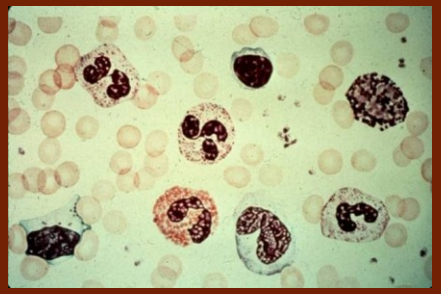
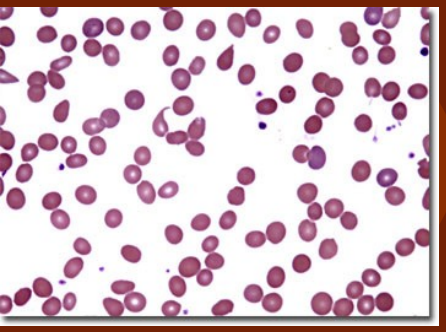
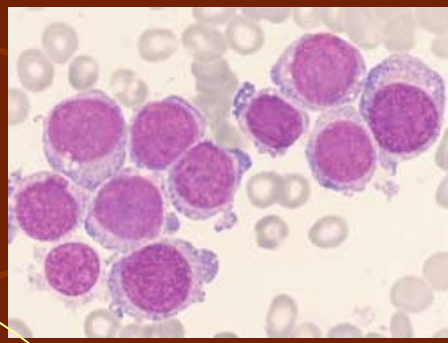
Hematology: Anemias

MUDr. Stanislav Matoušek, PhD





HEMATOLOGY



Anemias and -penias
(not having enough elements)
thrombocytopenia

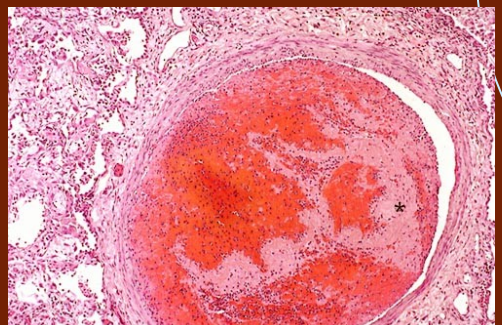
Leukemia etc.
(oncology – lympho- and myeloproliferative disorders)
E.g. chronic myeloid leukemia

Disorders of blood clotting

Primary hemostasis

Secondary hemostasis

Bleeding disorders/ thrombo-embolism



Clinical symptoms of hematologic disease

- Anemia
  - → signs of hypoxia – tiredness, weakness, dyspnea
  - → signs of low levels of hemoglobin - paleness
  - → cardiovascular symptoms – palpitation

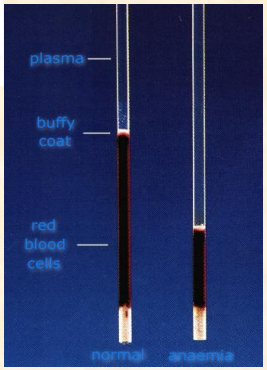
- Polycytemia → hyperviscose blood → risk of thrombosis

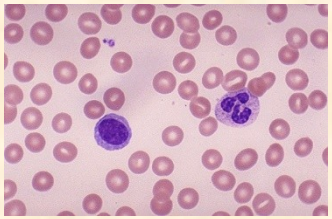
- Bleeding, spontaneous bleeding, unceasing bleeding

- Thrombosis → embolism – local symptoms of swelling or ischemia – DVT -, pulmonary embolism
- Frequent infections

Anemia

- Hb
  - M: < 135 g/L
  - F: < 120 g/L
- Hct
  - M: < 40 %
  - F: < 37 %
- Ery
  - M: < 4,3 * 10^{12} /L
  - F: < 3,9 * 10^{12} /L





Principal criterion: Hb< 120 g/L in w or < 135 in m

Pathophysiology of anemia symptoms

low hemoglobin concentration → paleness

↓

Deficient oxygen delivery into tissues

↓

Tissue hypoxia → sympaticus activated

↓ ↓

weakness,dyspnea

palpitations ← hyperkinetic circulation

Causes of hypoxia

- Altitude hypoxia – lack of O_2 in the inspired air = low pO_2
- Respiratory insufficiency– hypoxic hypoxia
- Lack of hemoglobin – transport hypoxia = anemia
- Circulatory disturbance – circulatory hypoxia
- Impaired oxidation in mitochondria – histotoxic hypoxia

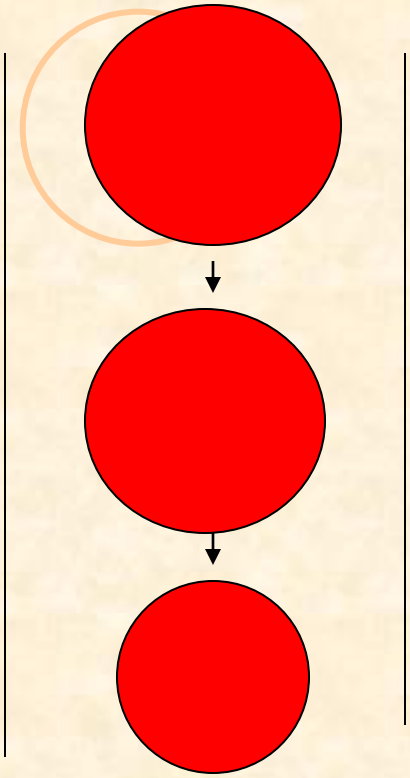
Laboratory tests:

- Principal:
  - Complete (full) blood count (CBC or FBC)
- Complementary:
  - Tests of iron metabolism
  - Erythropoietin levels
  - Detecting antibodies against RBC – Coombs test = antiglobuline test AGT
  - Osmotic fragility test
  - Historically: Ham's test (resistance in acidic environment)
  - Blood film/smear microscopic examination
  - **Bone marrow cytology/ aspiration (Sternal puncture)**

Anemias classified by RBC morfology (CBC)

- by MCV
  - microcytic - e.g. iron deficiency
  - normocytic - e.g. acute bleeding
  - macrocytic (megaloblastic) - pernicious
- by MCHC (color)
  - hypochromic – lack of iron
  - normochromic

BONE MARROW



Stem cells, growth factors
erythropoietin:

