HYPOCHROMIC ANAEMIAS,
ANAEMIAS OF CHRONIC DISEASES,
MEGALOBLASTIC ANAEMIAS

5 year, 9 and 10 semester
General Medicine, 2014/2015
ANAEMIA – definition (1)

- **ANAEMIA**
  
  Anaemia – may be defined as a state in which
  
  - the blood haemoglobin level is below the normal reference level for patients’ age sex and gravidity

- **NORMAL VALUES** for peripheral blood

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/l)</td>
<td>120-160</td>
<td>135-175</td>
</tr>
<tr>
<td>- Haematocrit (PCV) (L/L)</td>
<td>0.37-0.47</td>
<td>0.42-0.54</td>
</tr>
<tr>
<td>- Red cell count (RCC)x10^{12}/L</td>
<td>3.9-5.0</td>
<td>4.5 (4.2)-6.0</td>
</tr>
<tr>
<td>- MCV (fl)</td>
<td>80-96(100)</td>
<td></td>
</tr>
<tr>
<td>- MCH (pg)</td>
<td>27-32</td>
<td></td>
</tr>
<tr>
<td>- MCHC (g/dl)</td>
<td>32-36</td>
<td></td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>0.5-2.5‰ (50-100x10^{9}/l)</td>
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</tbody>
</table>

- **ALTERATIONS** in the Hb level

  - changes in plasma volume
    
    - hypervolemia/pregnancy → plasma volume ↑↑/Hb↓; dehydratation → PV ↓ / Hb ↑
CAUSES of anaemia

DECREASED or INEFECTIVE MARROW PRODUCTION
- Lack of iron, B_{12} or folate
- Hypoplasia/aplasia of the BM
- BM invasion by malignant cells

PERIPHERAL CAUSES
- Blood loss
- Haemolysis
- Hypersplenism

MORPHOLOGICAL CLASSIFICATION
- A. normocytic/normochromic – normal MCV (80-96 fl)
  - acute blood loss, a. of chronic diseases, renal anaemia, a. sideroblastic
- A. microcytic/hypochromic – low MCV (< 80 fl/MCHC < 32 g/dl)
  - iron deficiency anaemia, thalassemia, a. sideroblastic, etc.
- A. macrocytic – high MCV (> 96 (100) fl)
  - B_{12} or folate deficiency
  - a. autoimmune haemolytic, a. aplastic, MDS, hypothyreosis, etc.
ASYMPTOMATIC state
- slowly falling level of Hb – enhancement of the oxygen-carrying capacity
  - a rise in 2,3-DPG → a shift of the O_2 dissociation curve
  - hemodynamic compensation

SPECIFIC SIGNS of the different types of anaemia
- koilonychia – spoon-shaped nails → iron deficiency
- jaundice – haemolytic anaemia
- …….. will be discussed within the different types of anaemias
NON-SPECIFIC SYMPTOMS and SIGNS

◊ SYMPTOMS
- Lassitude
- Fatigue
- Faintness
- Breathlessness
- Palpitations
- Throbbing in head and ears
- Headaches
- Dizziness
- Tinnitus
- Dimness of vision
- Insomnia
- Angina pectoris
- Intermittent claudication
- Paresthesia in fingers and toes

◊ SIGNS
- Pallor
- skin
- mucous membranes
- palms of hands
- Tachycardia
- Systolic flow murmur
- Cardiac dilatation
- Cardiac failure
- Oedema
- Rarely papilloedema
IRON DEFICIENCY ANAEMIA (IDA) (5)

- IDA
  - one of the most common chronic maladies in humans
  - is the most common cause of anaemia in the world
  - affecting 30% of the population
  - 1/3 – 1/2 healthy females in reproductive age have empty iron stores and ~ 10% have IDA
  - IDA – is the most important cause of a microcytic/hypochromic anaemia
  - All three red cell indices are reduced
    - MCV < 80 fl
    - MCH < 27 pg
    - MCHC < 32 g/dL
  - The blood film shows microcytic, hypochromic red cells
    - the appearance is due to a defect in Hb synthesis
IDA – morfologic changes in PB and the bone marrow
Perl’s reaction in the bone marrow in IDA and SA

IDA

SA

SA – sideroblastic anaemia
<table>
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<tr>
<td>Hb</td>
<td>7.5g/dl</td>
</tr>
<tr>
<td>RBC</td>
<td>4.05x10^{12}/l</td>
</tr>
<tr>
<td>PCV</td>
<td>26%</td>
</tr>
<tr>
<td>MCV</td>
<td>64fl</td>
</tr>
<tr>
<td>MCH</td>
<td>18.5pg</td>
</tr>
<tr>
<td>reticulocytes</td>
<td>2.6%</td>
</tr>
<tr>
<td>WBC differential</td>
<td>normal</td>
</tr>
<tr>
<td>platelets</td>
<td>530x10^{9}/l</td>
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CLINICAL FEATURES

- RE (Mo-Ma) stores i.e. haemosiderin and ferritin are completely depleted, before anaemia occurs!

