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Differential diagnostics of anemia

Red blood cells parameters - normal range in adults

Parameter	Men	Women
Hemoglobin (g/l)	140-170	120-150
Hematocrit (%)	39-49	33-43
Red blood cells (10^6/µL)	4.3-5.9	3.5-5.0
Reticulocytes (%)	0.5-1.5	
Mean cell volume (fl)	82-96	
Mean cell hemoglobin (pg)	27-33	
Mean cell hemoglobin concentration (g/dl)	33-37	
RBC distribution width RDW	11.5-14.5	

Anemia

- **reduction of total circulating red cell mass <u>below normal limits</u> according to sex, age and status (pregnancy)**
  - Men < 140g/l
  - Women < 120 g/l

adult vs. children, „dilution“ anemia in pregnancy

- **anemia is a syndrom of multifactorial etiology**
- **present in 1/3 patients of all medical disciplines**

- ***<u>Clinical manifestation</u>***
- severity of anemia - Hb>100 g/l - ~ asymptomatic, <80 g/l – „anemic“ syndroma
- development speed – acute anemia**“** *(bleeding, hemolysis)*, vs. gradual onset *(pernicious anemia)*
- **age/comorbidity –** for example ***atherosclerosis***

- **dehydration** *(sweating, diarrhoea)* ⇒ **„unmasking“ anemia after rehydration**

Anemic syndroma

Set of symptoms and signs developed as a result of tissue hypoxia caused by ↓ of transport capacity of blood for O_2

General signs derived from **:**

- tissue hypoxia (lack of O_2 in tissues)
- hypovolemia
- ↑ cardiac output with hyperkinetic circulation

Decrease of transport capacity for O_2

Symptoms

- tiredness, letargy
- weakness, malaise, fatique

- dyspnea on mild exertion
- headache, vertigo, syncope
- angina pectoris, shortening of claudications
- memory loss, decrease in cognitive function
- amaurosis fugax
- dyspepsia
- sensations of cold hands and feet

Signs

- tachypnea, paleness
- brittle nails
- loss of convexity (koilonychia)
- pale skin and mucous membranae, conjunctivae
- pale nail beds and palmar creases

↑ cardiac output

- Hyperkinetic circulation
- Decreased blood viscosity

- forceful heartbeat
- strong peripheral pulses
- systolic flow murmurs
- Sinus tachycardia
- Big pulse pressure (syst-diastol difference)

- Palpitations
- Tinnitus
- Sleeplessness

Compensatory mechanisms

- Efford to increase O_2 amount in blood
  - ↑ EPO level
  - Right shift of **oxygen–hemoglobin dissociation curve** – higher extraction of O_2

- Efford to increase cardiac output
  - Tachycardia
  - ↑ of myocardial contractility
  - ↓ of blood viscosity
  - ↓ of peripheral vascular resistance

„Specific“ signs of anemia

Iron deficiency anemia – stomatitis angularis /fissures/, hair loss, brittle nails, spooning of the fingernails (koilonychia)

Pernicious anemia – sore tongue – smooth and beefy red, dysphagia, vitiligo, numbness and paresthesia in the extremities, ataxia („neuroanemic sy“)

Hemolytic anemia – red-brown urine, fever, jaundice

Aplastic anemia – necrosis and ulcerations at mucous membranes and skin, bleedings

Classification of anemia according to underlying mechanism

1. impaired red cell production
2. increased rate of destruction of erytrocytes
3. blood loss
4. combined causes – multifactorial etiology

Blood loss

- Acute blood loss (MCV normal)
- Chronic blood loss (MCV↓) – iron defficiency anemia

- GIT
- Genito-urinary tract
- Hematuria
- Hemoptysis, epistaxis
- Trauma, surgery
- Hemodialysis

Classification of anemia according to underlying mechanism

Ineffective erytropoesis

- Iron defficiency
- Megaloblastic anemia
- Myelodysplastic syndroma
- Aplastic anemia (pure red cell aplasia)
- Hemoglobinopathy
- Anemia of chronic disorder

Increased destruction of erytrocytes

- Hypersplenism
- Corpuscular hemolytic anemia (inherited, acquired)
- Extracorpuscular hemolytic anemia
  - Immunologic causes (AIHA)
  - Non-immune causes (chemic and physical forces)

Morphologic classification

MCV

- Normocytic (MCV 78-100fl)
- Mikrocytic (MCV < 78fl)
- Macrocytic (MCV > 100fl)