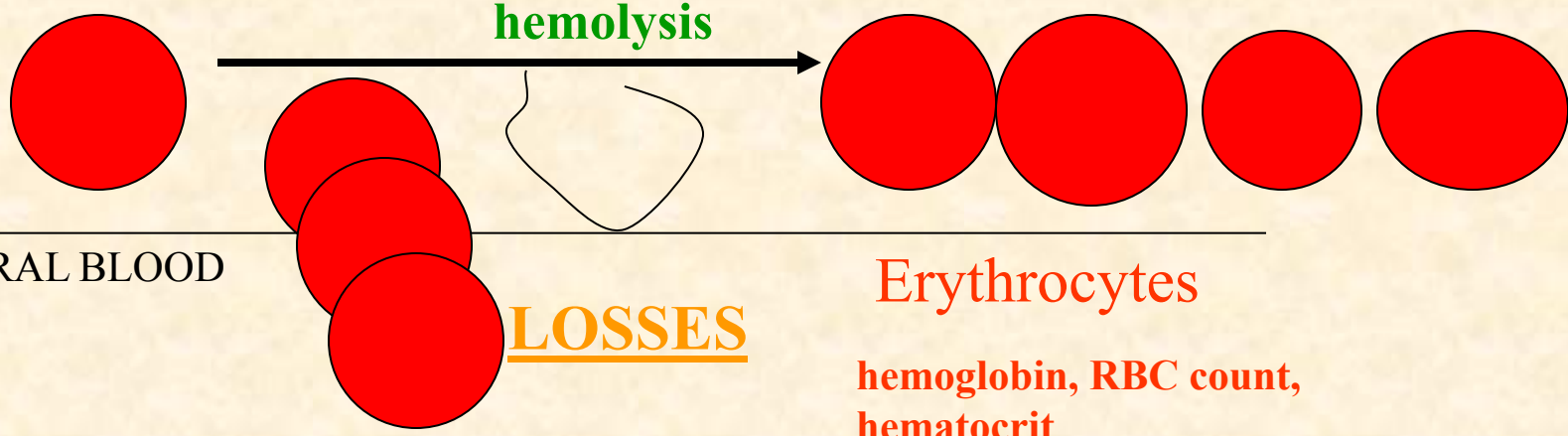
Cellular division, maturation:
DNA synthesis: vitamin B_{12}, folic acid

Hemoglobin synthesis: globin, porphyrine, Fe

Other factors

PRODUCTION

hemolysis



PERIPHERAL BLOOD

LOSSES

bleeding

Erythrocytes

hemoglobin, RBC count,
hematocrit
MCV, MCH, MCHC
shape etc.

Anemias by their etiology/patho

- **decreased production**
  - Stem cell failure or failure to differentiate
  - disorder in DNA synthesis
  - Disorder in hemoglobin synthesis
  - Lack of erytropoetin / renal failure
    - Complete loss of erythropoiesis – decrease of RBC count 10% / wk
- **increased destruction - hemolysis**
  - Defect of erythrocytes
  - Causes outside of RBC
- **increased loss - bleeding**
- **misdistribution and loss (hyperslenism, pooling in spleen)**

Reticulocyte count

- Daily replenishment rate
  - 0.5 – 1.5% of total RBC count
  - Mature during the 1st day in peripheral blood
- Criterion of bone marrow activity –
- Key test in distinguishing anemias
  - Reticulocytosis
    - Reaction of the BM to a blood loss (hemolytic anemias, severe bleeding)
    - Response to a correct anemia therapy (e.g. defic. B12 or Fe)
  - Reticulocytopenia
    - Defective erythropoiesis

Blood loss anemia

- **Acute** blood loss
  - shortly after massive blood loss Hb normal due to vasoconstriction
  - normochromic - normocytic
- **Chronic** blood loss
  - results in iron deficiency
- Excessive hemolysis (RBC destruction)

Excessive hemolysis (RBC destruction)

reticulocytosis, LDH is increased, unconjugated bilirubin accumulates

Extrinsic RBC defect (normocytic-normochromic RBC)

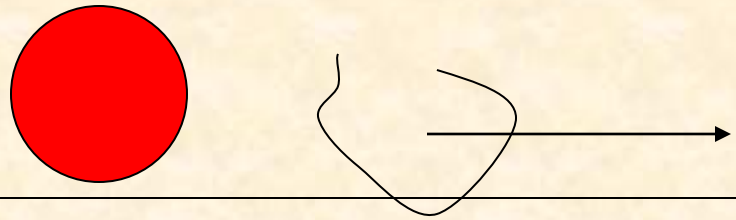
- Immunologic abnormalities (AIHA, PNH)
- Mechanical injury (trauma, infection)

Intrinsic RBC defect

- Membrane alterations
  - congenital (spherocytosis, elliptocytosis)
  - Aquired (hypophosphatemia)
- Metabolic disorders (G6PD deficiency)
- Hemoglobinopaties (Sicle cell disease, Thalassemia)

HEMOLYSIS

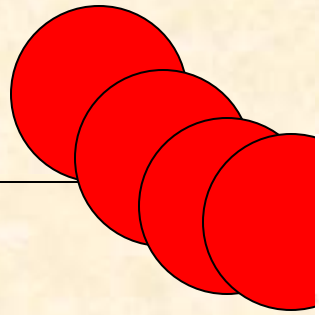
INTRAVASCULAR

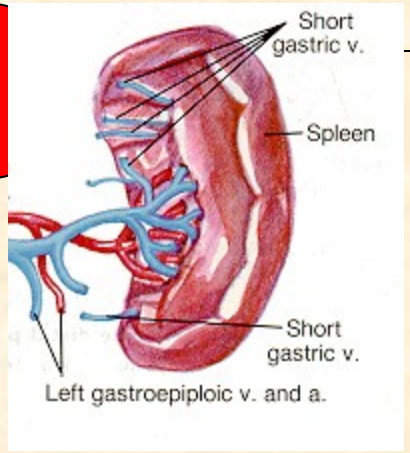


Hb

haptoglobin
kidneys

EXTRAVASCULAR





spleen, bone marrow, liver
(macrophages)