SYMPTOMS

- **Early stage** – there are usually no clinical symptoms
- **Later** – general symptoms and signs of anaemia (Hb < 90-110 g/l)
  - there is poor correlation between Hb levels and severity of symptoms
  - some patients with marked iron deficiency may deny the common symptoms of fatigue, weakness, or palpitations
  - Symptoms
    - shortness of breath particularly on exercise, weakness, palpitation, headaches, irritability
    - in older subjects angina pectoris, intermittent claudication
    - paresthesias and burning of the tongue
    - pica – craving to eat unusual substances such as clay or ice („classic“)
PHYSICAL EXAMINATION

- Nonspecific signs
  - pallor (skin, mucous membranes)
  - tachycardia, cardiomegaly, systolic flow murmur, congestive heart failure

- Specific signs
  - smooth red tongue (painless glossitis → atrophy of the papillae), angular cheilitis, stomatitis, brittle and spoon nails (koilonychia), dysphagia due to pharyngeal webs (Patterson-Kelly or Plummer-Vinson syndrome), brittle hair, hair loss
  - retinal hemorrhages/exsudates (severe anemia)
  - splenomegaly (occasionally?)

- The cause of the epithelial cell changes (atrofie gastritis) may be related to reduction of iron in iron-containing enzymes
IDA – pallor of mucous membranes (lips) and skin.
IDA – pallor of conjunctival mucosa
IDA – angular cheilosis, fissuring and ulceration at the corners of the mouth

IDA – atrophic glossitis; the bald, fissured appearance of the tongue is due to flattening and loss of papillae
IDA – pallor of palmar skin

IDA – koilonychia
The nails are concave, ridged and brittle
IDA – develops when there is inadequate iron for Hb synthesis – „latent“ iron deficiency – a normal Hb level is maintained

- the most frequent

- menorrhagia (≥ 80 ml/cycle) is a common cause of iron deficiency

- rarely haematuria

- in men and in postmenopausal women
  - esophageal varices, hiatus hernia, peptic ulcer, aspirin or NSA drugs ingestion, carcinoma- of the stomach, colon or rectum, hookworm, angiodysplasia, colitis, piles, diverticulosis, etc.
  - negative iron balance is usual in chronic blood loss despite the increased absorption of food iron

- chronic blood loss

- gastrointestinal (occult) bleeding - the most common cause of iron deficiency

- in men and in postmenopausal women
  - esophageal varices, hiatus hernia, peptic ulcer, aspirin or NSA drugs ingestion, carcinoma- of the stomach, colon or rectum, hookworm, angiodysplasia, colitis, piles, diverticulosis, etc.
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- respiratory tracts – pulmonary haemosiderosis

- phlebotomy for blood donation or laboratory testing

- self – induced autodamage bleeding (genitourinary or GIT)
CAUSES (2)

- **Malabsorption of iron** – decreased iron absorption
  - subtotal/partial gastrectomy – rapid GIT transit, bleeding from anastomotic ulcer
- **increased demands** – growth, pregnancy (iron loss is 900 mg) and lactation (30 mg/month)
- **intravascular hemolysis with Hb-uria**
- **inadequate dietary intake of iron** – „contributory factor“
  - primarily in infants and children
  - unsupplemented milk diet
    - intestinal parasites
    - vegetable diet – background of latent iron deficiency
IRON DEFICIENCY occurs in stages:

- iron depletion: storage iron decreased or absent
- iron deficiency: low serum iron concentration and transferin saturation
- IDA: low Hb level and reduced HTC
- Iron stores
  - ~ 2/3 of the total body iron is in the circulation as Hb (2.5 – 3 g)
  - iron is stored in RES, hepatocytes and skeletal muscle (0.5 – 1.5 g) as ferritin (2/3) and haemosiderin (1/3)
  - haemosiderin – insoluble iron – protein complex in RES \(\rightarrow\) Perl´s reaction

Requirements

- each day 0.5-1.0 mg of iron is lost in the faeces, urine and sweat
- blood loss through menstruation in excess of 100 mL \(\rightarrow\) iron deficiency, ↑ absorption
- the demand for iron increases – during growth and pregnancy
- iron content of the body – remains in normal adult relatively fixed
IRON ABSORPTION

- The average daily diet contains 15-20 mg of iron → normally only 10% is absorbed
  - in iron deficiency/pregnancy → ↑ to 20-30%
  - haem iron (Hb, myoglobin in red meats) is better absorbed than non-haem iron

- Factors influencing iron absorption
  - ferrous iron (Fe\(^{2+}\)) is absorbed better than ferric (Fe\(^{3+}\)) iron
  - gastric acidity – keeps iron in the Fe\(^{2+}\) and soluble in the upper gut
  - ↓ iron absorption – complexes with phytate or phosphate
  - ↑ iron absorption - ↓ iron stores, ↑ erythropoietic activity, e.g. bleeding, etc.
  - ↓ absorption – in iron overload

