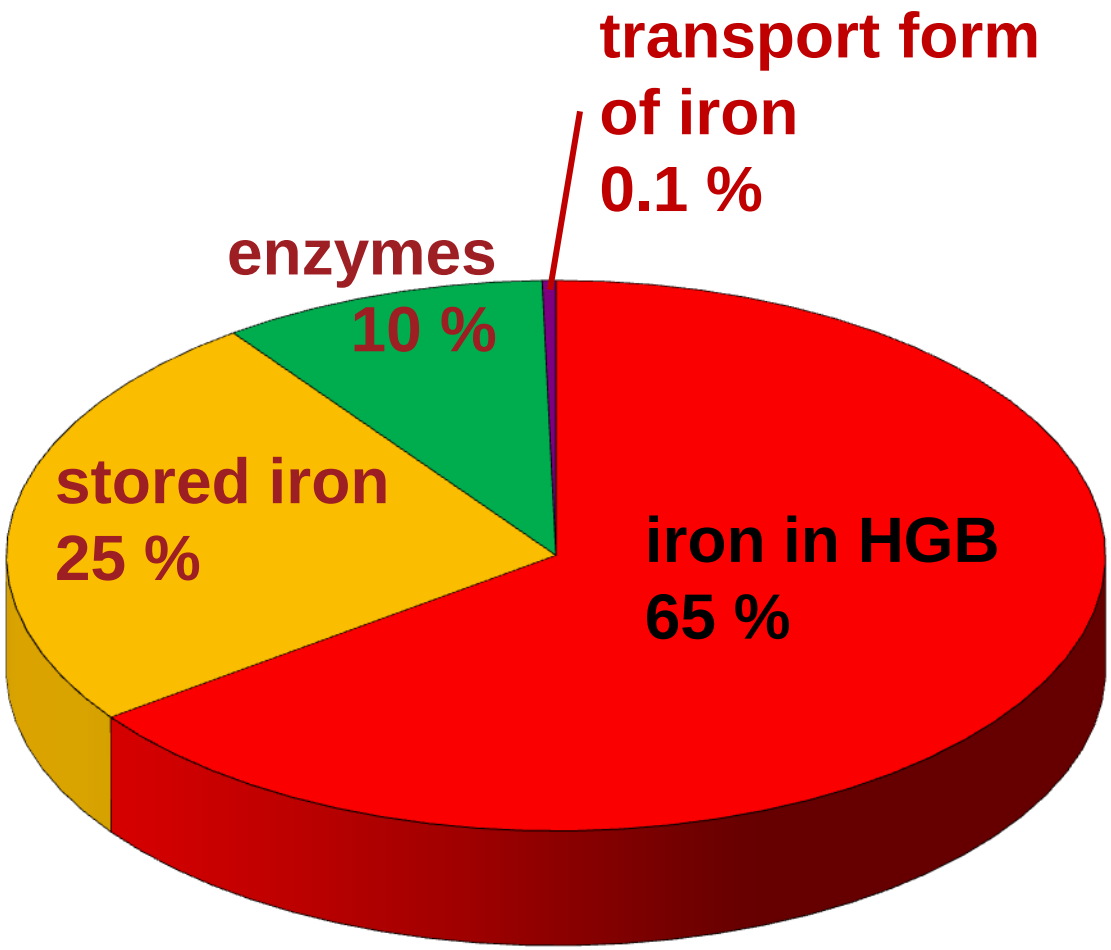


IRON DEFICIENCY ANEMIA

- **the most prevalent anemia in the world**
- **iron deficiency in one third of the world´s population (WHO)**

IRON DISTRIBUTION IN THE BODY

TOTAL AMOUNT 4 G



DISTRIBUTION OF IRON IN THE BODY (MAN, 70 KG)

protein	localization	iron content (mg)
hemoglobin	erythrocytes	3000
myoglobin	muscles	400
cytochroms	all tissues	50
transferin	plasma and a extravascular fluid	5
feritin and hemosiderin	liver, spleen and bone marrow	100-1000

IRON DEFICIENCY

3 levels of iron deficiency:

prelatent sideropenia- reduction of storage iron, but the supply of iron to erythroblasts is not affected

latent sideropenia- storage iron is exhausted, decreased iron supply for erythropoesis, but no anemia

manifest sideropenia= iron deficiency anemia

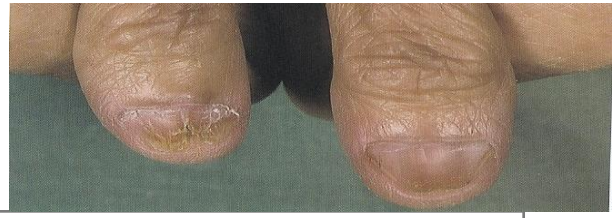
IRON DEFICIENCY STAGES

	prelatent	latent	manifest
serum iron *μmol / l*	normal	< 12	< 10
transferin	normal	> 70	> 74
transferin saturation %	normal	< 15	< 10
ferritin *normal 20-200 μg / l*	< 20	< 15	< 10
storage iron in BM	slightly ↓	moderately ↓	significantly ↓
MCV	normal	78-83	<78
MCH	normal	25-28	<25
MCHC	normal	normal	<320

CAUSES OF IRON DEFICIENCY

A. Chronic blood loss	
	Gastrointestinal- hemerhoids, diverticulosis, peptic ulcers, oesophageal varices, carcinomas, gastritis, colitis, drugs (aspirin, non-steroidal antiinflammatory drugs, anticoagulants), parasites, angiodysplasia Gynecologic- menorrhagia, metrorrhagia Urinary tract- hematuria, hemoglobinuria Hemodialysis Iatrogennic causes- blood donors, frequent blood sampling Self-inflicted blood loss
B. Inadequate iron intake	
	Poor diet Malabsorption- gluten-induced enteropathy, gastrectomy,…
C. Increased demands	
	Pregnancy, breast feeding Growth Erythropoetin therapy

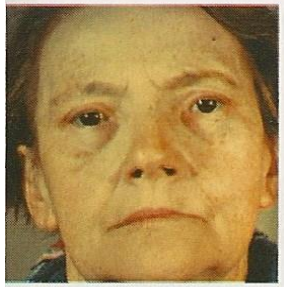
CLINICAL FEATURES OF IRON DEFICIENCY



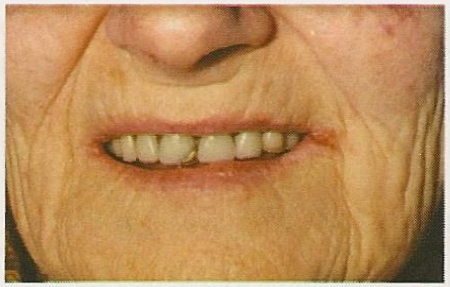
Nonspecific symptoms of anemia	Fatiquability, weakness, dyspnoe on exertion, palpitation, pallor, reduced load tolerance
Neuromusculatory system	Increased lactate production durin the exertion, muscle weakness, neurastenia, neuralgia,reduces sensitivity, parestesia, cognitive behavioral disorders
Epitel	Nail fragility, nail thinning…koilonychia (rare and limited to severe chronic deficiency), hair loss, atrophy of lingual papillae, glositis, angular cheilitis, dysphagia
Immune system	Defects of specific cellular immunity, defective phagocytic functions
Pica	Pagophagia (ice), geophagia (clay), amylophagia (starch)
Skeletal system	Growth disorders in children
Other	Reduced sensitivity to cold Mild splenomegaly

CLINICAL FEATURES OF IRON DEFICIENCY ANEMIA

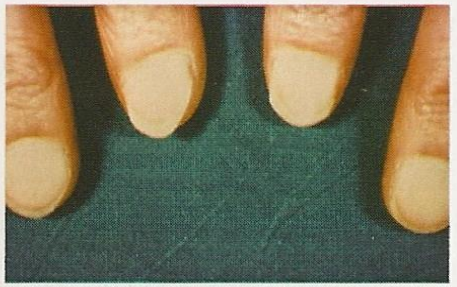
Clinical Signs of Iron Deficiency Anemia (source: Hoffbrand et al. 2003)