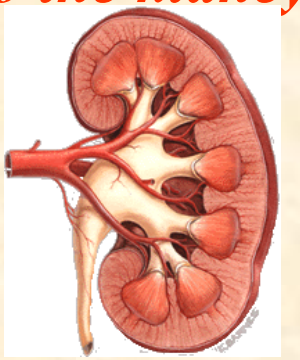
SYMPTOMS OF HEMOLYSIS

loss of red blood cells → loose Hb

intravascular

hemoglobinemia,
hemoglobinuria
hemosiderinuria

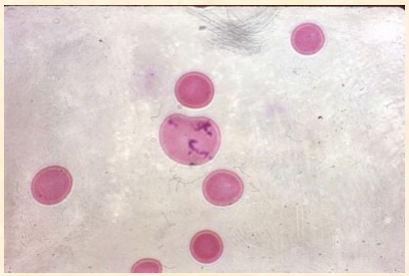
damage to the kidneys



anemia

BM activation

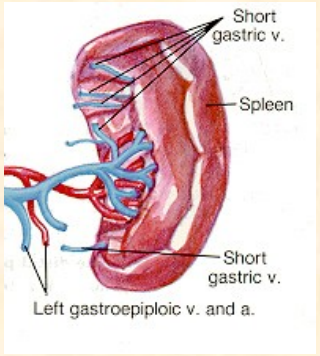
reticulocytosis



extravascular

increased prodution of ***bilirubin***
jaundice (icterus)

splenomegaly



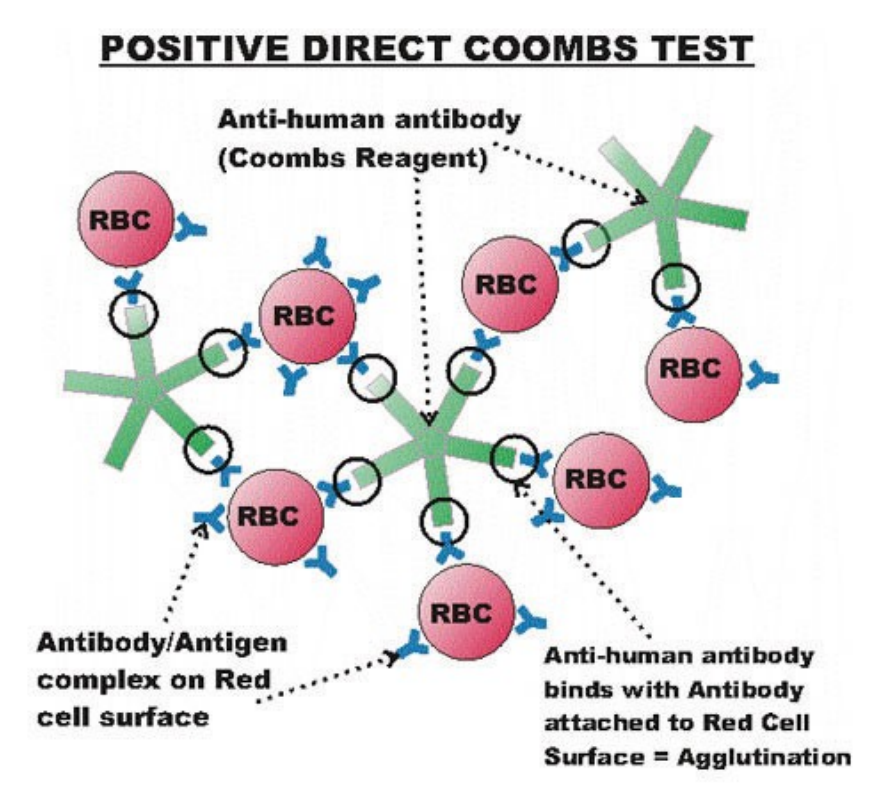
TESTS FOR HEMOLYSIS

immune mechanisms – direct Coombs (antiglobulin) test

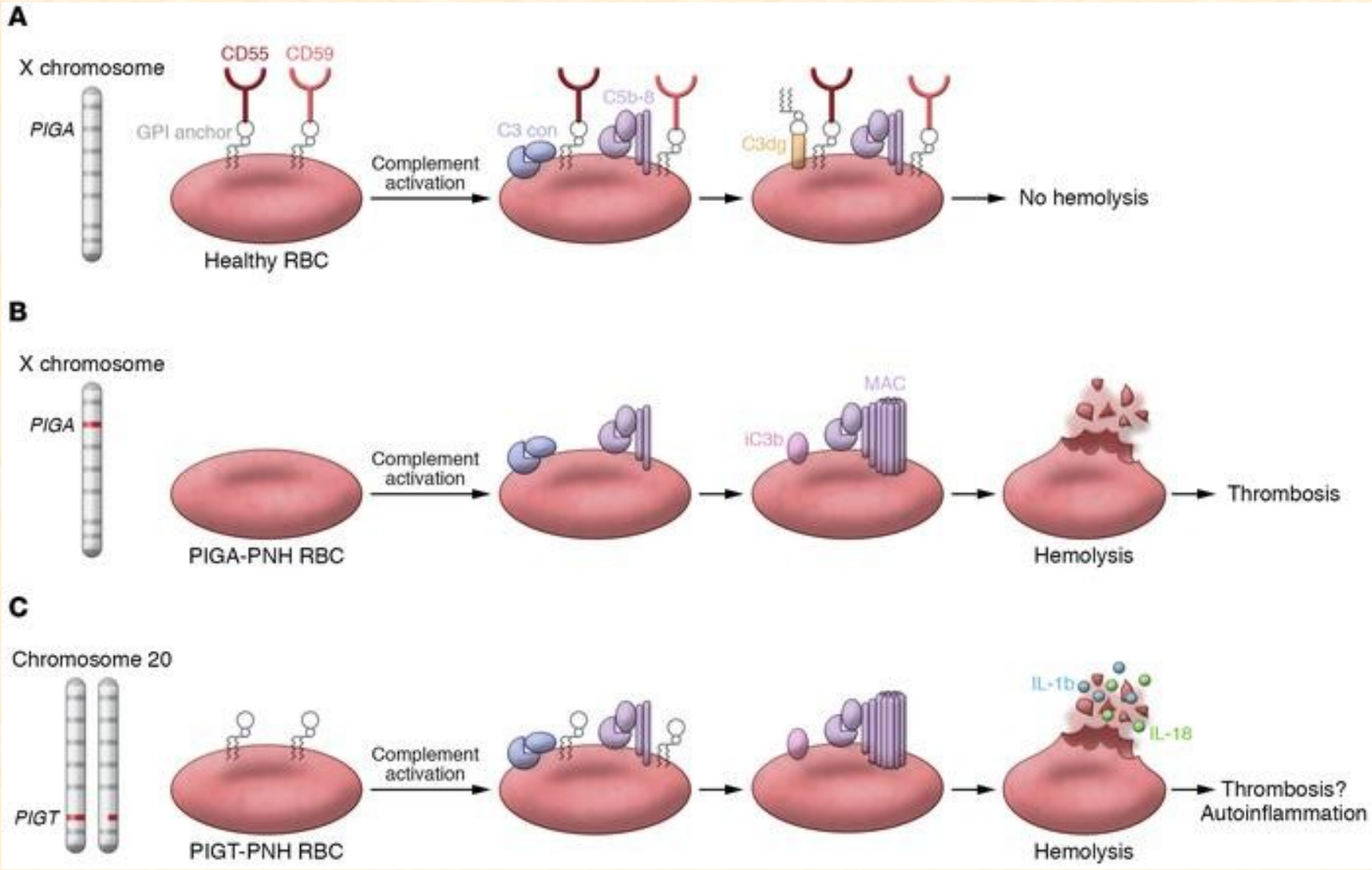
Search for antibodies against proper RBC

