I. PATHOPHYSIOLOGY OF ANEMIAS

Definition:
Anemia is defined as a low Hb level (< 13 g Hb/dL in men and < 12 g Hb/dL in women), decreased number of circulating RBC (< 4.4 millions/mm³ in men and < 3.8 millions/mm³ in women), or both, resulting in diminished oxygen-carrying capacity of the blood.

General manifestations:
Are due to: i) decreased Hb concentration (pallor of skin, conjunctiva and nail beds), ii) tissue hypoxia due to deficient oxygen transport (weakness, easy fatigability, exertional dyspnea), and iii) recruitment of compensatory mechanisms (cardio-circulatory, biochemical and medullar) aimed at increasing cardiac output and oxygen delivery to the tissues (Fig. 1).

![Figure 1. Compensatory changes and complications in anemia.](Modified after Huether & McCance: Understanding Pathophysiology, 5th ed)
Classification:

**Morphologic classification** (according Hb, Ht red cell indices and the peripheral blood smear) subdivides anemias into:

1. **Microcytic hypochromic anemias**
2. **Macrocytic normochromic anemias**
3. **Normocytic normochromic anemias**

**Functional classification** (according the reticulocyte count) comprises:

1. **Regenerative anemias** (> 3%)
2. **Aregenerative anemais** (< 3%)

These 2 classifications are addressed within the labs.

**Etiopathogenic classification:**
Anemias may result from:

A. **Decreased red cell production** (decreased erythropoiesis)
B. **Increased red cell destruction** (increased hemolysis)

**A. ANEMIAS DUE TO DECREASED ERYTHROPOIESIS**

Decreased erythropoiesis is due to:

A. **Nutrients' deficiency:**
   1. Deficiencies in **hemoglobin synthesis:**
      a. Iron deficiency anemia
      b. Sideroblastic anemia
   2. Deficiencies in **DNA synthesis:**
      a. Vit. B12 deficiency
      b. Folate deficiency

B. **Bone marrow failure:**
   1. Pluripotential stem cell failure:
      a. Aplastic anemia
      b. Anemia of leukemia and of myelodysplastic syndromes
   2. Erythroid progenitor cell failure:
      a. Anemia from chronic disease/inflammation
      b. Anemia from chronic kidney disease

1. **Iron Deficiency Anemia**

Definition: the most common form of anemia worldwide characterized by microcytosis, hypochromia, decreased iron stores (low ferritin) and low serum iron.

**Iron metabolism:**

Iron balance depends on adequate intake, absorption, recycling and loss. The total body iron content is about 3.5 g in men and 2.5 g in women and is divided into functional and storage compartments. Functional iron (80%) is found in hemoglobin, myoglobin and iron-containing enzymes whereas the storage compartment is represented by ferritin (rapid available iron, a combination of iron and apoferritin), and hemosiderin. Hepatocytes are the main site of ferritin storage and minute quantities are present in plasma in equilibrium with the intracellular ferritin. Hemosiderin is mainly stored in the macrophage-monocyte system as aggregates or crystals of ferritin with the apoferritin partially removed.

**Intake.** Dietary iron (10-20 mg/d) is present as heme- in animal products and non-heme iron in vegetables, respectively.
**Absorption.** Iron is primarily absorbed in the proximal duodenum as Fe$^{2+}$ (ferrous iron), being transported across the apical membrane by the divalent metal transporter 1 (DMT1). A low pH of the chyme is essential for absorption. Plasma transfer of iron from enterocytes to the plasma occurs through specific iron channels called ferroportins at the basolateral membrane, and is coupled to the oxidation of Fe$^{2+}$ to Fe$^{3+}$ (ferric iron), by a protein (with ferroxidase activity) called hephaestin. Iron is transported in blood bound to its carrier protein, transferrin (Fig. 2).