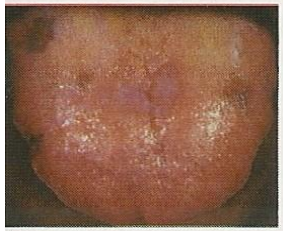
Dirty brown skin color



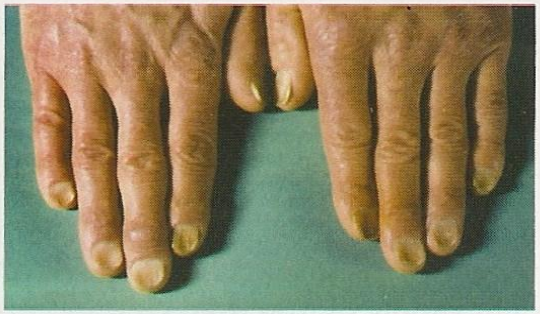
Angular stomatitis



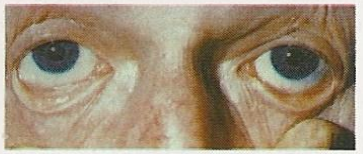
Longitudinal grooves in finger nails



Smooth tongue



Typical spoon nails

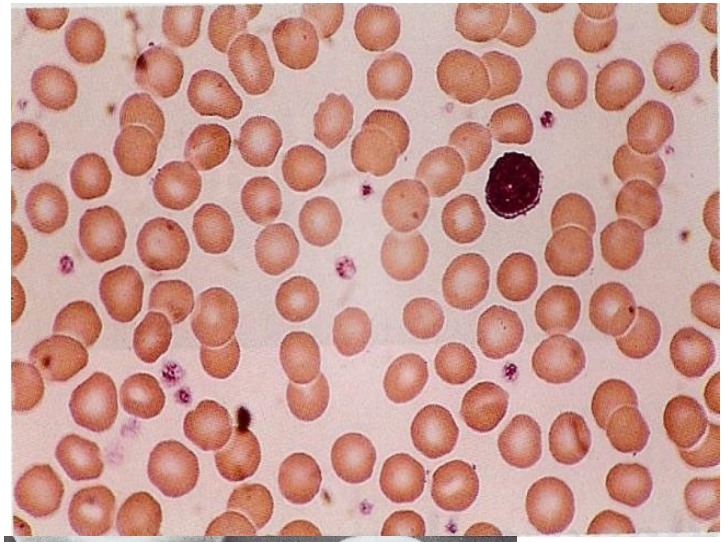


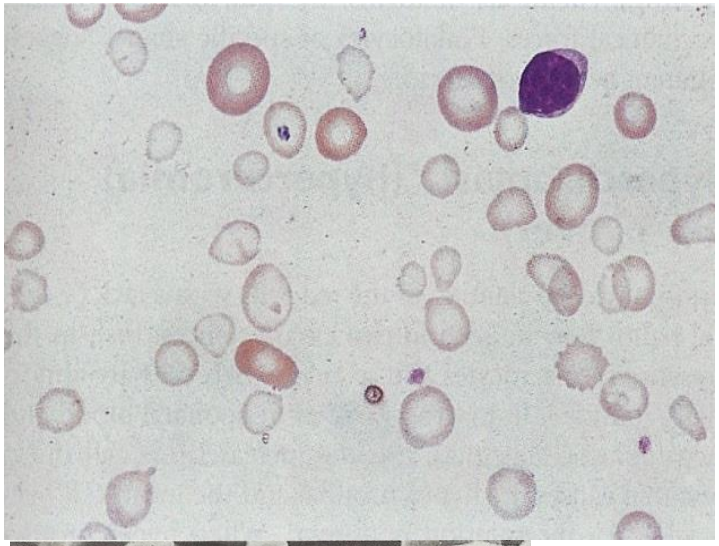
Pallor of conjunctiva

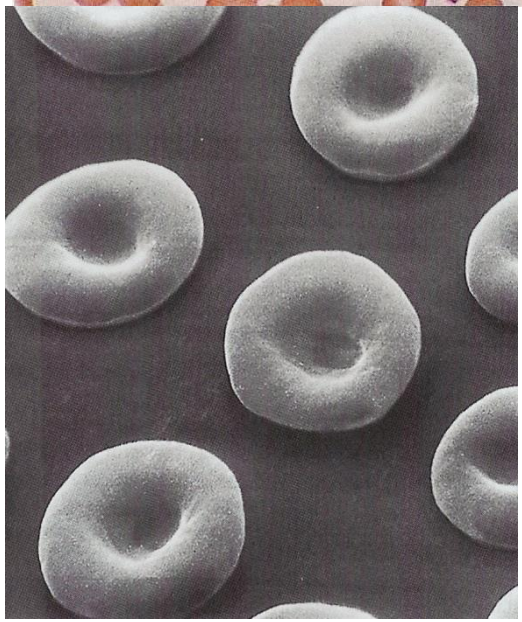
LABORATORY CHANGES OF IRON DEFICIENCY ANEMIA

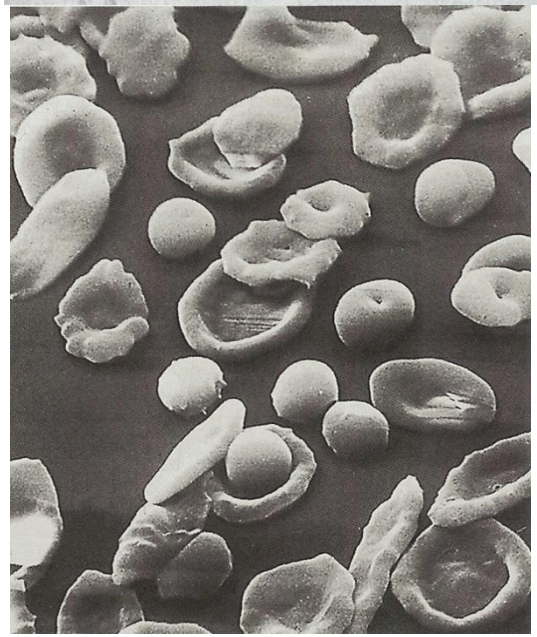
- microcytic hypochromic anemia
- anizocytosis, poikilocytosis, anulocytes
- normal or reduced reticulocyte count
- reduced number of siderophages and sideroblasts in bone marrow
- increased level of soluble transferin receptors (not affected by the acute phase reaction)
- mild thrombocytosis is found
- in small number of patients is leukopenia found

PERIPHERAL BLOOD – NORMAL FINDING X IRON DEFICIENCY ANEMIA









SERUM FERRITIN

- **Serum ferritin is the most important parameter for diagnosis of iron deficiency**
- **Ferritin is acute phase protein!**

  may be elevated in concomitant inflammatory diseases

  ferritin > 100 ug/l makes iron deficiency inlikely

- **Diagnosis of iron deficiency in inflammation or tumor**

  decreased transferin saturation

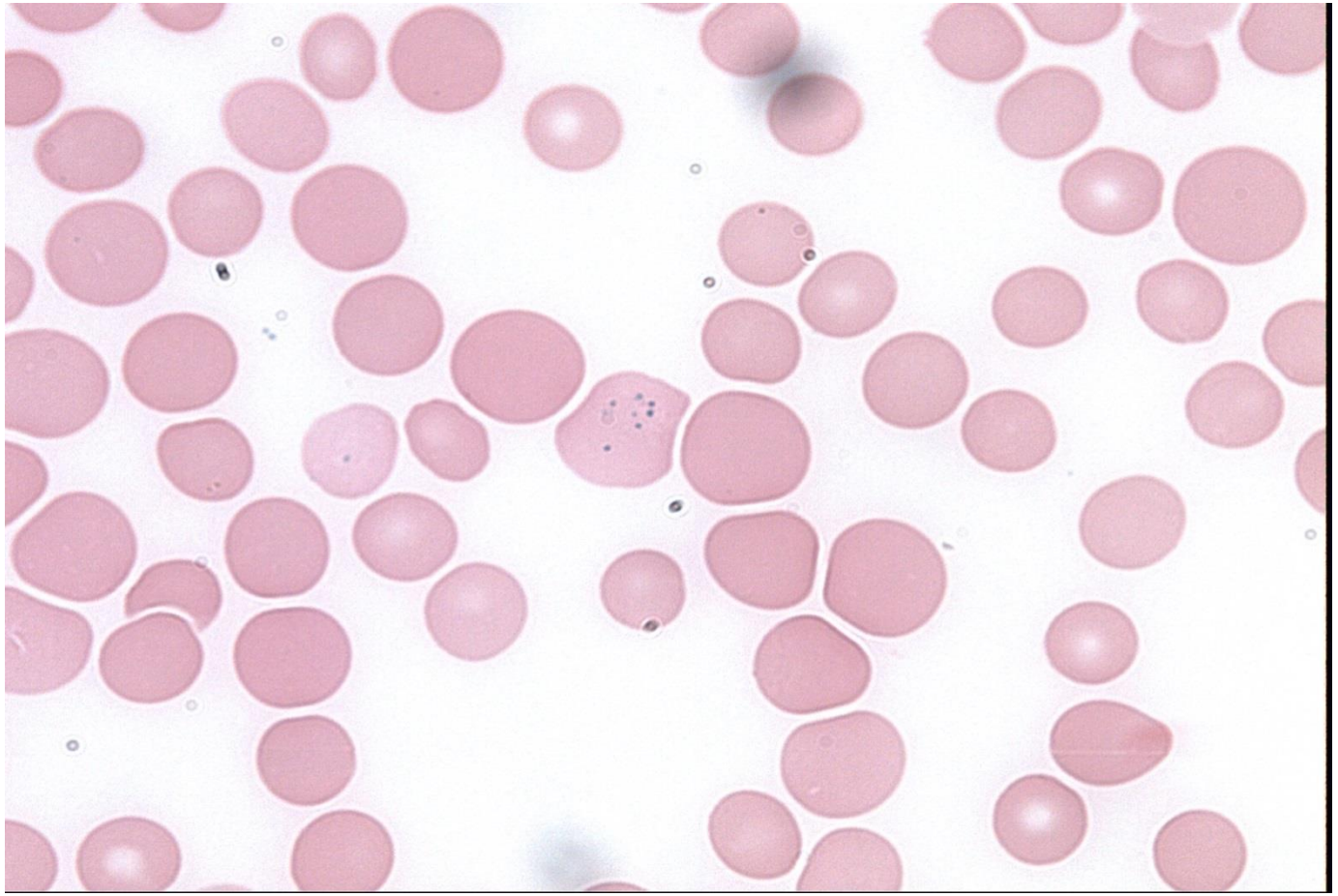
  bone marrow examination

  therapeutic test: iron therapy for 3 weeks

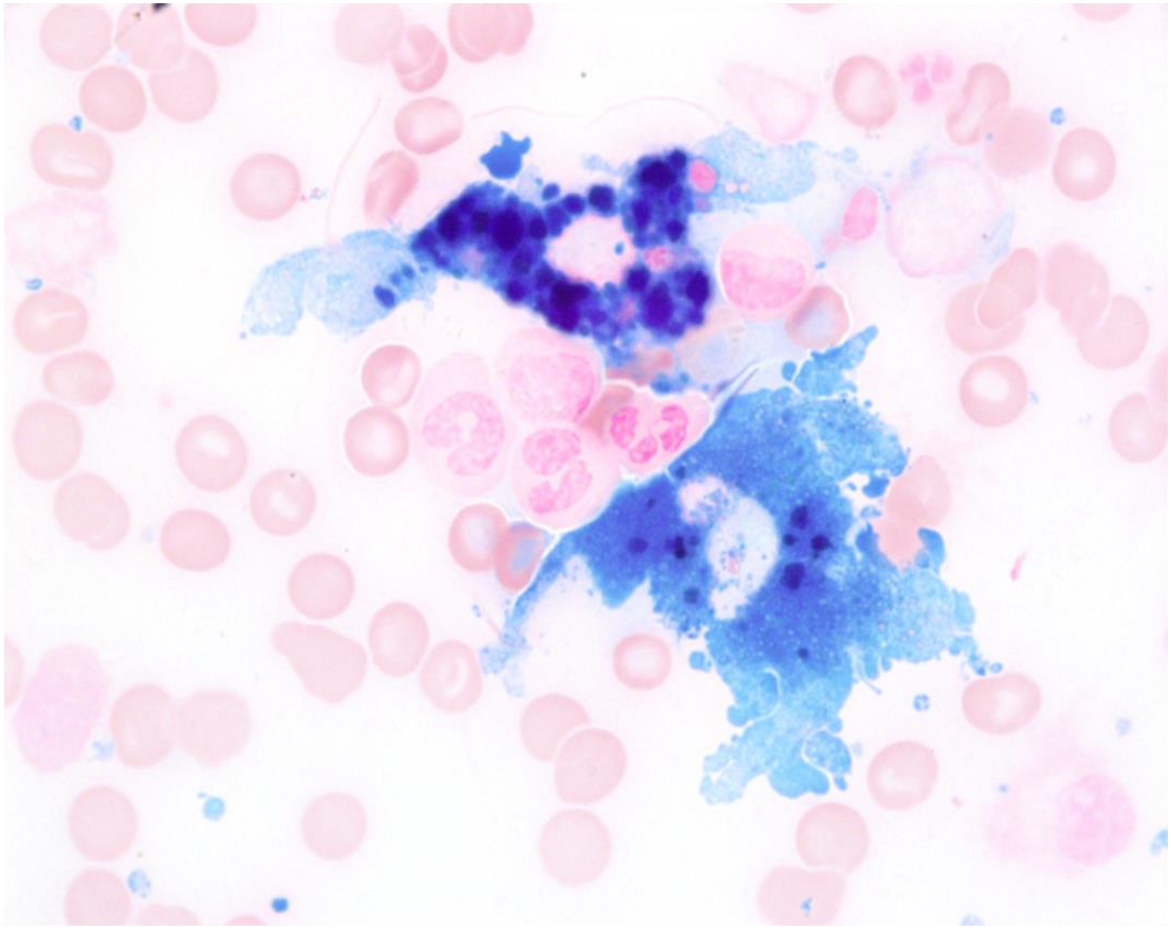
EVALUATION OF STAINABLE IRON IN BONE MARROW

siderocytes	erythrocytes with blue-green granules in cytoplasma
sideroblasts	erythroblasts with 1-3 granules (normal 20-60%)
ring sideroblasts	numerous granules form a ring around the nucleus
siderophages	macrophages
extracelular iron	present, rarely or absent