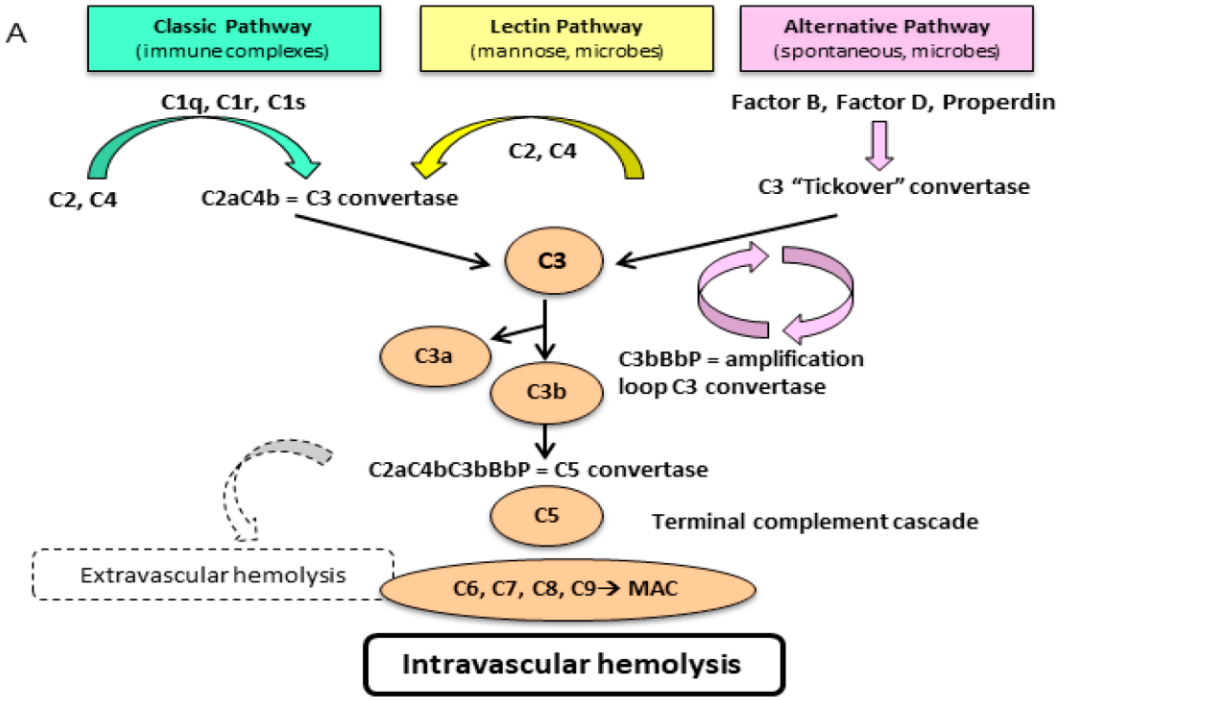
Antibodies other than AB0

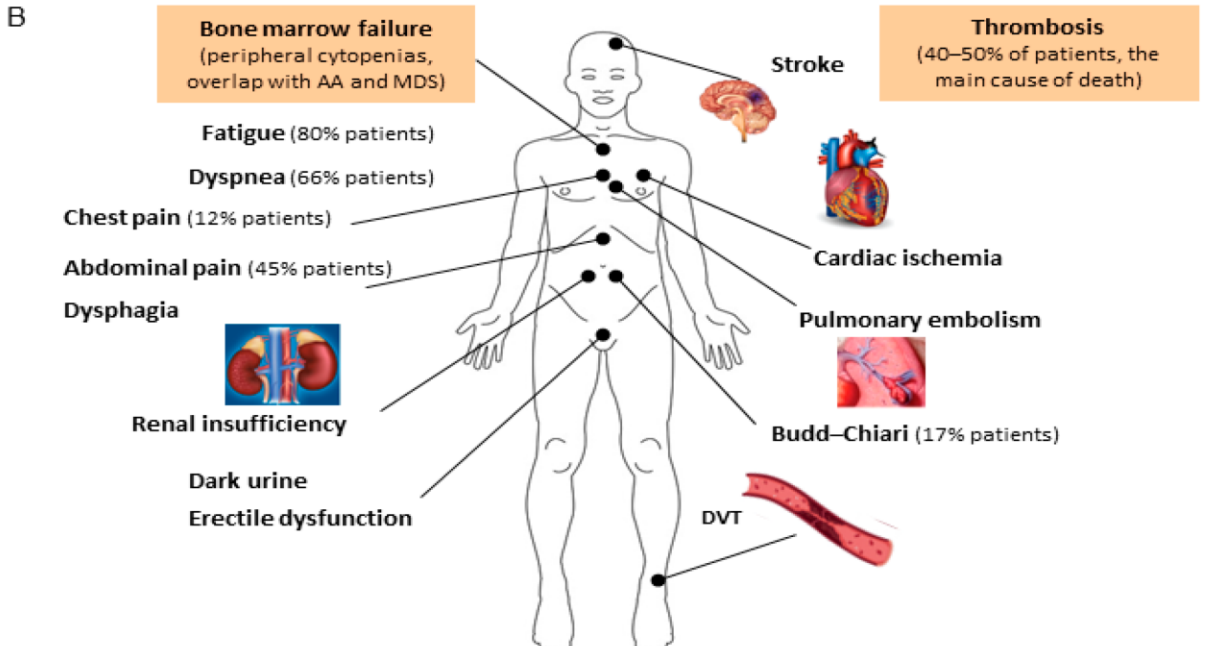
These Abs are responsible for hemolysis



Paroxysmal nocturnal hemoglobinuria







Paroxismal Nocturnal Hemoglobin-uria

From: Fattizzo B, Serpenti F, Giannotta JA, Barcellini W. Difficult Cases of Paroxysmal Nocturnal Hemoglobinuria: Diagnosis and Therapeutic Novelties. *J Clin Med*. 2021;10(5):948. Published 2021 Mar 1. doi:10.3390/jcm10050948

Direct antiglobulin (Coombs') test (DAT)

- Detection of antibodies to erythrocyte surface antigens
- AIHA
- Antiglobulin serum is added to washed RBC from the patient ------ agglutination indicates presence of immunoglobulins or complement components bound to RBC

TESTS FOR HEMOLYSIS

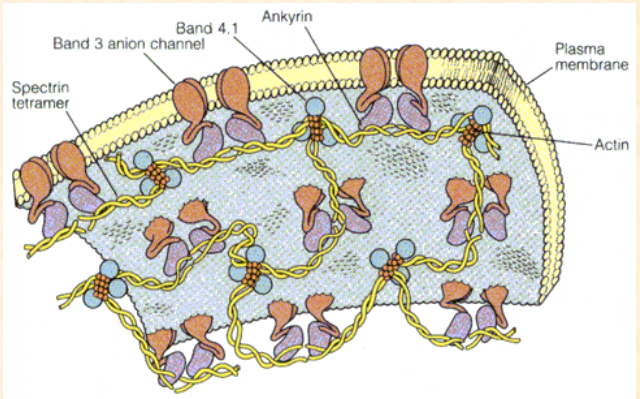
Test of osmotic resistence

RBC survive only in isotonic surrounding but have some toleration to its changes

RBC in some hemolytic states have decreased tolerance

Special tests

membrane properties (electrophoresis of proteins)
properties of hemoglobin
genetic tests



Deficient erythropoiesis

- **Iron deficiency**
  - microcytic-anisocytosis, ↓ reticulocytes
- **Vitamin B_{12} or Folate deficiency**
  - macrocytes-anisocytosis