•**MCH:** anemia normo- and hypochromic

MCH (pg)	< 26	27 – 32	> 32
MCHC (g/dl)	< 0.31	0.32 – 0.36	> 0.36

•**RDW:** homogenous distribution vs. pathologic anisocytosis

•**Reticulocyte production**

•Increased x normal

IRON DEFICIENCY ANEMIA

- **Microcytic, hypochromic**
- imbalance between intake and utilization of iron in the body
- **Consequences of iron deficiency**
  - ↓biosynthesis of hem/Hb ⇒ ↓ intensity of erytropoesis
  - ↓synthesis of DNA, cytochromes in mitochondria ⇒ ↓ production of energy
  - ↓ content of ferritin, hemosiderin, myoglobin
  - gradual onset, protracted course
- **IRON DEFICIENCY: 35-85% of women in childbearing age**
  - **Daily loss of iron**: M cca 1mg, W cca 2-3mg (blood loss during period is about 80ml/month≅1,1mg/day, pregnancy 5-6mg/day
  - M-16mg, resorption cca 6%
  - W-11mg, resorption cca 12% (⇒optimal iron intake 18mg)
  - resorption in iron deficiency – up to 20% (prerequisite is meat in food)
- **Resorption**
- iron „built in“ hem – very good – there is a special transport mechanism
- facilitation of resorption by HCl – soluble iron, ascorbic a. $Fe^{3+} \rightarrow Fe^{2+}$

IRON DEFICIENCY		Ist stage	IInd stage	IIIrd stage
LABORATORY TESTS	NORMAL CONDITION	NEGATIVE IRON BALANCE	LATENT	OVERT ANEMIA
Iron supply par. **Marrow iron** **Ferritin ug/l M/W**	II-III 30-400/30-150	0 (-+) <20	0 <15	0 <15
Marrow sideroblasts	40-60%	20-30%	<10%	<10%
Saturation of transferin(%)	20-45	N	<16	<10
Soluble Rc for transferrin	1,3-3,3			↑ (> 3,3)
TfR/log ferritin	3,74±3,4			↑
Transferrin ug/l	45-75			> 80
Serum iron	N	N	<50	<30
Hemoglobin	N	N	N	↓
Erythrocytes	Normochromic Normocytic RDW 12-14,5			Hypochromic Microcytic RDW > 14,5

Causes of iron deficiency – BLOOD LOSS

- **GIT** (60% of all IDA cases)
  - Often occult losses: hiatal hernia, esophageal varices, peptic ulcers, gastropathy, hemorrhoids, polyps, diverticula, tumors GIT, angiodysplasia
  - less often: IBD, Meckel´s diverticulum, helminthism
  - Side effects: Salicylic acid, NSAIDS, corticosteroids, antikoagulants, potassium chloride
- **Genito-urinary tract:** hypermenorhea, IUD, metrorhagia, hematuria
- **Hemodialysis**
- **Hemoglobinuria:** PNH, mechanical fragmentation of erythorocytes by myxoma, prostethic valve
- **Respiratory tract:** recurrent epistaxis, hemoptysis, Goodpasteur sy.,idiopatic pulmonary hemosiderosis
- **Blood donation + trauma**

Causes of iron deficiency –
decreased iron intake or decreased absorption

a) **Inadequate diet:** ↓meat intake, ↑intake of phytates and phosphates ⇒ ↓ iron resorption up to 50%

a) **Malabsorption from surgery:** ↓ release of iron from food, ↓availability of soluble iron
   - **Post-gastrectomy**

b) **Malabsorption from disease:** sprue, lactose intolerance, Crohn´s d.

c) **Lack of transferrin**
   - Inherited or acquired (nefrotic syndroma)

INCREASED DEMAND FOR IRON OR HEMATOPOIESIS

a) **Adolescence**

b) **Pregnancy (fetus´s body contains ≅ 1g of iron)**

c) **Sport – erythropoetin therapy**

Iron deficiency anemia

- The most common anemia, 10% of women of childbearing age
- Rarely as independent condition

CLINICAL PRESENTATION

- Usual signs of anemic syndroma + signs related to iron def.
- Symptoms
  - Anemic syndroma – fatigue, reduced exercise capacity
  - Symptoms of lack of iron in tissues
    - sideropenic dysphagia (Kelly- Patherson´s sign)
    - dyspepsia (atrofic gastritis as underlying cause)
- Signs:
  - Skin atrophy, premature hair greying and excessive baldness, cheilosis, atrofic glossitis
  - ↓ nail growth, koilonychia
  - Non-specific – defect of cellular immunity ⇒ ↑ risk of infection

Iron deficiency anemia - THERAPY

- Precise identification and fixing of underlying cause
- Correction of anemia
- Saturation of tissue iron stores