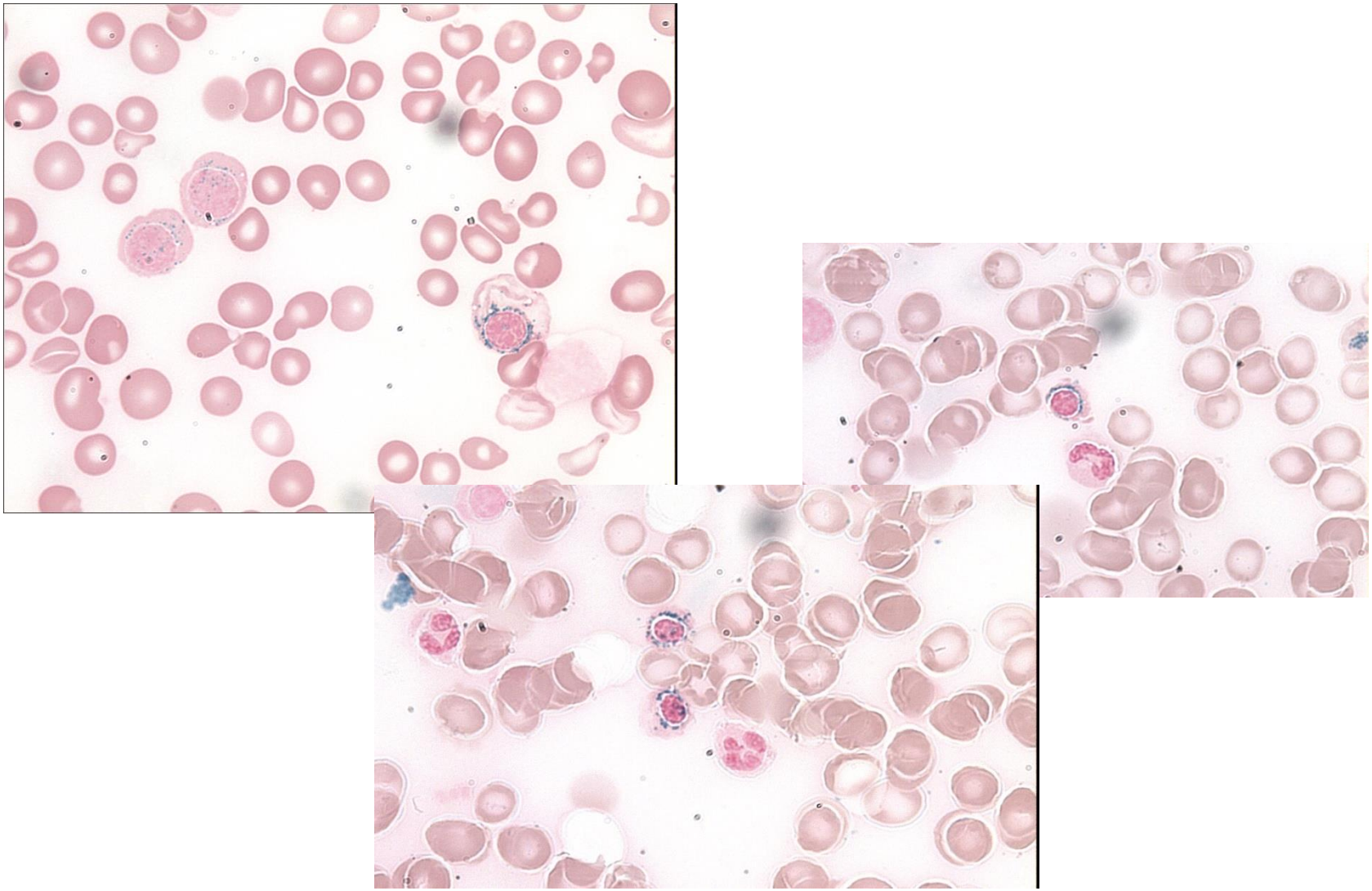
SIDEROCYTES



STORAGE IRON (ACD)



RING SIDEROBLASTS



SOLUBLE TRANSFERIN RECEPTORS

- **level of soluble transferin receptors is directly proporcional to transferin receptor expression on erythropoetic precursors**
- **in case of iron deficiency induction of these receptors occurs**
- **no affected by inflammation**
- **increased level in iron deficiency anemia and anemia of chronic disease**
- **ferritin index (FI)=sTfR/log ferritin (increased in iron deficiency anemia, decreased in ACD)**

DIAGNOSIS OF IDA

LABORATORY FEATURES

- **Microcytosis:** **MCV < 84 fL**
  - may be absent in more rapid loss of iron
- **Low mean cell HGB:** **MCH < 28 fL**
- **Hypochromia:** **MCHC < 320 g/L**
- **Low serum iron:** **< 10 μmol/L**
  - similar to ACD (not useful for differential dg)
- **High TIBC (serum transferrin)**
- **Low iron saturation of TIBC** **< 20 %**
- **Low serum ferritin: approx.** **< 20 mg/L**
- **Low marrow sideroblasts** **< 20 %**
  - not necessary for diagnosis

THERAPY OF IRON DEFICIENCY ANEMIA

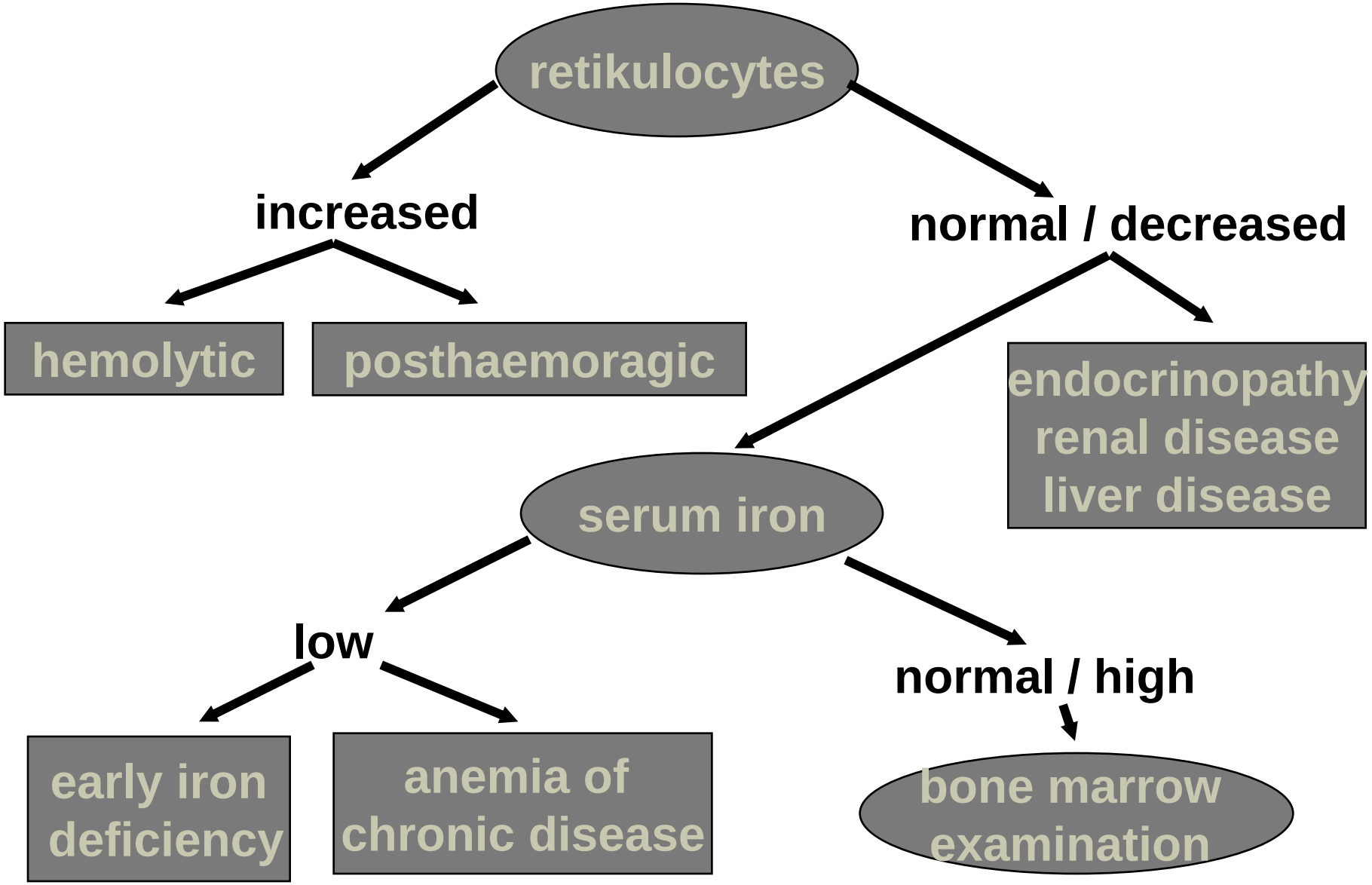
- **the basic rule is eliminating the causes of iron deficiency**
- **oral iron therapy**
  - **daily total of 150-200 mg elemental iron**
  - **in case of intolerance- dose reduction to 100 mg/day (adverse effects comparable to placebo)**
  - **therapy is long-term (3-6 months after hemoglobin normalisation)**
  - **the patient should be instructed: do not give with meals**
  - **inhibition of resorption by tea, coffee, milk**