- **BM failure -** chronic diseases, aplastic anemia, myelodysplasia, leukemia
  - normochromatosis-normocytosis
  - BM hypoplasia

Tests for iron

- iron concentration in serum (age , sex)
- TIBC (total iron binding capacity for Fe)
- transferrin saturation (N 20-55 %)
- serum ferritin
- serum (solubile) transferrin receptor (sTfR)

Tests of iron metabolism

Serum iron (SI)

- F: 600-1400 μg/L, 11-25μmol/L; M: 750-1500 μg/L, 13-27μmol/L
- Low in Fe deficiency and chronic disease
- High in hemolytic syndromes and iron overload

Total iron binding capacity (TIBC)

- 2500 – 4500 μg/L , 45-82 μmol/L
- High in Fe deficiency
- Low in chronic disease

Serum ferritin (30-300 ng/mL)

- Fe storage glycoprotein
- Closely correlates with total body Fe stores
- <12 ng/mL Fe deficiency
- Elevated in Fe overload, liver injury, tumors (Acute phase protein)

Tests for iron metabolism

Serum transferin receptor

- Increase in increased erythropoiesis and early Fe deficiency

RBC ferritin

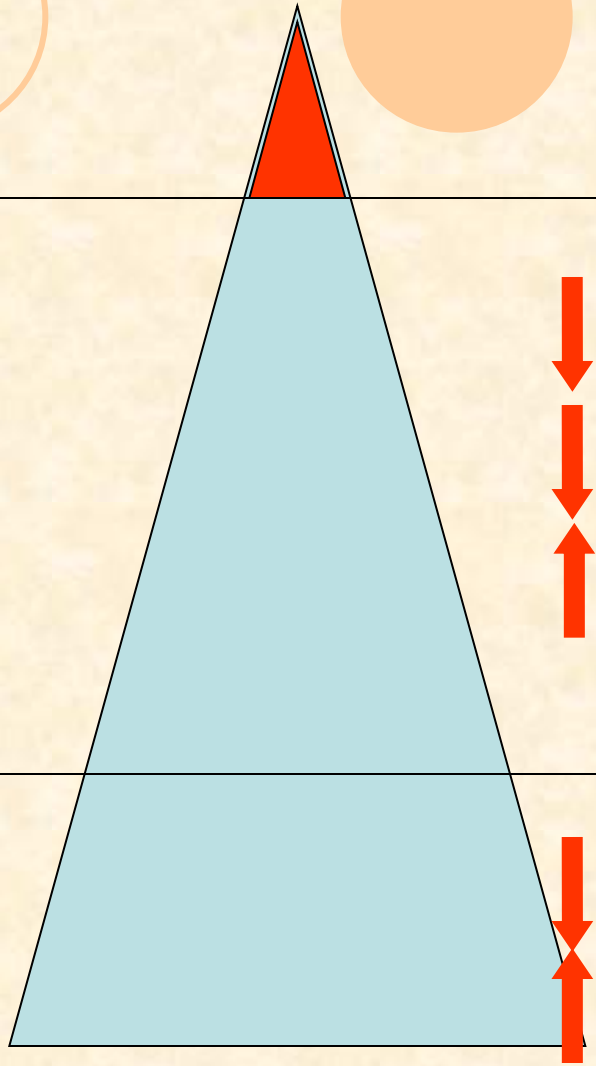
- storage status over the previous 3 month (Fe deficiency/overload)
- unaffected by liver function or acute illness

Free RBC porphyrin

- increased when heme synthesis altered

Manifest

anemia



Latent

iron deficiency erythropoiesis

serum iron

transferin satur.