- Transport in the blood
  - iron is transported bound to transferrin → most iron comes from RES
  - transferrin – bound iron becomes attached by specific receptors to erythroblasts in the BM and the iron is removed
  - in average, 20 mg of iron is incorporated into Hb every day
Iron metabolism

- Most body iron is in Hb in circulating red cells.
- Macrophages store Fe released from Hb as ferritin and haemosiderin.
- Transferrin takes Fe to tissues with TfR → BM.
- Iron is incorporated into Hb.
- Small loss of iron in urine, faeces, skin, and in menstruating females as blood.
- This loss (1-2mg/day) is replaced by iron absorbed from the diet.
IDA – diagnosis (12)

**IDA DIAGNOSIS**
- good clinical history
  - dietary intake, self-medication (NSA), blood in the faeces
  - in women – careful enquiry about the duration of periods, the occurrence of clots and the number of sanitary towels or tampons

**LABORATORY INVESTIGATIONS**
- **Red cell indices and blood film**
  - red cell indices (MCV < 80 fl, MCH < 27 pg) fall even before IDA occurs
  - blood film shows
    - Earliest change is anisocytosis and increased RDW
      - hypochromic/microcytic cells, occasionally target cells and poikilocytes
    - reticulocyte count is low or normal
    - platelet count is raised (~ 50%), particularly when chronic active blood loss continues
- **Bone marrow iron**
  - Iron staining (Perls' stain by Prussian blue) is carried out routinely on all BM aspiration smears
  - IDA – complete absence of iron from stores (macrophages) and absence of siderotic iron granules from erythroblasts (no sideroblasts)
  - The erythroblasts are small with marrow rim of ragged cytoplasm and poor haemoglobin formation
Bone marrow aspirate smears

IDA
• Perls' stain
  - absence of stainable iron in the bone marrow

Sideroblastic anaemia
• gross increase in iron
• multiple ring sideroblasts and increased iron (haemosiderin) in siderophages
Sideroblastic anaemia (primary acquired): bone marrow aspirate showing erythroblasts with complete or nearly complete rings (or collars) of iron granules around their nuclei. The rings are best seen in late erythroblasts but, in severe cases, also occur in the earliest recognizable erythroblasts. Perl’s stain.
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<th>ACD</th>
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<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>S-ferritin</td>
<td>↓ (&lt;20μg/L)</td>
<td>↑ - N</td>
<td>N</td>
</tr>
<tr>
<td>S-TIBC</td>
<td>↑</td>
<td>↓ (N)</td>
<td>↓ - N</td>
</tr>
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<td>↑</td>
<td>N - ↓</td>
<td>↓</td>
</tr>
<tr>
<td>S-sTfR</td>
<td>↑</td>
<td>0 - ↓</td>
<td>↑</td>
</tr>
<tr>
<td>BM-sideroblasts (%)</td>
<td>↓ - 0</td>
<td>N - ↓</td>
<td>0 - ↓</td>
</tr>
<tr>
<td>(iron in erythroblasts)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BM-siderophages (iron in marrow)</td>
<td>0</td>
<td>N - ↑</td>
<td>↓ - 0</td>
</tr>
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<td>Iron absorption test (10-20 mg p.o.)</td>
<td>Positive</td>
<td>Negative</td>
<td></td>
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IDA – Investigations of the cause of IDA (14)

- IDA is established – obligation to determine the site and cause of haemorrhage! (1)
  - Gynaecological examination – previously in premenopausal women
  - GIT examination
    - clinical history of blood loss
    - physical and rectal examination
      - GI-endoscopy/gastroduodenoscopy, colonoscopy and rectoscopy
      - X-rays or MRI of GIT
    - hookworm ova in the stool
    - rarely – a coeliac axis angiogram may be helpful if active bleeding (>0.5 ml/min) (e.g. angiodysplasia)
    - occult multiple blood tests – chemical detection of the halm ring
      - sensitive test – 2-3 ml daily blood loss
    - Cr-labelling of red cells with a 5-day collection of stools is a more accurate method of quantitative assessing foecal blood loss
    - pertechnate uptake studies may detect a Meckel diverticulum
    - exploratory laparotomy if source is not apparent
IDA – Investigations of the cause of IDA (15)

- IDA is established – obligation to determine the site and cause of haemorrhage! (2)

- **Negativity of these tests**
  - intermittent GI-blood loss
  - loss of iron in the urine
    - hematuria or haemosiderinuria (*intravascular haemolysis*)
  - x-ray of the chest and haemosiderin-laden macrophages in sputum – exclusion of idiopathic pulmonary haemosiderosis or intrapulmonary bleeding
  - self-induced haemorrhage (psychiatrically disturbed individuals)
  - a long-lasting poor diet or malabsorption

- **Differential diagnosis**
  - Anaemia of chronic disease
  - Thalassemia
  - Sideroblastic anaemia
IDA – angiogram of coeliac axis showing numerous „blushes“ due to angiodysplasia of the terminal ileum
IDA is not a diagnosis per se

- The correct management of iron deficiency is
  - to find and treat the underlying cause as fast as possible
  - iron therapy to correct the anaemia
  - replace iron body stores

- The response to iron therapy can be monitored using
  - the reticulocyte count peck at 1 to 2 weeks
  - Hb level (expected rise in Hb of 10 g/l per week)
    - Hb level normal at 2-4 months
Oral iron is all that is required in most cases

- The best is oral ferrous sulphate – safest, cheapest (Aktiferin, Sorbifer, Ferronat R etc.)