ORAL IRON THERAPY

- Ferrous sulfate, ferrous fumarate, ferrous gluconate, polysaccharide iron (150-300mg of elemental Fe/day-empty stomach – it should result in resorption up to 50mg Fe/day
- ↑reticulocyte count in 4-7days after initiation, restoring Hb levels after 1-3months, provision of iron stores – sustained treatment for 6-12months
- Side effects: gastrointestinal distress (15-20% of patients)

PARENTERAL IRON THERAPY

Ind: intolerance of oral iron, altered resorption of iron, needs for iron are „acute“

- Necessity to calculate the dose
- Iron dextran, sodium ferric gluconate, iron sucrose

Iron deficiency anemia - THERAPY

RED CELL TRANSFUSION

- symptoms of anemia, cardiovascular instability, excessive blood loss
- failure of iron therapy, preoperative management

PROPHYLACTIC IRON THERAPY

- growing children and adolescents
- pregnancy, lactation, hemodialysis
- those with inadequate dietary intake of iron

FAILURE OF THE THERAPY
WRONG DIAGNOSIS OF IDA or LOSS > INTAKE

Anemia of chronic disease

- **ACD –** mild/moderate anemia
- chronic infection, inflammation or cancer (common denominator- proinflammatory cytokines)
- inadequate iron delivery to the marrow, despite the presence of normal or increased iron stores
- hypoproliferative marrow ↑production cytokines and ↑activation of macrophages
- **Reflected by:** ↓s-Fe, ↑RBC protoporphyrin, transferrin sat.-15-20%, siderophagi N-↑ s-ferritin ↑↑-N
- high prevalence – the 2nd. most common anemia (3- 10%)
- often mistaken for iron deficiency anemia and therefore also inappropriately treated (Warning: iron administration)

Anemia of chronic disease

- **CHRONIC INFECTIONS –** pulmonary, infectious endocarditis, osteomyelitis, chronic infections of genitourinary tract, TBC, AIDS, mycoses
- **AUTOIMMUNE DISEASES-** rheumatic fever, RA, SLE, SS, PN, GCA etc.
- **Chronic non-infectious inflammatory diseases**: IBD
- **NEOPLASMS**: solid tumors, malignant lymphoma and leukemias
- **OTHER CONDITIONS –** st.p.Tx, postoperative or posttraumatic state

NOT EVERY CHRONIC DISEASE IS ASSOCIATED WITH ACD

- Patophysiology is complex: ↑ TNF, INFγ, IL-1,IL-6, ↓ EPO production, resistance to EPO **Hepcidin** – master regulator of iron homeostasis - **Inhibits iron transport by binding to the iron channel ferroportin** (gut enterocytes, macrophages)
- Partially responsible for iron sequestration in anemia of chronic disease and in people with renal failure

Therapy of ACD

- special treatment often unnecessary
- recovery usually when underlying disease is appropriately treated

- Red cell transfusions
  based on patients symptoms – be aware of iron overload
- Erythropoietin
  - Prerequisite – evaluation of iron status – if necessary repletion of iron
  - rHu-EPO 50-150U/kg 3times a week s.c.
  - Responsivness evaluated based on ↓s-ferritin and ↑S-sTfR and than Hb increase

Parameter	IDA	ACD	IDA & ACD
MCV (fl)	↓/↓↓↓(<80)	N-↓(> 72)	↓
MCH (pg)	↓/↓↓	N-↓(> 26)	↓
RDW (%)	↑	↑-N	↑
Serum iron (umol/l)	↓/↓↓↓	↓	↓
Serum-Ferritin (ugl/l)	↓(<20)	↑↑-N	↓-N
S-TIBC (umol/l)	↑/ ↑↑	↓-N	↓-N
S-transferrin	↑	↓-N	↓
Saturation of transferrin (%)	↓	N-↓	↓
S-sTFR (0,8-3,1)	↑(2,0-20,0)	N-↓	N-↑
sTfR/log ferritin(0,3-2,5)	(> 2,0)	(<1,0)	(> 2,0)
S-hepcidin	↓	↑	↓
BM-sideroblasts (%)	↓	↓ (<20)	↓
BM-siderophagi	↓-0	N- ↑	↓-0
Iron absorption test	Good resorption	Negative (S-<80ug/dl)	resorption

Megaloblastic anemia

- disorders caused by impaired DNA synthesis in ery
- cell divison is ↓, but cytoplasma develops normally
- megaloblastic cells are large with ↑ratio of RNA to DNA
- „ nuclear - cytoplasmic asynchrony
- ineffective erytropoiesis
- deficiency of cobalamin and/or folic acid in most cases
- other causes: drugs that impair DNA metabolism + metabolic disorders (rare)