transferin

Prelatent

no stores

serum ferritin

TIBC ~ transferin

iron in BM

Regulation of iron

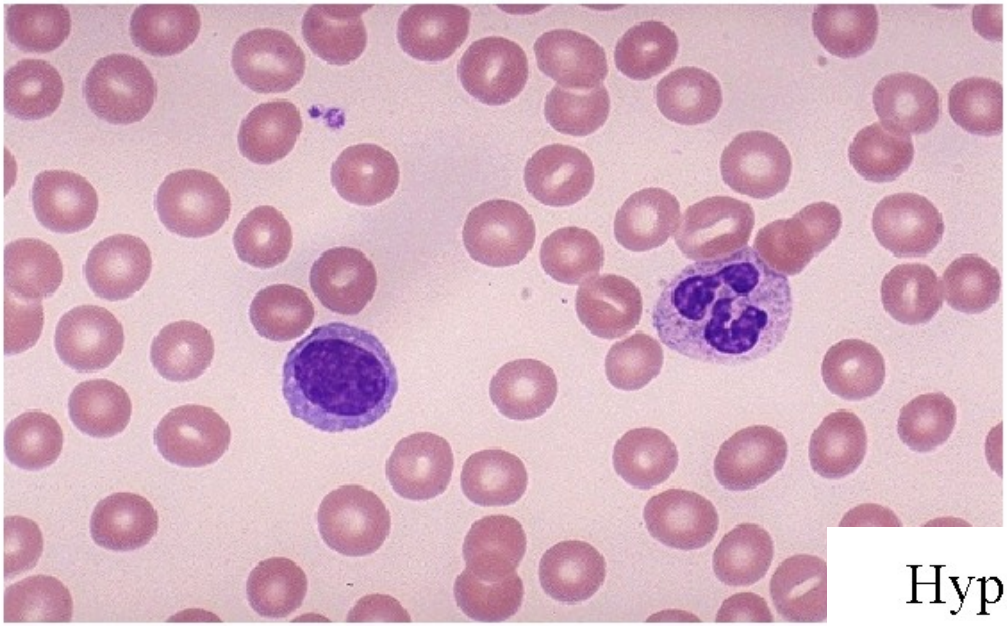
- Hepcidin – signal molecule – keep your iron inside the cell! - anemia of chronic disease

Microcytic Hypochromic Anemia (MCV<83; MCHC<31)

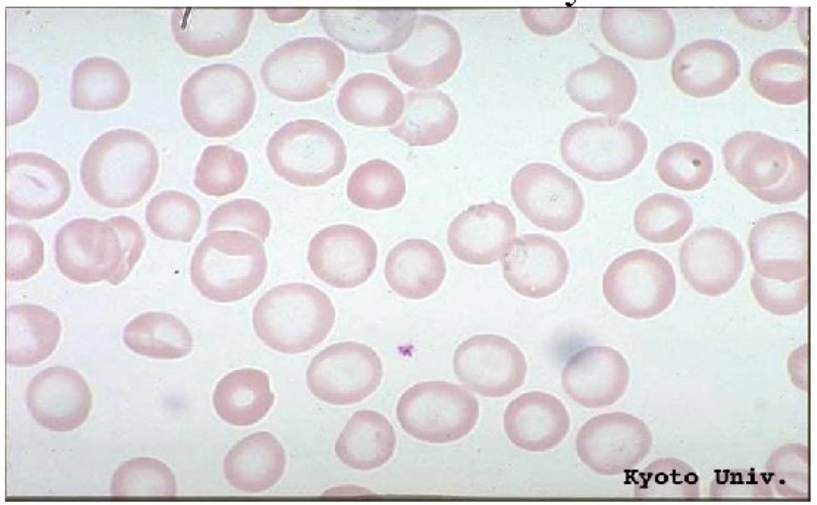
Iron deficiency	Responsive to iron therapy
Chronic inflammation	Unresponsive to iron therapy
Thalassemia major	Reticulocytosis and indirect bilirubinemia
Thalassemia minor	Elevation of fetal hemoglobin, target cells, and poikilocytosis

Lead poisoning	Basophilic stippling of RBCs
Sideroblastic	Ring sideroblasts in marrow
Hemoglobinopaties	Hemoglobin electrophoresis

Normal Smear



Hypochromic/Microcytic Anemia
Iron Deficiency



Microcytic Hypochromic Anemia (MCV<83; MCHC<31)

	Serum Iron	Total Iron-Binding Capacity (TIBC)	Bone Marrow Iron	Comment
Lead poisoning	N	N	++	Basophilic stippling of RBCs
Sideroblastic	⬆	N	++++	Ring sideroblasts in marrow
Hemoglobinopaties	N	N	++	Hemoglobin electrophoresis

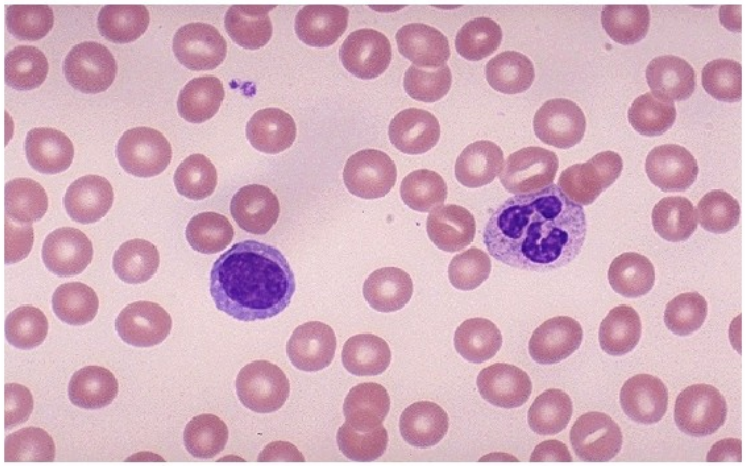
Microcytic Hypochromic Anemia (MCV<83; MCHC<31)

	Serum Iron	Total Iron-Binding Capacity (TIBC)	Bone Marrow Iron	Comment
Iron deficiency	⬇	⬆	0	Responsive to iron therapy
Chronic inflammation	⬇	⬇	++	Unresponsive to iron therapy
Thalassemia major	⬆	N	++++	Reticulocytosis and indirect bilirubinemia
Thalassemia minor	N	N	++	Elevation of A of fetal hemoglobin, target cells, and poikilocytosis