NORMOCYTIC ANEMIA

Anemia with normal mean cell volume of erytrocytes (MCV) resulting from:

- **reduced production of erythrocytes (anemia of chronic disease, aplastic anemia)**
- **increased destruction or loss of erythrocytes (hemolysis, posthemoragic anemia)**
- **uncompensated increase in plasma volume (excess fluid)**
- **combination of conditions leading to microcytic and macrocytic anemia**

NORMOCHROMIC NORMOCYTIC ANEMIA



CAUSES OF NORMOCYTIC ANEMIA

- anemia of chronic disease
- nutritional anemia (inicial stage of iron deficiency anemia)
- anemia in chronic renal failure
- anemie in chronic heart failure
- hemolytic anemia
- primary bone marrow disorder
  - aplastic anemia, pure red cell anemia
  - myelodysplastic syndrom
  - paroxysmal nocturnal hemoglobinuria
- secondary bone marrow disorder
  - drugs, toxins, radiation, viral infections
  - myelofibrosis
  - bone marrow infiltration (hematologic and solid tumors)
  - liver disease
  - endocrinologic disease

ANEMIA OF CHRONIC DISEASE

ANEMIA OF CHRONIC DISEASE, ACD

specific group of acquired anemias associated with number of chronic diseases (lasting for more than 1-2 months)

- **does not include posthemoragic anemia, hemolytic anemia, bone marrow infiltration**
- **anemia associated with liver, renal or endocrinologic diseases are not usually classified as ACD (multifactorial etiology, ACD is only one of causes)**
- **the most prevalent anemia in inpatients and old people**
- **occurs in more than half oncologic patients, incidence decreases in inflammatory conditions**
- **interdisciplinary problem**
- **usually confused with iron deficiency anemia and treated uncorrectly**

ETIOLOGY OF ANEMIA OF CHRONIC DISEASE

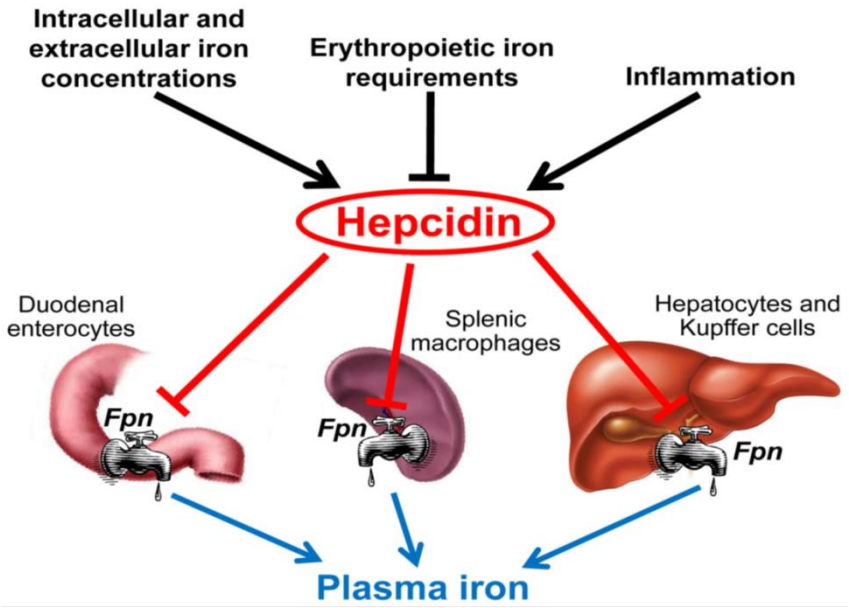
- ***chronic infections*** **(osteomyelitis, chronic kidney and urinary tract infections, HIV, chronic skin disorders- decubits, leg ulcers …)**

- ***chronic non-infectious inflammatory conditions*** **(connective tissue disease, inflammatory bowel disease, nephritis, rheumatoid arthritis…)**

- ***malignancy*** **(solid tumors and hematologic malignancy)**

- ***traumatic and postoperative conditions*** **(burns, post-transplant conditions)**

PATHOGENESIS OF ANEMIA OF CHRONIC DISEASE



- ***Increased production of inflammatory cytokines*** **(TNF α, IL-1, IL-4, IL-6, IL-10 a IFN γ)**
- ***Increased production of hepcidin in the liver*** **(regulatory protein of iron metabolism)**

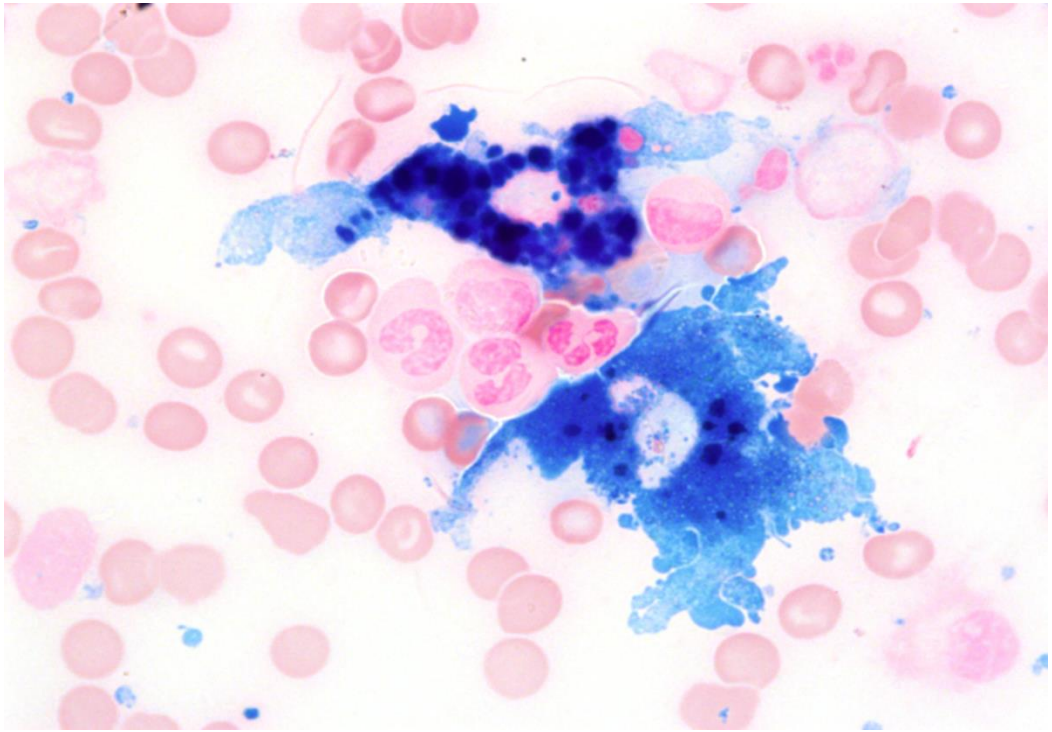
- **relative iron deficiency**
- **suppression of erythroid progenitors (BFU-E) and precursors (CFU-E)**
- **decreased production of erythropoetin and impaired ability of erythroid precursors to respond to EPO**

HEPCIDIN
- KEY ROLE IN ACD

- **plays role in regulation of iron homeostasis**
- **hepcidin binds to and leads to ferroportin degradation (the primary cell surface iron exporter) in cells (macrophages, enterocytes, hepatocytes)**
- **is produced in liver**
  - in tumors and inflammation (IL-6)
  - in high intake of iron
- ***negative regulator of iron absorption in enterocytes and iron release in monocyte-macrophage system***
- **decrease of serum iron can be a natural immunity mechanisms – antimicrobial peptid**

CHARACTERISTICS OF ACD

- no marked anizocytosis (normal or slightly increased RDW)
- normal or low reticulocytes
- normal cellularity of bone marrow, no increase of erythropoesis
- marrow contains increased storage iron

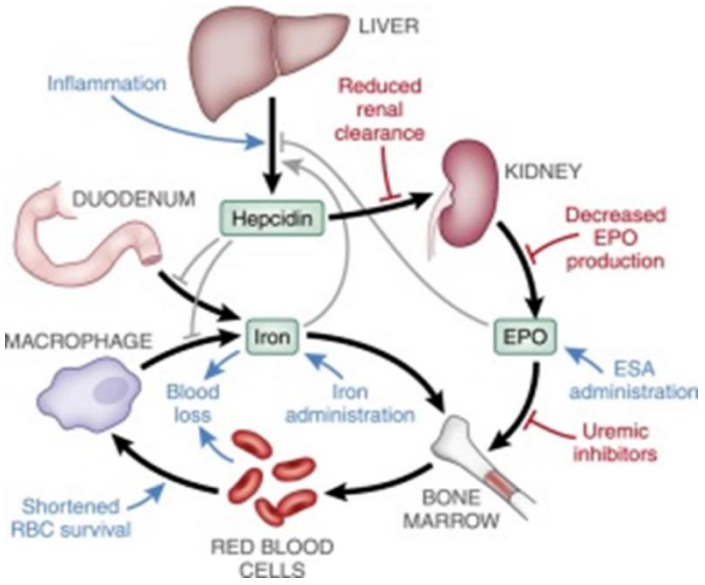


DIFFERENCIAL DIAGNOSIS OF ACD

	ACD	IDA	ACD and IDA
MCV (fl)	N-↓ (>72)	↓/↓↓↓	↓
RDW	↑- N	↑	↑
serrum iron	↓	↓↓/↓↓↓	↓
serum ferritin (ug/l)	N- ↑	↓ (<20)	< 30→ sideropenia
serum transferin	↓- N	↑	↓
transferin saturation(%)	N- ↓	↓	↓
sTfR (0,8- 3,1 mg/l)	N	↑ (2,0-20,0)	N- ↑
sTfR/log.ferritin (0,3-2,5)	< 1,0	> 2,0	> 2,0
serum hepcidin	↑	↓	↓
BM- sideroblasts	↓ (< 20%)	↓	↓
BM- siderophages	N- ↑	↓- 0	↓- 0

ANEMIA IN CHRONIC RENAL FAILURE

- ❑normocytic normochromic anemia
- ❑hypoproliferative (low reticulocytes)
- ❑must be considered in decreased glomerular filtration under 30 ml/min



(Babitt JL, Herbert YL. Mechanismus of anemia in CKD. J Am Soc Nephrol 2012)

MACROCYTIC ANEMIA

MACROCYTIC ANEMIAS (MCV > 96 FL)