- Daily total 200 mg elemental iron in 3 doses, each 1h before meals (optimal absorption in fasting patient)

- Do not give with meals or antacids

- ~20% patients may have GIT-intolerance
  - Pyrosis, constipation, diarrhoea, abdominal pain or metallic taste
  - Require: total daily dose reduction change of oral (parenteral?) iron preparation (ferrous genconate) and/or give iron with food
  - The use of expensive iron compounds, which release iron slowly

- Oral iron should be given for a long time, usually 6-12 months after Hb level is normal to replenish iron stores

- Avoid multiple hematotics (a.g. combination iron+ vit. B₁₂ + folic acid)

- Dietary iron sources may not be sufficient for treatment

- Therapy may be needed indefinitely if bleeding continues
IDA – haematological response to ferrous sulphate therapy
IDA – treatment III (18)

- **PROPHYLACTIC** iron therapy
  - throughout pregnancy (in combination with folic acid)
  - patients undergoing regular haemodialysis

- **PARENTERAL** iron therapy
  - routine use rarely justified
  - **Indications are**
    - malabsorption
    - in contraindication of p.o. therapy – severe gastric/intestinal inflammatory
    - intolerance to oral iron preparations
    - need for highen doses that can be given orally
    - patient uncoopertive or unavailable for follow-up
    - need of uncooperative rapidly replenish of body iron
  - **Parenteral iron can be given**
    - as repeated deep intramuscular injections (Ferrum Lek i.m.) of iron-sorbitol (1.5 mg of iron/kg)
    - by slow intravenous infusion of iron-sucrose (Ferrlecit, Ferrum Lek i.v.)
    - be aware of danger of anaphylaxis or other systemic side effects
FAILURE to respond to therapy

- bleeding not controlled
- therapy not long enough to show response
- patient not taking medication
- concomitant deficiencies (vit. B$_{12}$, folate, thyroid)
- concomitant illness
  - inflammation, infection, malignancy, hepatic disease, renal disease

Diagnosis is incorrect: thalassemia, etc.
ANAEMIA OF CHRONIC DISORDERS

5 year, 9 and 10 semester
General Medicine, 2014/2015
ACD – Anaemia of chronic disorders (1)

ACD definition

- **it is a chronic, mild or moderate, mostly asymptomatic anaemia associated with chronic infection, inflammatory, autoimmune or neoplastic disease**
  - it is one of the most common types of anaemia and the most frequent in hospital patient

- **1-2 months** of sustained disease is required for anaemia to develop
  - **chronic infections**, e.g. *pulmonary abscess or pneumonia*, *osteomyelitis, infective endocarditis, tuberculosis*, etc.
  - **chronic inflammatory diseases** such as *Crohn’s disease, ulcerative proctocolitis, sarcoidosis*, etc.
  - **autoimmune diseases** e.g. *rheumatoid arthritis, SLE, polymyalgia rheumatica*, etc.
  - **neoplastic diseases** such as *carcinoma, malignant lymphomas, sarcoma*, etc.
ACD – Anaemia of chronic disorders (2)

- ACD pathogenesis
  - impaired release of iron from BM macrophages leads to a low level of serum iron and consequent low saturation of transferrin – this change occurs early
    - there is a decreased amount of „functional iron“ for developing erythroblasts
  - red cell survival is reduced by 20-30%
  - inadequate endogeneous EPO production in anaemia (absolute deficiency)
  - impaired ability of erythroid precursors to respond to eEPO (relatively deficiency and resistency)

- The exact mechanisms are not clear, but
  - they seem to be mediated by inflammatory cytokines (IL-1, TNF-α, γ, IL-6, etc.)
Multiphactorial etiopatogenesis of ACD

- IL-1α/β
- TNF-α
- TGF-β
- INF-γ
- IL-6

- Neopterin

- NO
  - cytokine (Th1 a Th2)
  - APR
  - Lactoferin → Fe/Mo-Ma

- BFU / CFU
- prol./maturation
- m. expresion TfR

- Acceptability of functional iron for erythropoiesis

- RBC survival (↑ MoMa system)

- ↓ eEPO

- ↑ Fe (metabol.act.) Mo-Ma system (siderophages)
The role of Hepcidin in Fe metabolism