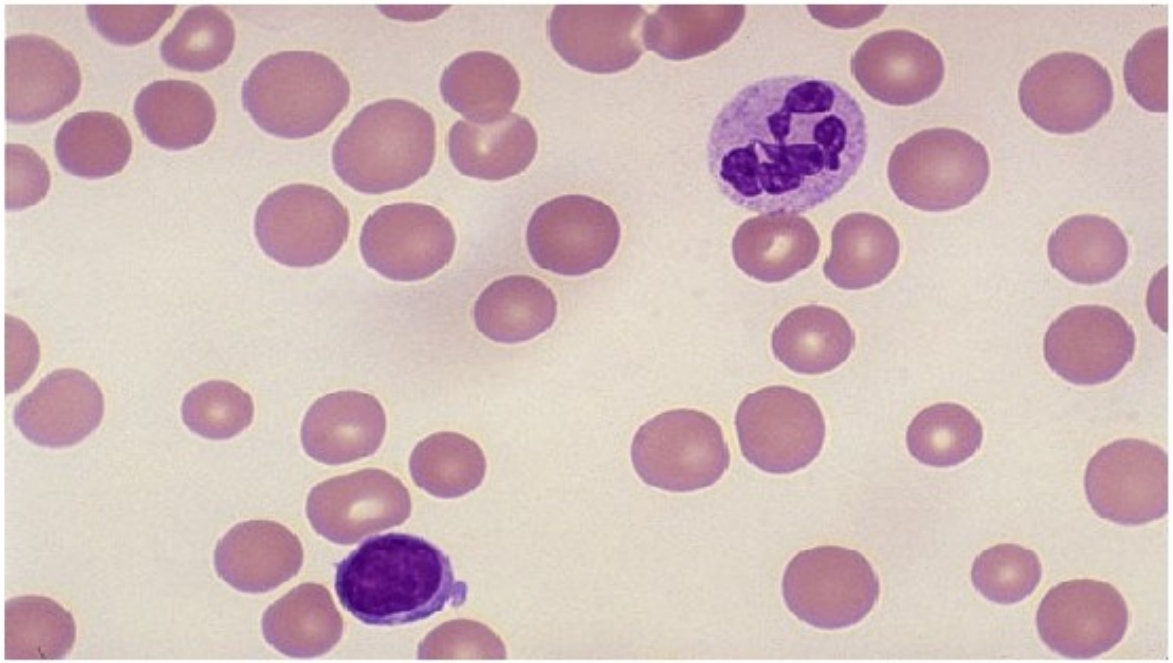
Macrocytic Anemia (MCV, >95)

Megaloblastic bone marrow	Deficiency of vitamin B-12
	Deficiency of folic acid
	Drugs affecting DNA synthesis
	Inherited disorders of DNA synthesis
Nonmegaloblastic bone marrow	Liver disease
	Hypothyroidism and hypopituitarism
	Accelerated erythropoiesis (reticulocytes)
	Hypoplastic and aplastic anemia
	Infiltrated bone marrow