The ‘master regulator’ of iron metabolism is a peptide of hepatic origin, hepcidin that binds to the iron channels, ferroportins (Fpn) in hepatocytes, enterocytes and macrophages. By inhibiting Fpn, hepcidin prevents these cells from secreting iron into the plasma, thus reducing iron availability for erythropoiesis. Hepcidin synthesis is suppressed by erythropoietic activity, ensuring a sufficient supply of iron to the bone marrow when demand for erythrocytes is high. During inflammation, hepcidin production is stimulated and thus iron entry into plasma is inhibited, causing the hypoferremia and anemia of inflammation (Fig. 3).
**Loss.** Only about 1 - 2 mg of iron is lost from the body per day (via the shedding of mucosal and epithelial cells) and must be replaced through diet. Importantly, iron released from the physiological hemolysis within the macrophages of the reticulo-endothelial system is almost completely recycled (>95%), thus being the major source for daily Hb synthesis.

**Etiology:**
Iron deficiency can result from:

1. **Chronic blood loss from gastrointestinal** (inflammatory bowel disease and malignancy in men/postmenopausal women) and **genitourinary tract** (increased menstrual blood loss in premenopausal women, the population with the highest incidence) or **is the major cause** worldwide.
2. **Decreased iron recycling in chronic disease and inflammation** (see mechanisms below) **is the second common cause.**
3. **Dietary deficiency** (malnutrition in the impoverished or elderly in developing countries, teenagers subsisting on “junk” food), **impaired absorption** (postgastrectomy, iron-binding food such as tannates, oxalates, carbonates) or **increased demands** (in infants, adolescents, women with multiple, closely spaced pregnancies) can also occur.

**Pathogenesis:**
Iron deficiency occurs in 3 stages:

1. **Iron-stores depletion** characterized by:
   - decreased serum ferritin < 20 μg/L and iron stores in macrophages (assessed by Prussian blue stain on smear of aspirated marrow)
   - increased plasma transferrin levels
   - normal serum iron, Hb, Ht, RBC
2. **Iron-deficient erythropoiesis** characterized by:
   - depletion of iron stores (disappearance of stainable iron from macrophages in the bone marrow)
   - decreased serum iron < 60 μg/dL
   - increased plasma transferrin
   - transferrin saturation < 20%
   - normal Hb, Ht, RBC
   or Hb = 10 -12 g/dL (normochromic normocytic anemia)
3. **Iron-deficiency anemia** characterized by:
   - decreased serum iron < 30 μg/dL
   - transferrin saturation < 15%
   - Hb < 10 g/dL (hypochromic microcytic anemia)
   ± clinical overt anemia

**Clinical manifestations:**

*Anemia develops insidiously* and remarkably low levels of hemoglobin can be tolerated with minimal symptoms. In addition to general manifestations (common to all anemias), patients with iron deficiency anemia might present: **brittle hair, nail changes** (brittle, koilonychia), **glossitis, angular stomatitis, pica** (appetite for bizarre substances e.g. ice, flour, clay).

Diagnosis usually rests on the above mentioned laboratory tests. In uncomplicated disease, oral supplementation with iron leads in 5 to 7 days to an increase in reticulocytes.

2. **Sideroblastic Anemia**

Definition: hypochromic, microcytic anemia with **increased iron stores** (high ferritin) and **elevated serum iron.**
Pathogenesis: alteration of hem synthesis and/or vitamin B6 (pyridoxine) deficiency

Etiology:
I. Primary sideroblastic anemia (hereditary)
   - rare, genetically induced by the deficit/absence of enzymes involved in hem synthesis

II. Secondary sideroblastic anemia (acquired)
   - more frequent, induced by the inhibition of enzymes involved in hem synthesis in:
     - lead poisoning ( saturnism)
     - alcoholism
     - cancers or pre-leukemic states (myeloproliferative disorders)
     - chronic inflammation
     - after drug consumption: ex., tuberculostatics (isoniazide)

Features:
- In the bone marrow:
  - ↑ number of sideroblasts with typical ringed sideroblasts
  - ↑ pathological stores as haemosiderin
  - ineffective erythropoiesis

- In the peripheral blood:
  - ↑ ferritin
  - ↑ serum iron > 190 μg/dL
  - ↑ transferrin saturation > 100%
  + pathological iron stores (hemochromatosis) in liver, heart, pancreas, spleen, skin.
  + pathological iron deposition (hemosiderosis) in different organs (liver, pancreas, heart, skin) → their dysfunction.