- **Hepcidin** – antimicrobial peptide
  - regulator of Fe metabolism
    - ↑ when tissues are overloaded with Fe
    - regulation of Fe absorption from GIT and its utilisation from Mo Ma for erytropoesis
  - feedback between the level of hepcidin and GIT absorption/tissue deposition of Fe

Diagram:

- Macrophage
  - Iron release
  - Enterocyte
  - Dietary iron absorption
  - Anemia
  - Hypoxia
  - Body iron stores ↓
  - Hepcidin ↓

- Hepatocyte
  - Iron release
  - Enterocyte
  - Dietary iron absorption
  - Body iron stores ↑
  - Hepcidin ↑
  - Inflammation
  - Iron accumulation

- Body iron stores
  - ↓ when tissues are overloaded with Fe
  - ↑ when tissues are underloaded with Fe
ACD – clinical and laboratory features (3)

- ACD – characteristic features
  - Anaemia is usually overshadowed by symptoms of the primary disease
    - anaemia is frequently asymptomatic, or with mild anaemic symptoms and/or signs
    - anaemia severity is related to the severity of the disease
    - anaemia is mild, usually nonprogressive (Hb rarely < 90 g/l)
  - Diagnosis depend on laboratory findings
    - initially normochronic, normocytic anaemia
    - hypochronic, microcytic features develop as disease progresses
    - low serum iron level and somewhat decreased S-transferrin concentration; decreased percent of saturation of transferrin (TIBC is reduced)
    - level of serum ferritin, i.e. an acute phase protein is inappropriately elevated with respect to storage iron, sTfR level is normal
    - bone marrow contain - increased storage iron (↑ siderophages)
      - the percentage of sideroblasts is decreased!
### IDA and ACD – differential diagnosis (4)

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ACHN – Perls’ reaction in the BM smear

- Defected mobilization of iron from Mo-Ma system and its incorporation into erythroblasts
- Defected iron transport in the situation of abundance of tissues' iron store
S - Ferritin

IDA
(n-48)

COMB.
(n-17)

ACD
(n-64)
S – s TfR levels
(s TfR)

95 percentil

IDA   COMB.   ACD
TfR – Ferritin index levels
(TfR / log feritin)

IDA

COMB.

ACD

5.4

3.2

0.8
DIFFERENTIAL DIAGNOSIS

• IDA, renal anaemia, a. aplastic due to carcinoma or lymphoma replacing marrow, drug-induced BM-supression

THERAPY

• successful treatment of the underlying disease
• no treatment may be often necessary
• iron is contraindicated → there i no response to iron therapy despite the low serum iron level
• packed red cell transfusions may be given, if the anemia is symptomatic
• therapeutical correction of the iron, vit. B$_{12}$, folate deficiency or renal failure etc. is necessary

➢ rHuEPO is effective in most cases
  • 100-150 U/kg of rHuEPO three times weekly sc or iv.
  • the more severe anaemia and lower baseline eEPO concentration, the better results
PERNICIOUS ANAEMIA

5 year, 9 and 10 semester
General Medicine, 2013/2014
MA – Pernicious anaemia (I) (1)

MA

- Anaemias in which the erytroblasts in the BM have signs of delayed maturation of the nucleus to that of the cytoplasm
  - characteristic are megaloblastic cells present in the erythroid series as large cells with immature – appearing nuclei but with increasing hemoglobinisation of the cytoplasm → „nuclear – cytoplasmic asynchrony“
    - giant metamyelocytes, giant stab forms and abnormally large megakaryocyte with hypersegmented nuclei are in the BM

- Disorders caused by impaired synthesis of DNA due to
  - mostly the deficiency of vit. B$_{12}$ or folate
  - less commonly
    - metabolic abnormalities of vit B$_{12}$ or folate
    - transcobalamin deficiency
    - anti-folate drugs (e.g. Metotrexate)
    - nitrous oxide (anaesthesia)
    - other defects of DNA synthesis (alcohol, etc.)
Cobalamin deficiency

- The vitamin B₁₂ deficiency is usually due to pernicious anaemia (PA)
- As a result from impaired absorption
- Other causes of vitamin B₁₂ deficiency
  - Nutritional – occurs rarely in vegetarians who avoid dairy products and eggs („vegans“)
  - Malabsorption
    - Gastrectomy syndromes – vitamin B₁₂ deficiency develops within 5-6 years often total or subtotal gastrectomy → loss of IF secretion and failure to absorb cobalamins
    - Zollinger-Ellison syndrome – acid may inactivate pancreatic proteases necessary for cobalamin absorption
    - „Blind-Loop“ syndrome – intestinal stasis – may lead to intestinal colonisation with bacteria which bind B₁₂ before absorption
    - Intestinal causes – jejunal diverticulosis, stricture, chronic tropical sprue, ileal resection, Crohn’s disease, etc.
    - Diphyllobothrium latum infestation – these parasites bind cobalamin and prevent absorption (only 3% of infested people are anaemic)
    - Pancreatic disease – pancreatic proteases are necessary for B₁₂ absorption
    - Congenital lack of IF (Gräsbeck-Immerslund syndrome)
- The deficiency takes at least 2 years to develop, the rate of consumption is 1-2 μg each day
Absorption of $B_{12}$