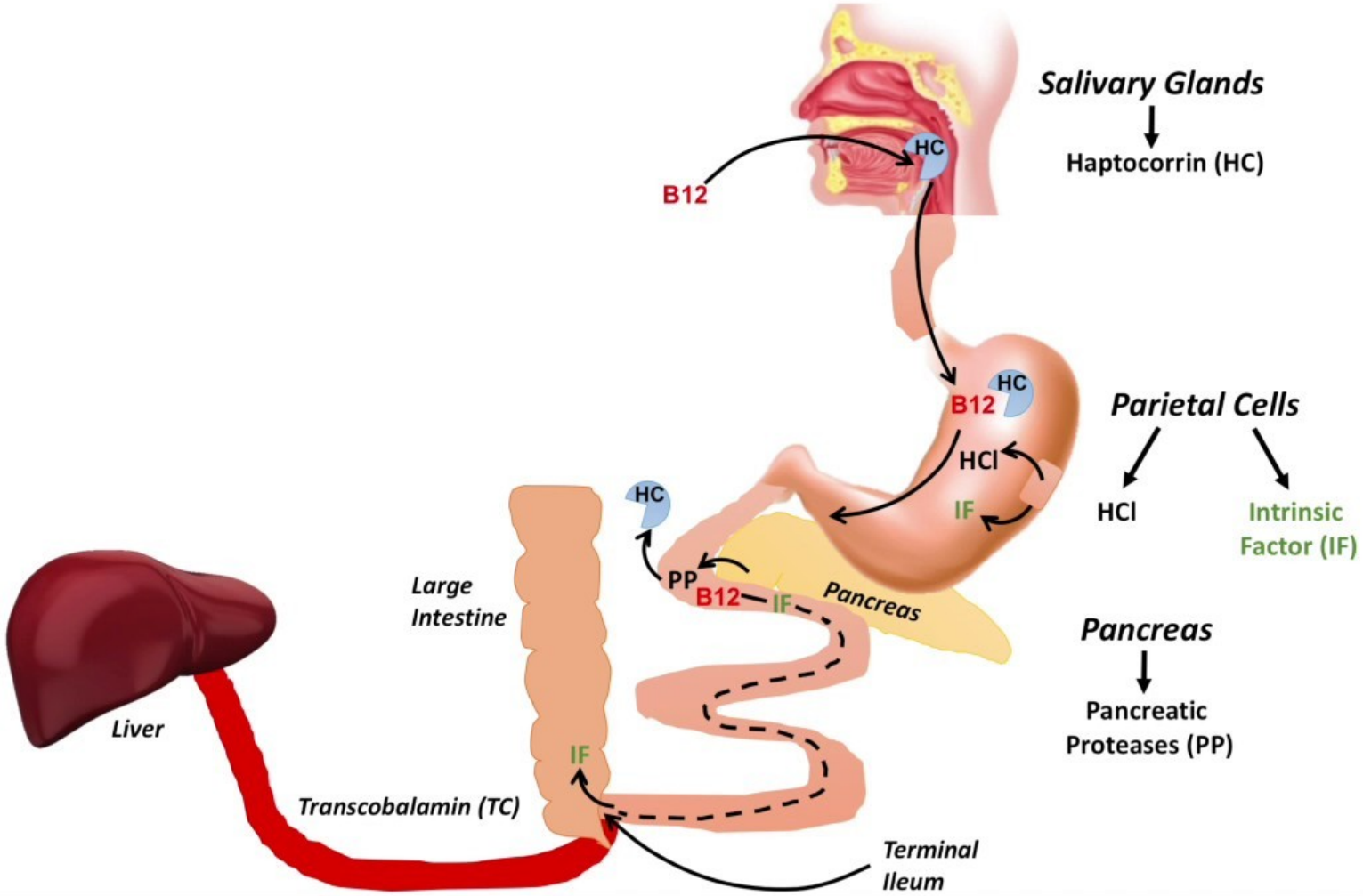
Normal Smear



Megaloblastic Anemia
B-12 Deficiency



Absorption of vit. B12



Lack of vit. B12 - causes

- Not enough ingestion – strict vegans if they do not take care
- Autoimmune inflammation of gastric mucosa (atrophic gastritis) leading to deficiency in intrinsic factor
- Diseases of terminal ileum (celiac disease, Crohn disease)

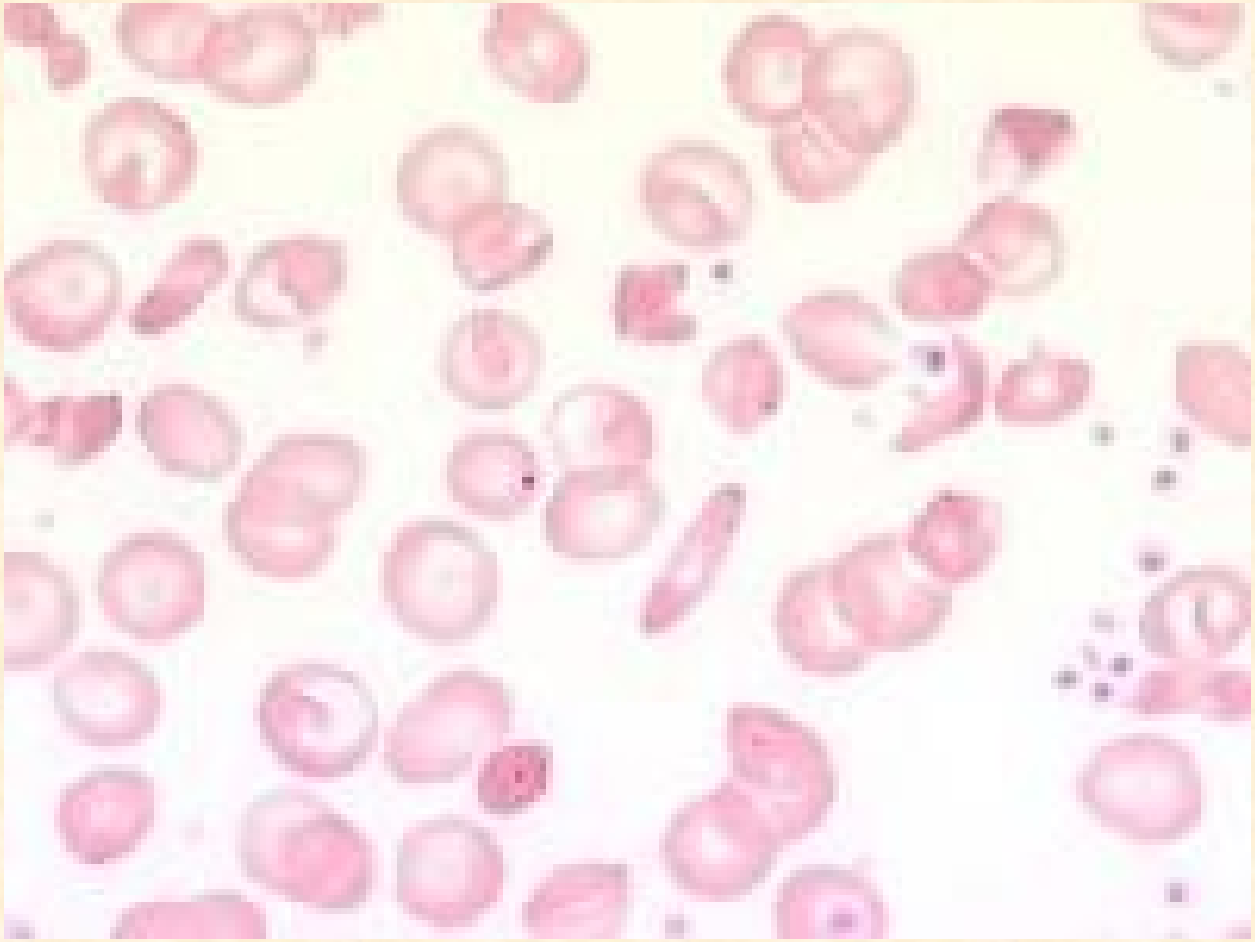
Blood smear

- **Morphology** of blood elements
  - Anisocytosis = variation in size
  - Poikilocytosis = variation in shape (schistocytes=RBC fragments; ovalocytes; spherocytes)

Various Forms of RBCs

Spherocyte	Loss of central pallor, stains more densely, often microcytic. Hereditary spherocytosis and certain acquired hemolytic anemias.
Target cell	Hypochromic with central "target" of hemoglobin. Liver disease, thalassemia, hemoglobin D, postsplenectomy.
Elliptocyte	Oval to cigar shaped. Hereditary elliptocytosis, certain anemias (particularly vitamin B-12 and folate deficiency).
Schistocyte	Fragmented helmet- or triangular-shaped RBCs. Microangiopathic anemia, artificial heart values, uremia, malignant hypertension.
Stomatocyte	Slitlike area of central pallor in erythrocyte. Liver disease, acute alcoholism, malignancies, hereditary stomatocytosis, and artifact.
Sickle cell	Elongated cell with pointed ends. Hemoglobin S and certain types of hemoglobin C and I.

Sicle Cell Disease



When to do CBC?

- **suspected** hematologic, inflammatory, neoplastic, or infectious disease
- **screening** of infants (<1 yr.), pregnant women, elderly patients, and patients with nutritional abnormalities
- routine patient evaluation, admission to hospital