- **Bone marrow - hypercellular**
  - decreased myeloid/erythroid ratio and abundant stainable iron
  - giant bands and metamyelocytes
  - number of megakaryocytes ↓, hypersegmentation

Causes of cobalamine deficiency

- **Inadequate intake (vegans) - rare**
- **Malabsorption**
- 1) defective release of cobalamin from food achlorhydria, part. gastrectomy, drugs blocking HCl
- 2) inadequate production of intrinsic factor pernicious anemia, total gastrectomy, congenital absence of IF
- 3) disorders of terminal ileum tropical sprue, nontropical sprue, regional enteritis, ileal resection
- 4) competition for cobalamin: fish tapeworm, bacteria – blind loop sy.
- 5) drugs: p-aminosalicylic acid, colchicine, neomycin, metformin
- **Other:** nitrous oxide, transcobalamin deficiency, congenital defects

Causes of folic acid deficiency

- Inadequate intake: unbalanced diet alcoholics, teenagers, some infants
- Increased requirements: pregnancy, infancy, malignancy, ↑ hematopoiesis, chronic exfoliative skin disorders, hemodialysis
- Malabsorption: tropical sprue, nontropical sprue, phenytoin,
- Metabolism disorder:

  1) dihydrofolate reductase inhibitors (MTX,pentamidin,trimetoprim)
  2) Alcohol
  3) Enzymatic deficits
- Drugs impairing DNA metabolism:
  - purine antagonists: 6-merkaptopurin, AZA
  - pyrimidin antagonists: 5-FU
  - others: acyclovir, zidovudine
- **Metabolic disorders:**
  - Lesch-Nyhan syndrome

Pernicious anemia

- Megaloblastic anemia
- cobalamin deficiency induced by the absence of IF due to atrophy or autoimmune destruction of parietal cells
- Autoimmune disease – antiparietal cell Ab in 90% and anti-IF Ab in 60% (blocking the bond between IF and cobalamin)
- Atrophy affects pepsin and acid producing portion of stomach
- ↑ gastrin – effect of blocked feedback
- 2x ↑ incidence of gastric carcinoma
- Clear association with diseases of immunological origin: Graves disease, thyroiditis, T1DM, adrenocortical insufficiency, vitiligo

Pernicious anemia: Clinical presentation

- Insidious onset and slow progression – severe anemia could be „asymptomatic“
- Skin: pale + slightly icteric, rarely purpura, (vitiligo)
- Sore tongue – on inspection is smooth and beefy red
- Neurologic manifestations in cobalamin deficiency – may occur in a patient with normal RBC
- 1) Demyelination 2) Axonal degeneration 3) Neuronal death irreversible spinal cord – posterior and lateral columns
- Sensoric symmetric polyneuropathy lower extremities- numbness and paresthesia, weakness, spastic ataxia, n. opticus atrophy – visual impairment
- Psychiatric disturbances – mild irritability, forgetfulness, severe dementia, frank psychosis
- Anorexia with weight loss $\Leftarrow$ malabsorption
- Very rarely splenomegaly
- Tachycardia – rapid pulse – systolic flow murmur

Pernicious anemia: Lab studies

- **Blood count:** macrocytosis: **MCV 100-150fl**.
- Macroovalocytes – fully hemoglobinized ↑ **MCH**
- marked aniso/poikilocytosis (↑ **RDW**)
- occasional megaloblasts may be seen in blood smear, **RTC low**
- ↓ **WBC/neutrofils and thrombocytes ⇒ pancytopenia,** neutrofils: hypersegmentation of the nucleus
- **Bone marrow:** „hypercellular“,↓ myeloid/erythroid ratio, giant bands and metamyelocytes, hypersegmented megakaryocytes
- Abundant stainable iron
- **Biochemical changes:**
- ↑ indirect bilirubin, ↑ Fe, ↑ ferritin, ↑ LDH
- ↓ cobalamin, values < 200ng/l = clinically significant def./age related

Pernicious anemia: Ancillary diagnostic methods

- Schilling´s test – test of cobalamin absorption – administration of labeled ^{57}Co by mouth – proportion of administered radioactivity in the urine during the next 24hours
- achlorhydria, atrophic gastritis
- Antiparietal Ab 90% a anti IF antibodies in 60%

Pernicious anemia: Treatment

Cobalamin deficiency - parenteral replacement treatment - cyanocobalamin

Initial saturation phase - 1000ug i.m. per week for 8 weeks

Follow up phase – administration 300 ug i.m. 1xweek until the correction Hb a Hct