3. Vitamin B12 and folic acid deficiency anemias

Definition: megaloblastic, macrocytic, normochromic anemias that share as common feature an impairment of DNA synthesis.

General features:
Both B₁₂ and folate are cofactors in the synthesis of thymidine, one of the four bases in DNA. The inadequate DNA synthesis results in defective nuclear maturation and a delay in cell division (cell size increases) whereas cytoplasmic maturation and hemoglobin accumulation proceed normally, leading to nuclear-to-cytoplasmic asynchrony.

Bone marrow. Megaloblastic changes are detected at all stages of erythropoiesis (promegablasts, megaloblasts) with a decreased production of mature RBC and release into the peripheral blood, causing anemia. The megaloblasts may undergo autohemolysis in the marrow or are destroyed by phagocytic cells in the marrow (ineffective erythropoiesis). Megakaryocytes are abnormally large too.

Peripheral blood. The presence of abnormally large red cells (high MCV) - macrocytes and macro-ovalocytes is highly characteristic. Large and hypersegmented neutrophils may be seen secondary to the delay in mitotic division. The impairment of DNA synthesis is systemic, and affects other rapidly dividing cells in the body, e.g. gastrointestinal epithelial cells. Since the megaloblastic features are indistinguishable morphologically in folate and B₁₂ deficiencies, diagnosis is established by laboratory tests (serum B₁₂ and folate levels).

Etiology:
I. Vitamin B₁₂ deficiency
   1. Decreased intake: vegetarians
   2. Impaired absorption:
      - Intrinsic factor deficiency (pernicious anemia, gastrectomy)
Diffuse intestinal disease (lymphoma)
Decreased ileal absorption of vitamin B₁₂ (surgical resection, Crohn’s disease)
Bacterial overgrowth and competition for vitamin B₁₂ (blind loop syndrome, diverticula)
Malabsorption states

II. Folate deficiency
Decreased intake: alcoholism, inadequate diet
Impaired absorption: intrinsic intestinal disease, malabsorption states, anticonvulsivants
Increased loss: hemodialysis
Impaired utilization: folic acid antagonists (sulfa drugs, methotrexate, phenytoin)
Increased requirements: pregnancy, infancy, disseminated cancer

a. Anemia of vitamin B₁₂ deficiency

Vitamin B₁₂ (cobalamin) metabolism
Source. Vitamin B₁₂ is found in animal products. Humans are totally dependent on dietary intake, with a daily need of 2-3 micrograms.
Absorption. Dietary cobalamin binds intrinsic factor (IF), a protein produced by parietal cells of the gastric mucosa. The IF-cobalamin complex remains intact until it reaches the distal end of the ileum, where it binds with high affinity to specific receptors located on ileal enterocytes. Cobalamin then enters these cells and reaches the portal plasma, which contains three cobalamin binding proteins known as transcobalamin I, II, and III.
Transport. Vitamin B₁₂ circulates bound to transcobalamin I and II (that are secreted by white blood cells). However, only transcobalamin II is capable of delivering vitamin B₁₂ to the cells (liver, bone marrow and gastrointestinal tract).
Storage. Liver contains approximately 2 mg of stored vitamin B₁₂ and vitamin B₁₂ deficiency develops in 3 years up to 6 years (10 years after other authors) after vitamin B₁₂ absorption ceases.
Biochemical functions. Vitamin B₁₂ serves as a cofactor for two reactions in the human body: i) as methylcobalamin is a cofactor for methionine synthetase in the conversion of homocysteine to methionine, and ii) as adenosylcobalamin is a cofactor for the conversion of methylmalonyl coenzyme A to succinyl coenzyme A. (Fig. 4).

> In the first reaction, cobalamin accepts the methyl group from N⁵-methyl tetrahydrofolate (THF, the main form of folic acid in plasma) ⇒ generation of tetrahydrofolate. The latter will further generate N⁵,10-methylene-THF that is required for the synthesis of tymidine, one of the purinic base in DNA. In vitamin B₁₂ deficiency, the impairment in DNA synthesis is caused by the reduced availability of THF (which remains ‘trapped’ as N⁵-methyl THF, a form that cannot be polyglutamated for intracellular storage) and the relative folate deficiency ⇒ anemia improves with administration of oral folate!