Diagram showing the absorption process of vitamin $B_{12}$ in the digestive system, including the roles of R-binder, IF, and parietal cells.
# Causes of Megaloblastic Anaemia

## Causes of Megaloblastic Anaemia I

**Vitamin B12 deficiency:**
- Inadequate diet: veganism
- Malabsorption:
  - Gastric: pernicious anaemia: acquired (autoimmune) & congenital
  - Intestinal:
    - partial or total gastrectomy
    - stagnant-loop syndrome, e.g. jejunal diverticulosis, ileocolic fistulae
    - chronic tropical sprue
    - ileal resection & Crohn's disease
    - congenital specific malabsorption with proteinuria (Imerslund-Gräsbeck)
    - fish tapeworm
    - drugs, e.g. metformin

## Causes of Megaloblastic Anaemia II

**Folate deficiency:**
- Inadequate diet:
  - poverty
  - institutions
  - goat's milk
  - special diets
- Malabsorption:
  - gluten-induced enteropathy
  - dermatitis herpetiformis
  - tropical sprue
  - congenital specific
  - Excess losses:
    - dialysis
    - congestive heart failure
  - Increased utilization:
    - pregnancy
    - prematurity
    - excess marrow turnover, e.g. in haemolytic anaemias
  - Drugs:
    - anticonvulsants
    - barbiturates
  - Mixed:
    - alcohol
    - liver disease

## Causes of Megaloblastic Anaemia III

**Abnormalities of:**
- Vitamin B12 metabolism:
  - Congenital: transcobalamin II deficiency
  - homocysteinuria with methylmalonic aciduria
  - Acquired:
    - nitrous oxide anaesthesia
    - etc.
- DNA synthesis:
  - Congenital: orotic aciduria
    - Lesch-Nyhan syndrome
    - dyserythropoietic anaemia
    - thiamine-responsive etc.
- Folate metabolism:
  - Congenital:
    - inborn errors, e.g. 5-methyltetrahydrofolate transferase deficiency
  - Acquired:
    - antifolate drugs, e.g. methotrexate, pyrimethamine
PA – megaloblastic anaemia due to impaired absorption of cobalamin (vit. B$_{12}$) in distal ileum, due to failure of secretion of intrinsic factor by the gastric mucosa

- **disease of older age**, peak of occurrence is 60 years, M/F – 1:1.6
- **autoimmune disease** – destruction of the acid and pepsin – secreting cells of the stomach → atrophic gastritis with achlorhydria, absence of IF secretion in all patients
- **90% show** parietal cell antibody, 50% blocking antibody to IF (↓ binding IF to vit. B$_{12}$)
- **association** with blood group A, blue eyes and early greying, twofold increase of the incidence of gastric cancer
- **there may be association with** myxedema, Hashimoto´s disease, Addison´s disease, etc.
CLINICAL FEATURES of PA

- **Anaemia features** develops slowly, the presenting symptoms are those of severe anaemia, with weakness, palpitation, fatigue, light-headedness and shortness of breath.
- **Many symptomless patients** are diagnosed when a macrocytic anaemia is revealed.
- **The skin** often assumes a lemon-yellow hue because of pallor combined with slight jaundice.
- **The glossitis** (a buffy – red sore tongue), angular stomatitis, loss of weight, thrombopenic purpura, vitiligo may be often present.
- **Vit. B<sub>12</sub> neurologic abnormalities** – may occur in the absence of anaemia and may be irreversible.
  - **Peripheral sensory symmetrical neuropathy**, affects the lower limbs (tingling in feet) previously.
  - **Spastic ataxia** – demyelinization of the posterior and lateral columns of the spinal cord (difficulty in walking).
  - **Optic atrophy** (perversion of vision) or psychiatric symptoms (somnolence, hallucinations, delusions, perversion of taste, dementia or frank psychosis may occur („megaloblastic madness“).
Pernicious anaemia: this 38-year-old male shows premature greying and has blue eyes and vitiligo, three features that are more common in patients with pernicious anaemia than in control subjects.
Megaloblastic anaemia: typical lemon-yellow appearance of a 69-year-old female with pernicious anaemia and severe megaloblastic anaemia (Hb:7.0g/dl; MCV:132fl). The colour is from the combination of pallor, due to anaemia, and jaundice, due to ineffective erythropoiesis.

Megaloblastic anaemia: spontaneous bruising on the thigh of a 34-year-old female who presented with widespread purpura and menorrhagia. She was found to have megaloblastic anaemia due to nutritional folate deficiency and alcoholism. Hb:8.1g/dl; MCV:115fl; platelet count:2 × 10^9/l.
Megaloblastic anaemia: glossitis due to B₁₂ deficiency in a 55-year-old female with untreated pernicious anaemia. The tongue is beefy-red and painful, particularly with hot and acidic foods. An identical appearance occurs in folate deficiency because of impaired DNA synthesis in the mucosal epithelium.