- then every month for the rest of patient´s life

In persons with neurologic symptoms only: 1000ug per weeks during 6months

- Usually prompt improvement of strenght and sense of well being

Transfusion – only in cases of severe anemia with symptoms and cardiovascular status – risk of fluid overload $\Rightarrow$ 1-2 TU packed RBC slowly

Response to treatment – reticulocytosis 4-5thday after, peak 7th remission of anemia over 1-2 months, LEU and PLT soon

- **Prophylactic cobalamin treatment** – indication: after total/partial gastrectomy or ileal resection, blind loop sy – ATB + vit B_{12} supplementation
- **cobalamin treatment by mouth** – possible (1% absorption also in absence of IF) $\Rightarrow$ dose cca 1000ug/day + monitoring: blood count: Warning: inappropriate in neurologic signs

Other causes of macrocytosis

- Alcohol consumption
- Hepatic disease
- Myelodysplastic syndroma (sideroblastic a.)
- Hypothyroidism
- Aplastic anemia
- Reticulocytosis
- Hypoxia
- Multiple Myeloma
- Cytostatics
- Pregnancy

Hemolytic anemia

- Lifespan of RBC shortened
- Increase in reticulocyte level
- ↑ erytropoetin levels
- ↑ erytropoiesis in bone marrow or alternate sites
- Accumulation of products of hemoglobin catabolism
- Intravascular vs. extravascular hemolysis

Intravascular hemolysis

- hemoglobinemia
- hemoglobinuria
- jaundice
- hemosiderinuria
- ↓ haptoglobin in serum
- Oxidation of free Hb → methemoglobin ⇒ excretion in urine ⇒ red-brown urine

Red Blood Cell Morphology in the Diagnosis of Hemolytic Anemia

Morphology	Cause	Syndromes
SPHEROCYTES TARGET CELL SCHISTOCYTES	LOSS OF MEMBRANE ↑ RATIO of RBC S/V TRAUMATIC DISRUPTION OF MEMBRANE	SPHEROCYTOSIS,AIHA HEMOGLOBIN DISORD. MICROANGIOPATHY INTRAVASCULAR PROSTHESES SICKLE CELL SY
SICKLED CELLS	POLYMERIZATION OF HEMOGLOBIN S	SEVERE LIVER DISEASE
ACANTHOCYTES	ABNORMAL MEMBRANE LIPIDS	
AGGLUTINATED CELLS	PRESENCE OF IgM Ab	Cold agglutinin disease
HEINZ BODIES	PRECIPITATED HEMOGLOBIN	UNSTABLE Hb, OX STRESS

Classification of immune-mediated hemolytic anemia

Warm reactive (IgG) antibody
complement fixation present
idiopathic
secondary to:
Lymphomas - NH, CLL
SLE + other collagen-vasc.dis.
Drugs
Postviral infections
Other tumors

Cold reactive IgM antibody
(Cold agglutinin disease)

Highest level of activity at 0-4°C
Dissociation at >30°C
Agglutination and fixation of complement in acral parts of the body
Acute: inf. mononucleosis
Chronic: idiopathic, lymphomas

Cold reactive IgG antibody (hemolysin) (paroxysmal cold hemoglobinuria)
Reacting specifically with Ag associated with the membrane+ fixation of complement

⇓

Hemolysis at temperatures below 30°C

Causes of aplastic anemia

Inherited: Fanconi´s anemia, dyskeratosis congenita

Acquired:

Idiopathic: Primary stem cell defect - Immune mediated

Drugs and chemicals: *Regular effects* Alkylating agents, Antimetabolites, Benzene, Chloramphenicol, arsenicals

Idiosyncratic reactions: Chloramphenicol Phenylbutazone Organic Arsenicals Methylphenylethylhydantoin Streptomycin Chlorpromazine Insecticide (e.g., DDT, parathion)

Irradiation

Viral infections: Hepatitis (unknown virus) Cytomegalovirus Epstein-Barr virus, Herpes varicella-zoster

Immune diseases: eosinophilic fasciitis

When bone marrow biopsy?

- Bicytopenia, pancytopenia
- Severe macrocytosis
- Pathological WBC diff (lymphocytosis, young granulocytes, blasts, erythroblasts)
- Paraproteinemia
- Lymphatic nodes/splenomegaly
- No reason of anemia found

General anemia treatment

- Underlying cause treatment
- Iron therapy in IDA
- Vitamins (FA, B12) in megaloblastic anemia
- Imunosupression in hemolytic anemia

- Indication of EPO treatment
  - Renal failure - hemodialysis
  - Anemia in malignancies
  - Myelodysplastic syndrome