![Figure 4. Role of cobalamin (vitamin B₁₂) and folic acid in nucleic acid and myelin metabolism. Lack of either cobalamin or folic acid retards DNA synthesis (A), and lack of cobalamin leads to loss of folic acid, which cannot be held intracellularly unless polyglutamated. Lack of cobalamin also leads to abnormal myelin synthesis, probably via a deficiency in methionine production (B). (Reproduced from McFee S, Hammer G, Pathophysiology of Disease: An introduction To Clinical Medicine, 2010)](image-url)
The interruption of the second reaction is responsible for increased plasma levels of methylmalonic acid that could lead to the generation of abnormal fatty acids and their incorporation in neuronal lipids ⇒ the neurologic abnormalities seen in cobalamin deficiency. Lack of methionine also contributes to the neurologic complications (hereditary defects in methionine synthesis lead to neuropathy).

Clinical Manifestations:
1. Hematologic manifestations
   - anemia
   - thrombocytopenia
   - neutropenia with hypersegmented neutrophils
2. Gastrointestinal manifestations
   - sore tongue
   - anorexia
   - moderate weight loss
3. Neurologic manifestations
   - demyelization of peripheral nerves & posterior/lateral columns of spinal cord
   - axonal degeneration & neuronal death: irreversible
   - numbness/weakness in extremities
   - ataxia.

Pernicious anemia (Biermer anemia)
A common cause of cobalamin malabsorption is pernicious anemia, a disease of unknown origin in which the fundamental defect is atrophy of the gastric (parietal cell) mucosa eventually leading to the absence of IF and HCl secretion. Because cobalamin is only absorbed by binding to IF and uptake by ileal IF-cobalamin receptors, the net consequence is severe cobalamin malabsorption leading to cobalamin deficiency.

There is a significant association of pernicious anemia with other autoimmune diseases. There is a positive family history for about 30% of patients, among whom the risk of familial pernicious anemia is 20 times as high as in the general population. The histological appearance of the gastric mucosa (infiltration with plasma cells and lymphocytes) is also strongly reminiscent of autoimmune-type lesions. There is also a high incidence of anti-parietal cell IgG antibodies in the serum of 90% of patients with pernicious anemia.

Pathogenesis: autoimmune destruction of the parietal cells occurs via production of:
- Antibodies against the parietal cells (the major Ag is the proton pump), responsible for:
  - Atrophy of the gastric mucosa
  - Achlorhydria
- Antibodies against the intrinsic factor, responsible for:
  - Decreased absorption of vit. B12 at the terminal ileum
  - Megaloblastic anemia.

b. Anemia of Folate Deficiency
Folate is found in fruits and fresh vegetables (especially citrus fruits and green leafy vegetables); daily need = 50 micrograms. Folates in natural foods are conjugated to chains of polyglutamic acid. Enzymes in the lumen of the small intestine convert the polyglutamate forms of folate to the monoglutamate and diglutamate forms, which are readily absorbed in the proximal portion of the jejunum. Most of the folate in plasma is present as 5-methyl-tetrahydrofolate (5 methyl-THF). The majority is loosely bound to albumin, from which it is readily taken up by the high-affinity folate receptors present on cells throughout the body. Once inside the cell, 5-methyltetrahydrofolate must be converted to tetrahydrofolate by the cobalamin-dependent enzyme, methionine synthase, before it can be converted to the polyglutamate form and take part in
the other folate-dependent enzymatic reactions. Total body stores of folate are approximately 5000 mcg, enough to supply requirements for 2–3 months.

**Etiology:** Folate deficiency can result from: i) *increased requirements* (most frequent in pregnancy, where daily supplementation is mandatory to prevent neural tube defects in babies), ii) *decreased dietary intake* (alcoholics), and iii) *malabsorption* (intestinal disease, or drugs such as methotrexate used in cancer treatment).