Megaloblastic anaemia: angular cheilosis (same patient as in Fig. 3.5). This is also thought to be due to impaired proliferation of epithelial cells. It is unusual for this abnormality to be so marked.
Pernicious anaemia: marked vitiligo in a 67-year-old male.
Cross section of spinal cord of patient with severe vit. B$_{12}$ neuropathy (subacute combined degeneration of spinal cord) Demyelination of the lateral (pyramidal) and posterior columns.

Section of stomach
- **normal** (left)
- **in PA** (right)
  - atrophy of all coats
  - loss of gastric glands and parietal cells
  - infiltration of the lamina propria by Ly and plasma cells
LABORATORY FINDINGS

- **Anaemia is macrocytic**, with MCV of 100-150 fl or more
  - macrocytes are typically oval in shape (megalocytes)
  - marked aniso-and poikilocytosis, basophilic stippling and Howell-Jolly bodies
  - megaloblasts (erythrocytes with megaloblastic nuclei) may be present
  - the reticulocyte count is low

- **WBC and platelet counts may be reduced** (leucopenia and thrombocytopenia)
  - hypersegmented neutrophils (> 5 lobes)
  - thrombocytes are smaller than usual

- **BM – megaloblastic transformation**
  - erythroid hyperplasia with striking megaloblastic changes (large, immature nuclei, fine chromatin pattern but normal haemoglobinisation)
  - giant and abnormally shaped metamyelocytes are present
  - giant and hypersegmented megakaryocytes
  - treatment with $B_{12}$ or folic acid $> 12$ hours before BM biopsy may mask the megaloblastic changes (response to therapy is helpful in diagnosis)
LABORATORY FINDINGS

- **Serum changes**
  - Elevation – S-Bi (unconjugated), S-Fe, S-ferritin and S-LDH levels
  - S-vit. B\textsubscript{12} value is very low (< 160 μg/L)
  - S-folate levels may be high in PA
  - Methylmalonic aciduria and elevated serum levels of methylmalonic acid or homocysteine are reliable indicators of cobalamin deficiency
  - The Schilling test – vit. B\textsubscript{12} absorption determined by measuring urinary radioactivity after ingestion of an oral dose of radioactive cobalamin (\textsuperscript{57}Co-cyanocobalamin)
    - distinguishing malabsorption from an inadequate diet

- **Other findings**
  - assessing of gastric function (achlorhydria, gastric atrophy, exclusion of carcinoma)
  - antibodies to gastric antigenes (parietal cell antibodies in 90% cases)
## Blood Count in Severe Megaloblastic Anaemia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>5.1 g/dl</td>
</tr>
<tr>
<td>RBC</td>
<td>1.4x10^{12}/l</td>
</tr>
<tr>
<td>PCV</td>
<td>18%</td>
</tr>
<tr>
<td>MCV</td>
<td>129 fl</td>
</tr>
<tr>
<td>MCH</td>
<td>36.4 pg</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>2.5%</td>
</tr>
<tr>
<td>WBC</td>
<td>1.9x10^{9}/l</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>63%</td>
</tr>
<tr>
<td>Platelets</td>
<td>53x10^{9}/l</td>
</tr>
</tbody>
</table>

Typical blood count in severe megaloblastic anaemia
Megaloblastic anaemia: peripheral blood film in a severe case showing a circulating orthochromatic nucleated red cell. The presence of such circulating megaloblasts may be the result of extramedullary haemopoiesis in the spleen and liver.

Megaloblastic anaemia and splenic atrophy: peripheral blood film showing Howell-Jolly bodies (DNA remnants) and Pappenheimer bodies (iron and protein-containing). The patient had severe folate deficiency and splenic atrophy due to adult coeliac disease.
Megaloblastic anaemia: high power views showing (upper left) accumulation of early cells, mainly promegaloblasts; (upper right) megaloblasts at all stages – the nuclei have primitive open (lacy) chromatin patterns despite maturation of the cytoplasm with haemoglobinization (pink staining), and two cells have nuclear (DNA) fragments (Howell–Jolly bodies) in their cytoplasm; (lower left) two late megaloblasts with fully orthochromatic (pink-staining) cytoplasm – two large band-form neutrophils are also present; (lower right) the central orthochromatic cells have karyorrhectic pyknotic nuclei linked by a thin chromatin bridge.
Megaloblastic anaemia: high power views showing a number of giant abnormally shaped metamyelocytes
Megaloblastic anaemia: megakaryocytes of variable maturity. All show nuclei with abnormal open chromatin patterns.
TREATMENT