Megaloblastic (tzv. megaloblastic hematopoiesis)

- **impaired synthesis of DNA**
  - cobalamin/folate deficiency
    - 30-50% of all macrocytic anemias
  - congenital DNA synthesis disorders
  - drug- induced
    - methotrexate, cytosin-arabinosid, cyklophosphamide
  - toxic DNA synthesis disorders (arsenic)

Nonmegaloblastic

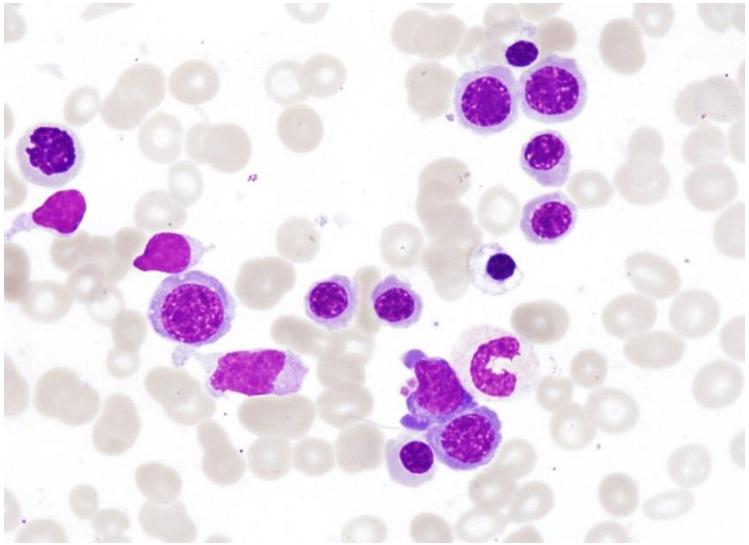
- **no impaired DNA synthesis**

NONMEGALOBLASTIC MACROCYTIC ANEMIAS

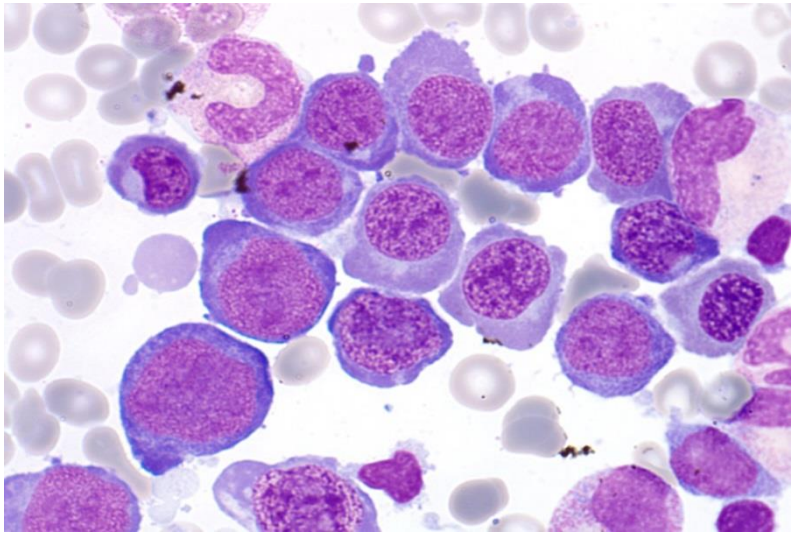
- **accelerated erythropoiesis**
  - hemolytic anemia
  - posthemorrhagic anemia
- **enlarged surface of red blood cells**
  - liver disease
  - splenectomy
- **dysplastic anemias**
- **alcoholism (macrocytosis without anemia)**
- **hypothyreosis**
- **chronic obstructive pulmonary disease**

COMPARISON OF NORMOBLASTIC AND MEGALOBLASTIC HEMATOPOIESIS

NONMEGALOBLASTIC



MEGALOBLASTIC



nuclear-cytoplasmic asynchrony

MEGALOBLASTIC ANEMIA- CAUSES- PART I

Cobalamin deficiency	**Decreased intake** - vegetarians/vegans - poor nutrition in older people **Malabsorption** - pernicious anemia - congenital deficiency of intrinsic factor - partial or total gastrectomy - celiac sprue (primary malabsorption) - selective cobalamin malabsorption with proteinuria (Imerslundové - Gräsbeckův syndrom) - blind loop syndrome - Ileal resection or disease - parasites (Diphylobothrium latum, Giardia intestinalis, Strongyloides stercoralis) - drugs- metformin - pancreatic insufficiency - Zollinger-Ellisonův syndrome (gastrinom)

MEGALOBLASTIC ANEMIA-CAUSES PART II

Folate deficiency	**Decreased intake** - poor nutrition - special diet **Increased loss** - congestive heart failure - hemodialysis **Drugs** - anticonvulsants - sulphalasine **Malabsorption** - gluten enteropathy - congenital malabsorption **Increased requirements** - pregnancy, breastfeeding - hemolytic anemia - premature infants - tumors (carcinomas, lymphomas, myeloma) - inflammatory disorders - skin disease (severe psoriasis or dermatitis exfoliativa) **Mixed** - alcoholism - liver disease

DRUGS CAN CAUSE MEGALOBLASTIC ANEMIA

Antimetabolits	antifolates	Methotrexate Pyrimethamine Trimetoprime Sulphasalazine
	purine analogues	6-merkaptopurine 6-thioguanine Azathioprine Acyklovir
	Pyrimidine analogues	5-fluorouracil 5-fluorodeoxyuridine Zidovudine
	Inhibitors of ribonukleosid reduktase	Hydroxyurea Cytosin arabinosid
Anticonvulsants	Difenylhydantoin Fenobarbital Karbamazepin Primidon	
Other drugs influencing folates	Oral contraceptives Cykloserine	
Inhibitors of proton pumps	Omeprazol	
Other	N_20 Metformin Kolchicin Neomycin Arzenic	

(According Lichtman MA et al. The Megaloblastic Anemias. Williams Manual of Hematology, 2011)

PERNICIOUS ANEMIA

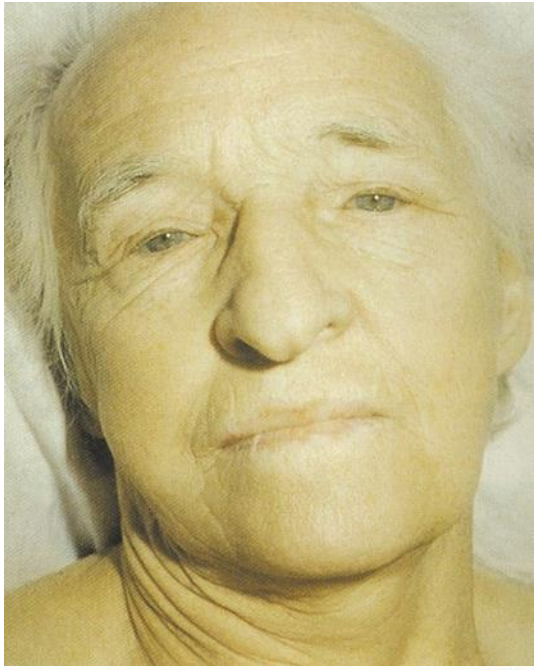
Autoimmune disease in which atrophy of the gastric mucosa of the stomach reduces the number of parietal cells that produce the intrinsic factor necessary for absorption of vitamin B12.

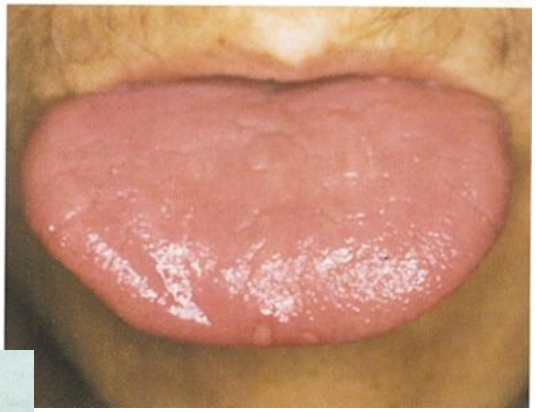
- **intrinsic factor antibodies**
- **parietal cell antibodies**