- Parenteral administration of vit. B₁₂ to replete tissue stores and provide daily requirements
  - A typical saturation schedule consists of 1000 μg of vit. B₁₂ i.m./daily for 10-14 days
    - maintenance therapy – weekly until Hb and HTK are normal, then monthly for life!
  - Patients with neurological abnormalities – should receive 1000 μg every 2 weeks for 6 months, but spinal cord damage is irreversible
  - Toxicity is nil, after vit. B₁₂ application there is often a prompt improvement in the sense of well being.
  - Blood transfusion may be required if the clinical picture requires prompt alleviation of anaemia, but it may cause circulatory overload. If it is essential (anoxia), 1-2 units of packed cells should be given slowly.
  - BM erythropoiesis converts from megaloblastic to normoblastic during ~ 48 hours.
  - Reticulocytosis („reticulocyte crisis“) appears on day 3 to 5, the Hb concentration should become normal within 1-2 months, WBC and Thr count normalize promptly.
  - Elevated S-Bi, S-Fe and S-LDH levels fall rapidly, potassium levels must be monitored → hypokalemia during therapy!
TREATMENT

- Inadequate response
  - Associated iron deficiency, infection or malignancy.
  - Incorrect diagnosis.

- Prophylactic application
  - All patients after total but also after partial gastrectomy and ileal resection.
  - Anaemia of the blind loop syndrome – vit. B\textsubscript{12} and oral antibiotic therapy.

- About 1% of an oral dose of vit. B\textsubscript{12} is absorbed even in absence of IF!
  - The patients with PA can be successfully treated with oral vit. B\textsubscript{12} in doses of 1000 μg/day. Such therapy should be carefully followed to ensure response.
FOLIC ACID DEFICIENCY

- Folate deficiency
  - decreased intake, poor nutrition, old age, poverty, starvation, alcoholism
  - ↑ elimination – hemodialysis
  - impaired absorption – nontropical sprue, etc.
  - increased requirements
    - pregnancy, lactation, increased cell turnover (hemolytic anaemia)
  - antifolate drugs – metotrexate, etc.

- An inadequate diet is the principal cause of folic acid deficiency
  - folic acid reserves are small → deficiency can develop rapidly
  - alcohol – depresses serum folate levels – accelerates MA manifestation
  - cooking causes a loss of 60-90% of the folate
MA – folic acid deficiency (IV) (10)

- CLINICAL FEATURES
  - MA – with laboratory evidence of folic acid-deficiency
  - full response to physiological doses of folic acid
  - patients with folate deficiency may be asymptomatic, neuropathy does not occur

- LABORATORY FEATURES
  - S-folate levels are reduced
  - red cell folic acid levels are reduced in folic acid and vit. B$_{12}$ deficiency on a deficiency diet – folate deficiency develops in about 4 months
  - the hematological findings are those of MA

- THERAPY
  - Folic acid administration of a dose of 1-5 mg/daily, for 4 months
  - Pregnant women should receive 1 mg of folic acid daily
Megaloblastic anaemia: (left) dimorphic peripheral blood film in iron and $B_{12}$ deficiencies following partial gastrectomy. There is a mixed population of microcytic hypochromic cells and well haemoglobinized macrocytes. Hb:8.0g/dl; MCV:87fl;MCH:27pg. In the bone marrow aspirate from the same case (right) giant metamyelocytes are present but megaloblastic changes in the erythroblasts are ‘masked’.
DIFFERENTIAL DIAGNOSIS

- **Acute megaloblastic anaemia**
  - BM megaloblastosis – rapidly developing thrombocytopenia and/or leukopenia, little change in the Hb level after nitrous oxide anaesthesia
    - NO – destroys methylcobalamin
    - administration of folic acid or vit. B₁₂ will accelerate early recovery
  - in patients in ICU, on dialysis or total parenteral nutrition → megaloblastic BM → treatment with parenteral folic acid and vit. B₁₂

- **MA caused by drugs**
  - Metotrexate – inhibition of dihydropholate reductase → treatment with folic acid
  - MA after: trimethoprim, sulfasalazin, azathioprine, acyclovir, AZT (zinovudin), hydroxyurea, phenytoin, etc.

- **MA in childhood**
  - vit. B₁₂ malabsorption in inherited disorder of childhood (Immerslund-Gräsbeck sy) with albuminuria → parenteral vit. B₁₂

- **Transcobalamin II deficiency** → vit. B₁₂ application

- **Megaloblastic changes in**
  - congenital dyserythropoietic anemias, MDS, erytroleukemia, etc.
<table>
<thead>
<tr>
<th>Other Causes of Macrocytosis</th>
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<tbody>
<tr>
<td>Alcohol</td>
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<tr>
<td>Liver disease</td>
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<tr>
<td>Hypothyroidism</td>
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<tr>
<td>Myelodysplasia, including acquired sideroblastic anaemia</td>
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<tr>
<td>Aplastic anaemia &amp; red cell aplasia</td>
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<tr>
<td>Raised reticulocyte count</td>
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<tr>
<td>Hypoxia</td>
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<tr>
<td>Myeloma &amp; other paraproteinaemias</td>
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<tr>
<td>Cytotoxic drugs</td>
</tr>
<tr>
<td>Pregnancy</td>
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</tbody>
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