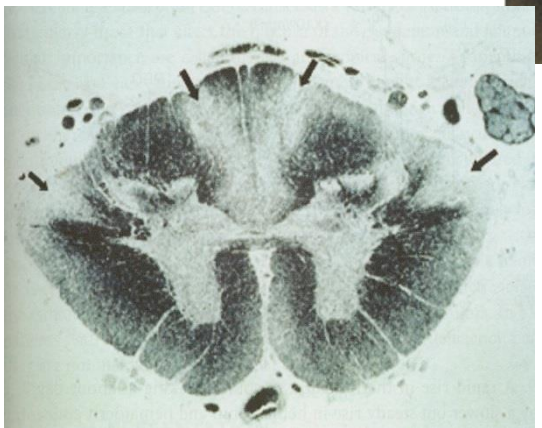
- **gastric body mucosal atrophy**

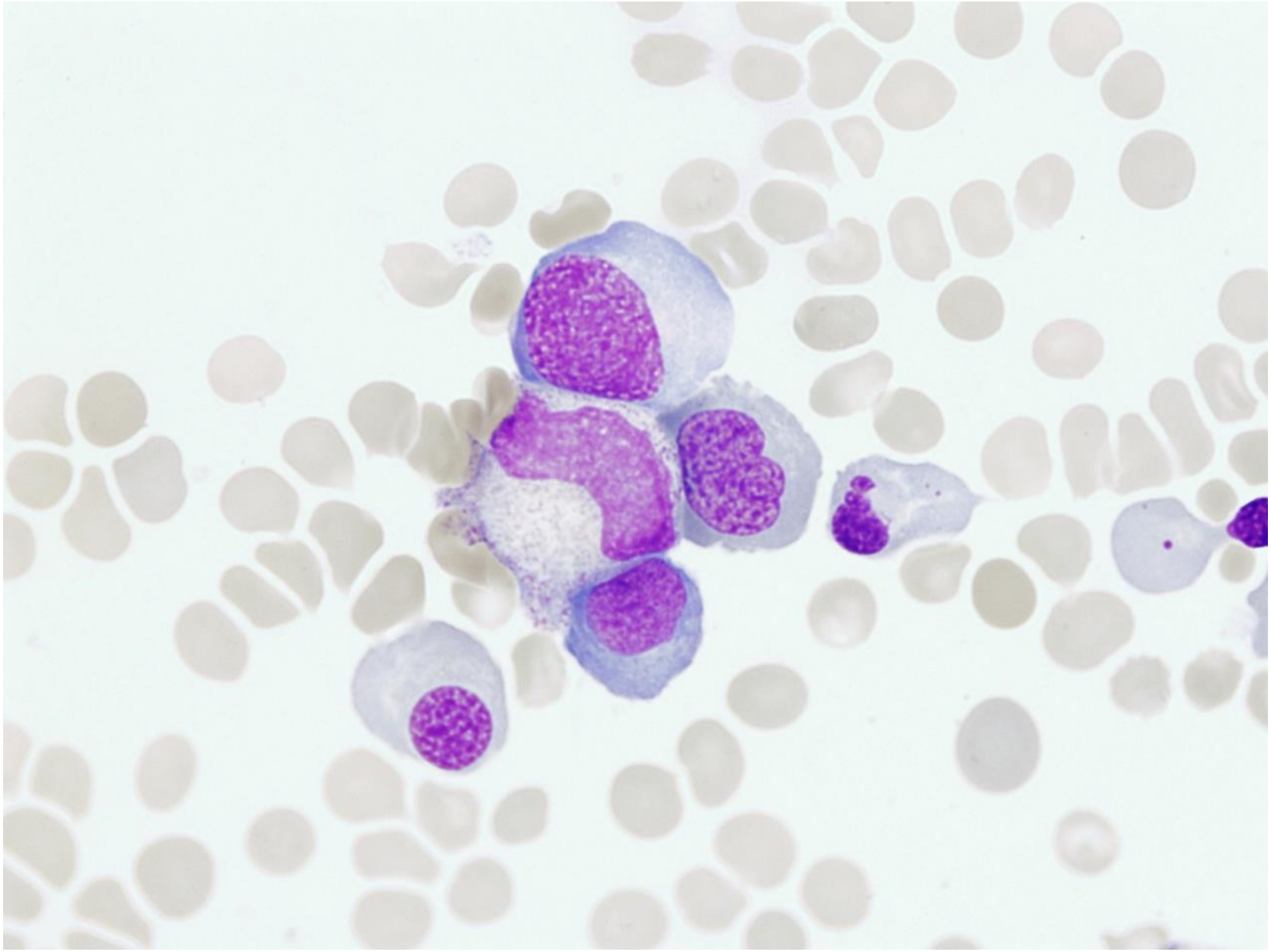
PERNICIOUS ANEMIA

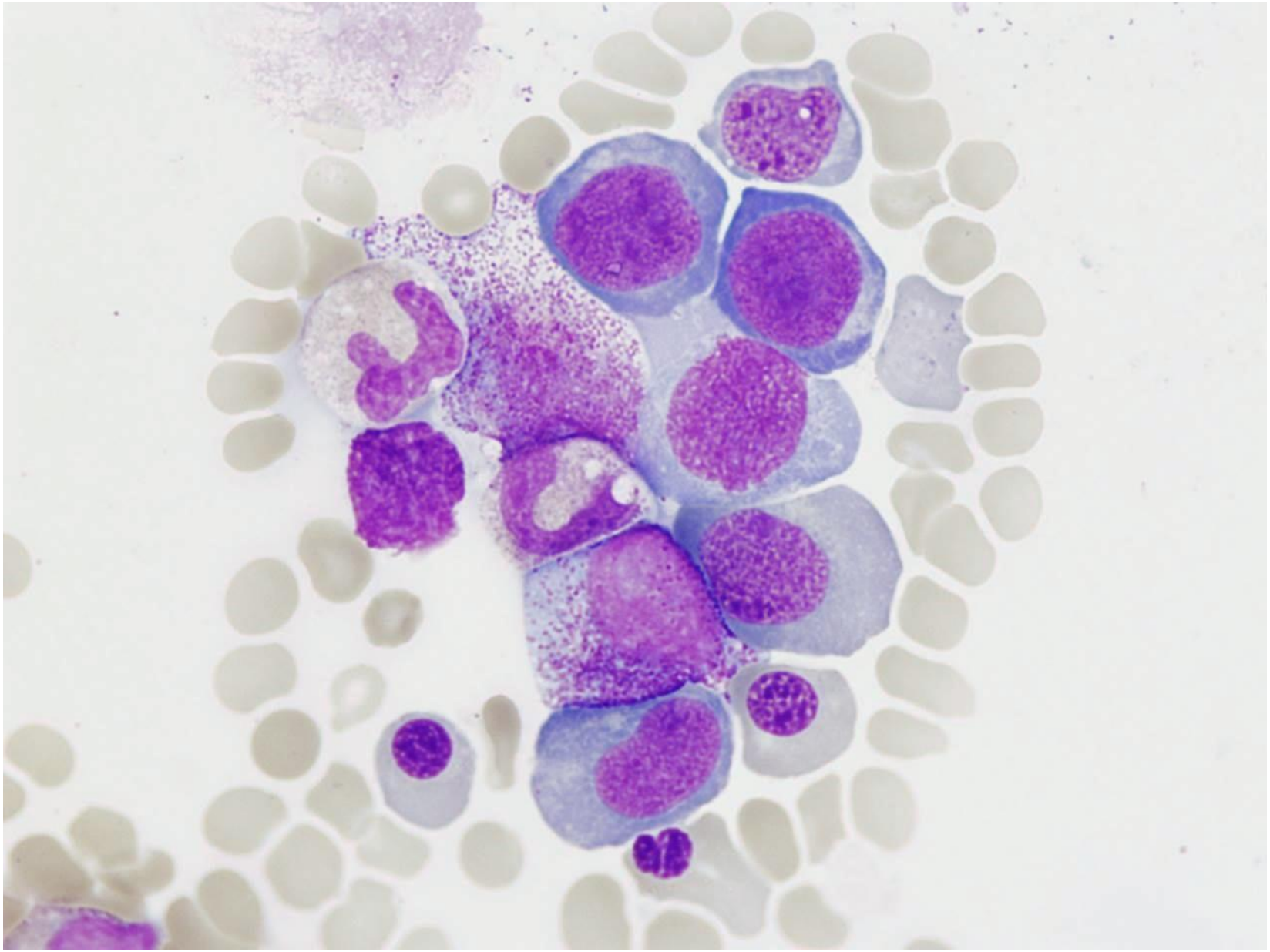
- **severe anemia acompanied by slow development of anemic syndrome**
- **often neurologic symptoms (not correlated with severity of anemia)**
- **macrocytosis often precedes anemia**
  - MCV 110-130 fl (up to 160 fl)
  - presence of macroovalocytes, hypersegmented neutrophils
  - normal reticulocyte number
- **neutropenia**
- **thrombocytopenia**
- **hyperplastic bone marrow**
  - megaloblastic erythropoiesis











DIAGNOSTIC CRITERIA – PERNICIOUS ANEMIA

Hemoglobin concentration < 130 g/l for men and < 120 g/l for women
Hematologic features of cobalamin deficiency (makroovalocytes, retikulocytopenia, hypersegmented granulocytes, megaloblastic bone marrow)
Laboratory proof of cobalamin deficiency
Gastric mucosal atrophy
Autoantibodies to intrinsic factor and/or to gastric parietal cells
Presence of clinical signs of myelopathy, neuropathy or cognitive dysfunction

TREATMENT OF PERNICIOUS ANEMIA

- **lifelong parenteral vitamin B12 substitution**
- **initial dose 1000 ug intramuscular injection daily or every other day for 1 week**
- **once a week 1-2 months**
- **maintenance dose usually once a months**

- **the most useful sign of a hematological response to therapy is an increase in the reticulocyte count on 7th to 10th day after B12 substitution start**

HEMOLYTIC ANEMIA

WHEN WE THINK ABOUT HEMOLYTIC ANEMIA?

- rapid onset of pallor and anemia
- icterus with increased indirect bilirubin concentration
- history of bilirubin lithiasis
- splenomegaly
- presence of circulating spherocytes (for example AIHA, hereditary spherocytosis)
- other red cell abnormalities
- increased lactate dehydrogenase level
- decreased or absent haptoglobin level
- direct antiglobulin test positivity
- increased reticulocyte count

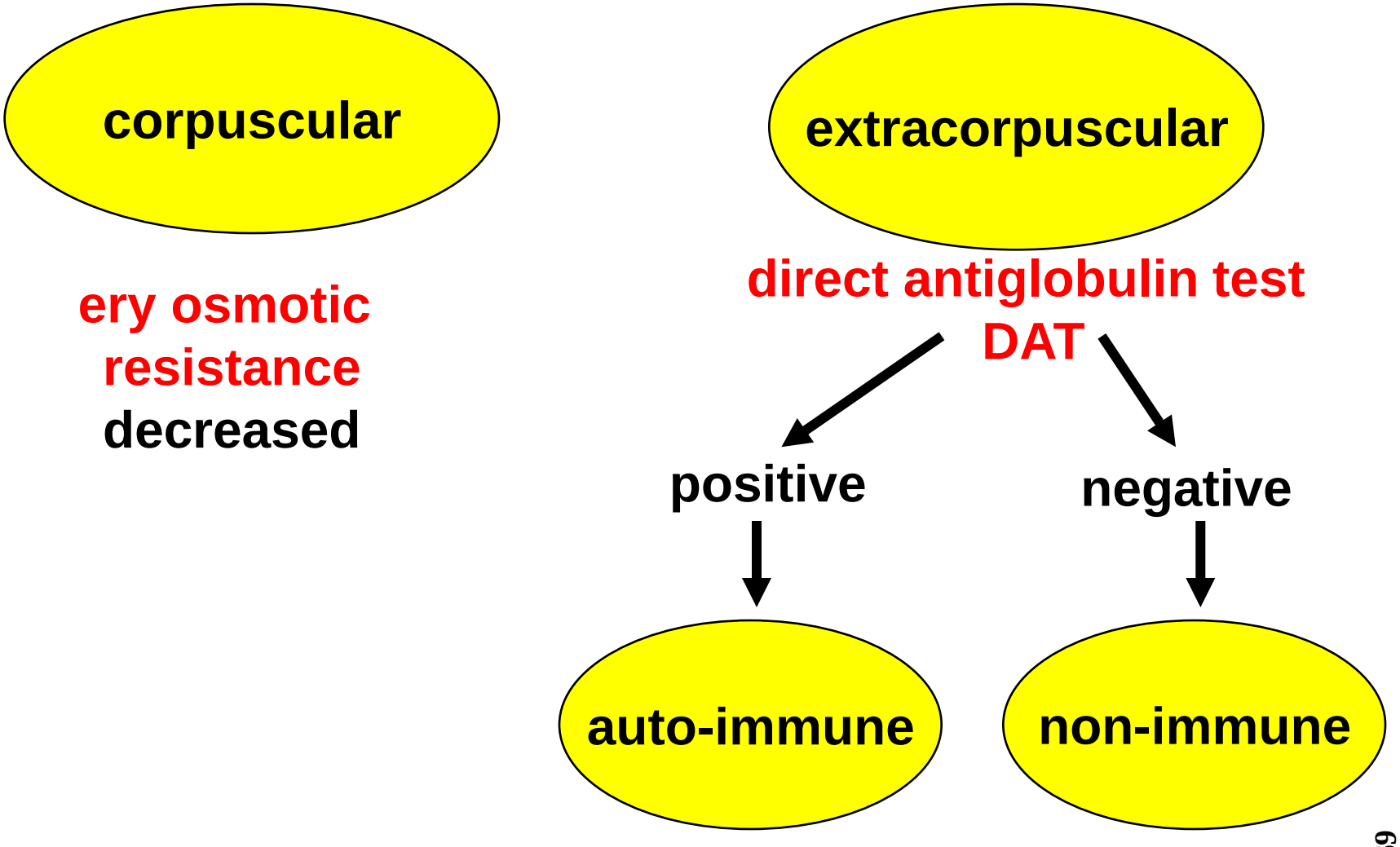
Anemia due to a shortened survival of circulating red blood cells (the bone marrow can replace the increased turnover up to 10 times)

CLASSIFICATION OF HEMOLYTIC ANAEMIAS

hereditary	acquired
Membrane deffects hereditární spherocytosis, hereditary elliptocytosis	**Immune** • *autoimmune* AIHA warm antibody type AIHA cold antibody type
Metabolism red cell deffects G6PD deficiency, pyruvate kinase deficiency	• *alloimmune* Hemolytic transfusion reactions Hemolytic disease of the newborn
Hemoglobinopathy (Hb S, HbC, unstable Hb)	• *Drug associated hemolytic anemias*
	Red cell fragmentation hemolytic anemias
	Infections malaria, clostridia
	Chemical and physical agents especially drugs, industrial/domestic substances, burns
	Secondary Liver and renal disease
	Paroxysmal nocturnal hemoglobinuria

HEMOLYTIC ANEMIAS

History, red cell count in smear



LABORATORY FINDINGS OF HEMOLYSIS

	extravascular hemolysis	intravascular hemolysis
reticulocyte count	increased	increased
indirect bilirubin	increased	increased
haptoglobin	can be low	low or absent
lactate dehydrogenase	increased	increased
free hemoglobin	normal	significantly increased
urine bilirubin	absent	absent
urine hemosiderin	absent	positive
urine hemoglobin	absent	positive in severe conditions

COMMON CHARACTERISTICS OF HEMOLYTIC ANEMIA

symptoms

Anemia-related symptoms (pallor, fatigue, exertional dyspnoe, palpitations) and signs of hemolysis (jaundice, dark urine)

laboratory findings

Various degrees of macrocytic anemia with reticulocytosis, increased LD, indirect bilirubin, decreased haptoglobin level.

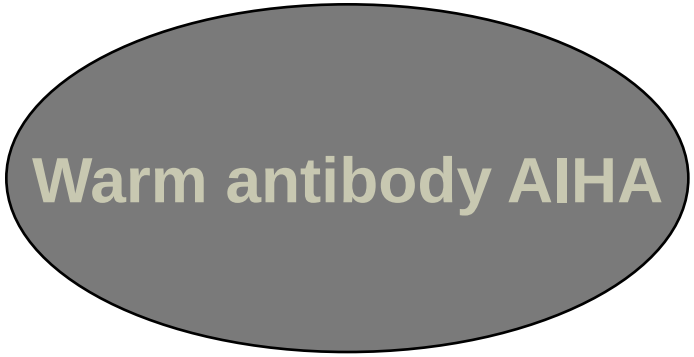
intravascular hemolysis

Sudden (acute) onset
Often severe and symptomatic anemia
Low back pain
Fever, chills
Hypotension, shock
Dark or reddish urine with hemoglobinuria, delayed hemosiderinuria (≥7 days), thrombocytosis, leukocytosis
Possible acute renal failure
Delayed jaundice

extravascular hemolysis

Progressive (subacute or chronic) and insidious onset
Mild to moderate anemia
Splenomegaly
History of gallstones
Leg ulcers
Dark urine
Variable MCV, presence of spherocytes
Hypocholesterolemia

AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA)



IgG
not monoclonal
do not bind complement
predominantly extravascular hemolysis
intravascular hemolysis at high titer

IgM
often monoclonal
bind complement
predominantly intravascular hemolysis

AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA)- LABORATORY FEATURES

- **Blood count:**

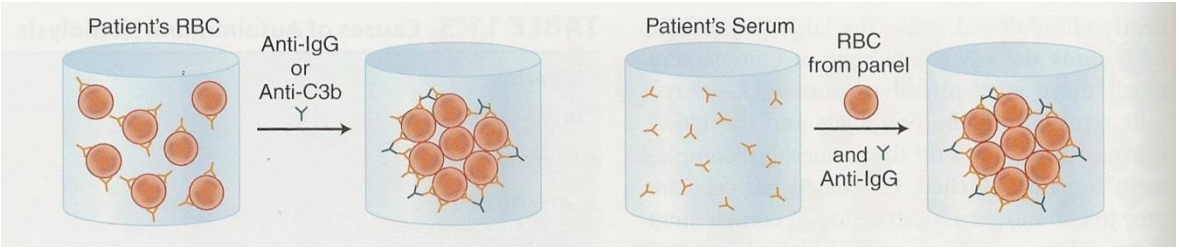
  usually macrocytic anemia with reticulocytosis

- **Biochemistry:**

  ↑ indirect bilirubin, ↑ LD

- **Special examination**

  direct and indirect antiglobuline test (Coombs)



Direct antiglobulin test

Undirect antiglobulin test

CLASSIFICATION OF AIHA WITH WARM ANTIBODIES

- **idiopatic form of AIHA**

- **secondary forms**
  - **lymphoproliferative diseases**
  - **autoimmune disease**
  - **drugs: penicillin, chinidin, methyldopa**

HAEMOLYTIC CRISIS
IN AIHA WITH WARM ANTIBODIES

- **rapid drop in HGB concentration**
  - severe anemia
- **jaundice**
- **abdominal pain, back pain**
- **fever**
- **splenomegaly**

Haematological emergency

MIKROANGIOPATHIC HEMOLYTIC ANEMIA, MAHA

Group of disorders resulting from the fragmentation of erythrocytes by the microvascular thrombi

DAT negative hemolytic anemia

- **Thrombotic thrombocytopenic purpura TTP, m. Moschkowitz**
- **Hemolytic-uremic syndrome, HUS**
- **HELLP syndrome in pregnant women**

PATOPHYSIOLOGY OF TTP / HUS

- **formation of platelet thrombi in microcirculation**
  - vWF + thrombocytes + small amount of fibrin
  - terminal arteriols and capillaries
  - subendotelial hyaline deposit
  - normal level of clotting factors
  - consuming thrombocytopenia
- **mechanic hemolysis, DAT negative**
  - schistocytes in peripheral blood

TTP / HUS

LABORATORY PICTURE

- **thrombocytopenia**
- **anemia**
- **schistocytes >4/1000 erythrocytes**
- **high lactatedehydrogenase (LD)**
- **elevated bilirubin**
- **increased free serum haemoglobin**
- **decreased haptoglobin**
- **normal blood coagulation tests**

TTP / HUS

- **young age: mean 42 years (18-72)**
- **previously healthy individuals**
- **acute onset**
- **fulminant course**
- **the disease can be fatal**

  most death occur within 48 hours
- **incidence is rising**

TTP / HUS
SYMPTOMATIC PENTAD

- **MAHA**
- **thrombocytopenia**
- **fever**
- **acute renal failure**
- **neurologic symptomatology**

APLASTIC ANEMIA

- **hematopoetic cell failure in the ability of self-renewal and maintain constant stem cell pool**
- **bone marrow hypocelularity**
- **cytopenia**
- **immune mechanisms- inhibition by T-lymphocytes, antibodies or lymphokines**

APLASTIC ANEMIA

- **by origin**
  - **inherited (Fanconi, Blackfan- Diamond)**
  - **acquired: idiopathic**
    secondary

- **by severity**
  - **chronic cytopenia**
  - **severe aplastic anemia**
  - **very severe aplastic anemia**

SEVERE APLASTIC ANEMIA PERIPHERAL BLOOD FINDINGS

- **granulocytes** **< 0,5 x 10^9/l**
- **reticulocytes** **< 1 %**

  < 40 x 10^9/l
- **thrombocytes** **< 20 x 10^9/l**

THROMBOCYTOPENIA

THROMBOCYTOPENIA

- **decrease of platelet count under 150 G/l**
- **in practice border 100G/l**
- **it is necessary exclude pseudotrombocytopenia (2% of patients)**
- **in case of true thrombocytopenia examine peripheral blood by microscope**

Decreased production	**Increased destruction**	**Sequestration**
Aplastic anemia MDS Leukemia Lymphoma Drugs (DITP) Immune (ITP)	DIC TTP HIT Drugs (DITP) ITP	Portal hypertension with splenomegaly Liver cirhosis with congestive splenomegaly Gaucher disease Myelofibrosis Viral infections with splenomegaly

SEVERITY OF THROMBOCYTOPENIA

(ACCORDING TO NATIONAL CANCER INSTITUTE)

PLT 75-150 G/l….. grade 1, mild thrombocytopenia

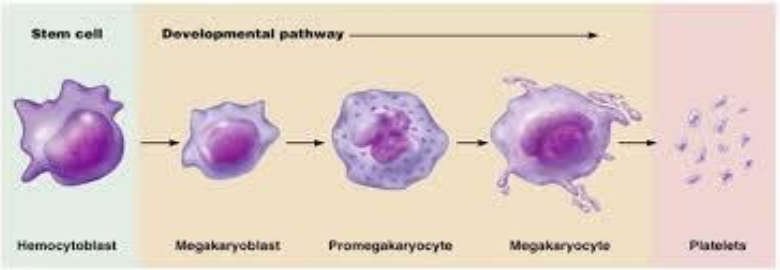
PLT 50-75 G/l……..grade 2, moderate thrombocytopenia

PLT 25-50 G/l…….. grade 3, severe thrombocytopenia

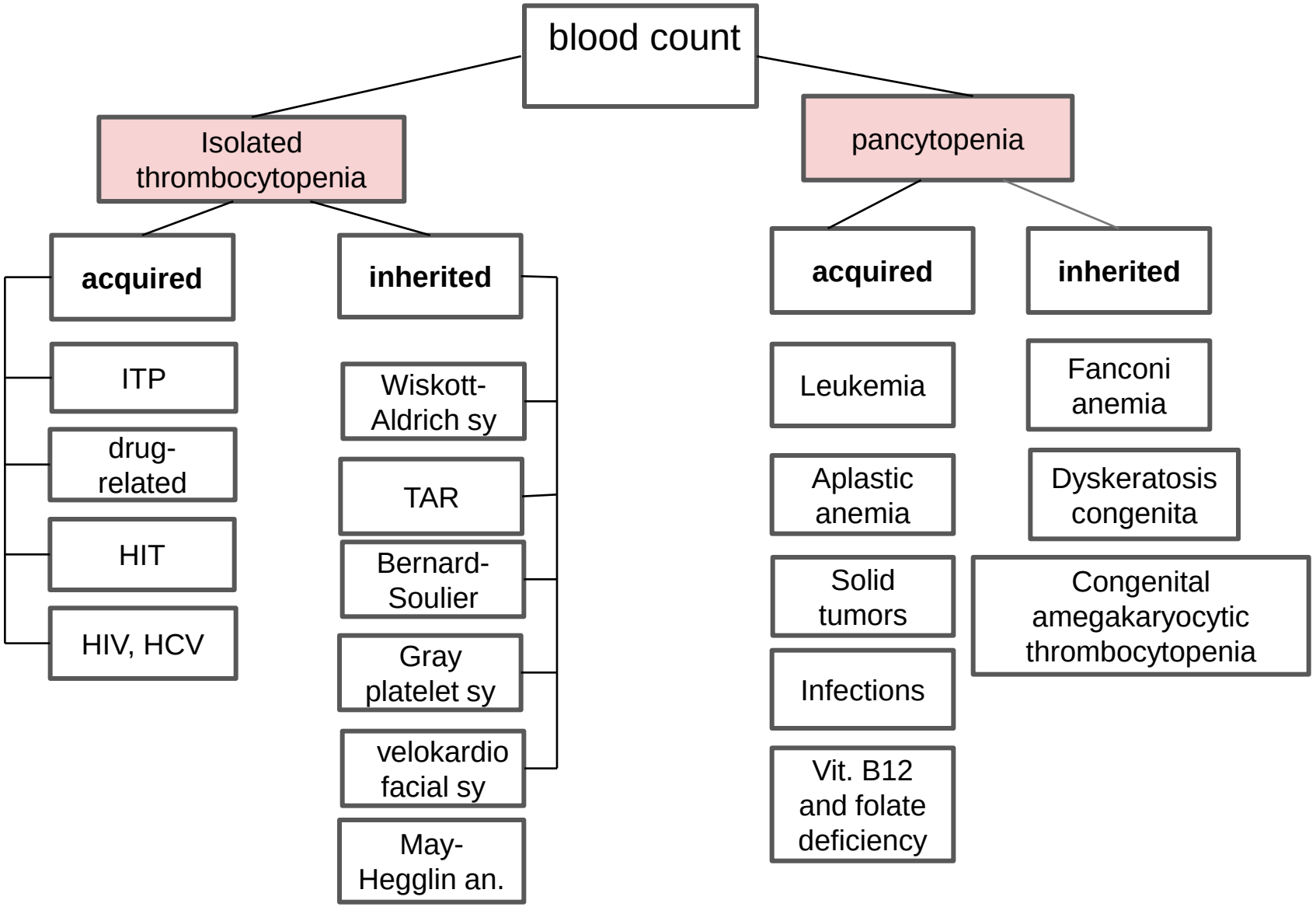
PLT bellow 25 G/l .. grade 4, life threatening thrombocytopenia

THROMBOCYTOPENIA
PATOPHYSIOLOGIC CLASSIFICATION

- **Arteficial (pseudothrombocytopenia, in vivo)**
  - clustering after the anticoagulant (EDTA)
- **Accelerated platelet destruction (the most frequent)**
  - immune
  - nonimmune (TTP)
- **Platelet formation disorders**
- **Abnormal platelet distribution in the body (pooling)**
  - disorders of spleen
  - massive transfusion delivery



THROMBOCYTOPENIA- DIF. DG.



DIAGNOSIS OF IMMUNE THROMBOCYTOPENIA IS PER EXCLUSIONEM

- **peripheral thrombocytopenia**
- **normal megakaryocyte count in bone marrow**
- **absent splenomegaly**
  - mild splenomegaly is possible

IMMUNE THROMBOCYTOPENIA

Idiopatic, ITP

Secondary

- drug-induced
  - heparin
  - chinidin, chinin, rifampicin, acetaminofen trimethoprim-sulfametoxazol, hydrochlorothiazid
- lymphoproliferation
- lupus erythematodes
- infections

Aloimmune

- newborn
- transfusion reaction

HEMORRHAGIC MANIFESTATION IN ITP

- **skin bleeding symptoms**
- **mucosal bleeding**
  - gingival
  - epistaxes
  - hematuria
  - menorrhagia
  - gastrointestinal bleeding
- **cerebral hemorrhage**
  - in 1% with severe thrombocytopenia(<$20x10^9$/l)
- **posttraumatic bleeding**
  - dental extraction, tonsilectomy, cutting wounds

LABORATORY FINDINGS IN ITP

- **often platelets of varying size and appearance**

- **abnormally large platelets 3-4 μm**
  - increased MPV
  - inverse MPV correlation with the number of platelets
  - contrast with low MPV in hypersplenism

- **abnormally small platelets and fragments of platelets**

- **platelet anizocytosis**
  - increased PDW
  - picture of accelerated production of platelets

- **antiplatelet antibodies are not specified for ITP**
  - often are increased in nonimmune thrombocytopenia and normal people
  - normal platelets have immunoglobulins in α-granules
    - release during platelet activation

MEAN PLATELET VOLUME, MPV NORMAL RANGE 8-11 FL

High level

Immune thrombocytopenia

Low level

Hypersplenism
MPN
Thrombocytopenia associated with chemotherapy
Septic trombocytopenia

BONE MARROW IN ITP

- **non-specific changes in megakaryocytes**
  - same morphology as in other types of accelerated platelet destruction
  - bone marrow examination is not necessary in ITP(under 60 years of age)
  - is usefull for the exclusion of other diseases

- **megakaryocytes**
  - large size, gigantic megakaryocytes
  - increased number
  - accelerated platelet production, increased young elements

ACUTE IMMUNE THROMBOCYTOPENIA

- **sudden appearance**

- **infection precedes 3 weeks before**
  - viral infection in children, respiratory infections
  - varicella zoster, EBV
  - vaccination

- **severe thrombocytopenia in children, bleeding symptoms usually mild**
  - spontaneous remision in 90% of children
  - usually duration 4-6 weeks in children

CHRONIC IMMUNE THROMBOCYTOPENIA

- **prolonged mild bleeding symptoms**

- **fluctuating course**

- **bleeding episodes last day to weeks**
  - can be cyclic course
  - spontaneous remision are incomplete
  - benign course

IMMUNE THROMBOCYTOPENIA

- diagnosis per exclusionem
- nonspecific proof of antiplatelet antibodies
- start therapy in platelet count under 30G/l
- 1-st line therapy- corticosteroids (prednison 1 mg/kg, dexamethazon 40 mg)
- immunoglobulins 0,4 mg/kg/day 5 days or 1g/kg 1-2 days
- 2-nd line therapy- splenectomy, immunosupression, rituximab 375 mg/m2 one a week for 4 weeks
- use of thrombopoetin receptor agonists (chronic ITP relapsed or refractery)

POST- TRANSFUSION PURPURA, PTP

- **severe thrombocytopenia occuring after a blood transfusion, it is caused by alloimmunisation against platelet antigens (about a week after transfusion)**

  - unclear pathophysiology

- **potencially fatal reaction**

- **rare occurence, usually**
  - multiparous women
  - previously transfused patients

DIAGNOSIS OF PTP IS CLINICAL

- **it is necessary to consider this diagnosis after blood transfusion, if the thrombocytopenia occurs in 3-14 days**

  - exeptionally after blood plasma

- **spontaneous regression in 1-3 weeks**

- **therapy**

  - IVIG
  - plazmaferesis
  - corticosteroids

DIFFERENCIAL DIAGNOSIS OF ITP

- acute leukamia
- myelodysplastic syndrome
- aplastic anemia

- thrombotic microangiopathy, TTP/HUS
- disseminated intravascular coagulopathy, DIC
- arteficial thrombocytopenia

HIT-4T SCORING SYSTEM(CLINICAL CRITERIA)

Category	2 points	1 point	0 points
1. thrombocytopenia	platelet count fall > 50% and platelet nadir ≥ 20G/l	platelet count fall 30-50% or platelet nadir 10-19G/l	platelet count fall < 30% or platelet nadir < 10G/l
2. timing of platelet count fall	clear onset between days 5-10 or platelet fall ≤ 1 den (prior heparin exposure within 30 days)	consistent with days 5-10 fall, but not clear (e.g. missing platelet counts) or onset after day 10 or fall ≤ 1 day (prior heparin exposure 30-100 days ago)	platelet count fall < 4 days without recent heparin exposure
3. thrombosis or other sequelae	new thrombosis (confirmed) or skin necrosis at heparin injection sites or acute systemic reaction after intravenous heparin bolus	progressive or recurrent thrombosis or nonnecrotizing (erythematous) skin lesions or suspected thrombosis (not proven)	none
4. other causes for thrombocytopenia	none apparent	possible	definite

0-3 low probability, 4-5 intermediate probability, 6-8 high

HIT- LABORATORY DIAGNOSTICS

category	immunologic tests	functional tests
principles	detect circulating antibodies against PF4/heparin	detect antibodies, which activate cell depending on heparin
examples	ELISA	serotonin release assay HIPA (heparin- induced platelet activation assay)
advantages	high sensitivity, simple design, widely available	high sensitivity and specifity
disadvantages	limited specificity	technically difficult and limited availability

THROMBOCYTOPENIA AND SURGERY (BCSH)

- stomatologic procedures ≥ 10G/l
- dental extraction ≥ 30 G/l
- small surgical procedures ≥ 50 G/l
- large surgical procedures ≥ 80 G/l
- lumbal puncture, epidural anesthesia, gastroscopy, biopsy, catheter insertion, liver biopsy ≥ 50G/l
- brain surgery, some eye surgery ≥ 100 G/l
- Caesarean section (SC) > 50G/l
- SC+ epidural anesthesia ≥ 80G/l
- vaginal delivery 30-50 G/l