**Symptoms and Signs.** The features are similar to those of vitamin B₁₂ deficiency, with *macrocytic anemia*. However, the *neurologic abnormalities are absent.*

### 4. Aplastic Anemia

**Definition:** A *normochromic normocytic anemia* due to a defect of the pluripotent stem cells responsible for primary hematopoietic failure with:

- marked bone marrow hypocellularity & fatty replacement of bone marrow
- pancytopenia (*anemia, neutropenia, thrombocytopenia*) in the peripheral blood

**Etiology:**

- in 2/3 of the cases - idiopathic aplastic anemia (no detectable initiating factor).
- the rest of the cases occur secondary to:
  1) *chemical toxins* (benzene, chlorinated hydrocarbons)
  2) *drugs*: alkylation agents and antimetabolites used in cancer therapy (dose related effect) and chloramphenicol, phenylbutazone (idiocyncratic mechanism)
  3) *viral infections* (hepatitis, AIDS, mononucleosis)
  4) *autoimmune disorders* (SLE, Hashimoto thyroiditis)

**Pathogenesis:**

Two major pathogenetic theories exist:

1. An *extrinsic* immune-mediated suppression of marrow precursors (triggered by exposure to chemicals, infectious agents, etc. with the production of cytokines (TNF and IFNγ) that are directly responsible for the suppression of hematopoiesis.
2. An *intrinsic* abnormality of stem cells (reduced proliferative capacity)

**Clinical problems** result from *anemia* (weakness, fatigue), *leukopenia* (infections) and *decreased platelets* (bleeding). Bone marrow transplantation has been successful, especially in patients less than 40 years old.

### 5. Anemia from Chronic Disease/Inflammation

**Definition:** normochromic normocytic anemia (in *mild forms*) microcytic and normochromic (in *moderate forms*), or microcytic and hypochromic (in *severe forms*) characterized by:

- low serum iron level
- increased ferritin level (reflecting the excessive iron stores of the organism).

**Etiology:**

1) Chronic infections (AIDS, osteomyelitis)
2) Chronic inflammations (inflammatory bowel disease)
3) Autoimmune disorders (lupus erythematosus, rheumatoid arthritis)
4) Cancer (Hodgkin’s disease)

**Pathogenesis.** Microorganisms, injured tissues, autoimmune dysregulation and tumor cells lead to T-cell activation and production of cytokines (IL-1, IL-6, TNF-α, IFNγ) that are responsible for:

- increased production of hepcidin by the liver (IL-6) which inhibits iron release from hepatocytes, enterocytes and macrophages
- macrophage sequestration of iron with decreased serum iron level & availability for erythropoiesis
- **macrophage activation** with *increased splenic destruction of red cells* (*hypersplenism* - RBC lifespan is decreased by 20 to 30%)
- **reduced production of erythropoietin** (EPO) and/or **resistance to EPO** resulting in **decreased erythropoiesis**.

6. **Anemia from Chronic Kidney Disease**

**Definition:** normochromic, normocytic anemia constantly associated with end-stage kidney disease

**Pathogenesis.**

Anemia is due to:
- decreased EPO production
- accumulation of toxic substances (uremic toxins) that:
  - blunt the response to EPO on red cell production
  - cause hemolysis (RBC lifespan = 60-90 days) and
  - cause bleeding tendencies (purpura, gastrointestinal hemorrhages)
- release of proinflammatory cytokines (IL-1, IL-6, TNF and IFNγ) that trigger apoptosis of RBC precursors and decrease iron availability via induction of hepcidin synthesis.

### B. HEMOLYTIC ANEMIAS

Hemolytic anemias are characterized by **shortened red cell survival**. Increased erythropoietin production results in increased red cell production with a **reticulocytosis** to compensate for the anemia.

Red cell destruction can occur within the spleen macrophages (**extravascular hemolysis**) or within circulation (**intravascular hemolysis**). Another feature is the **retention of Hb degradation products in the body** → increased serum levels of indirect bilirubin > 2.5 mg/dl → jaundice.

**Extravascular hemolysis:** destruction of red cells in the mononuclear phagocytic system (spleen, liver) – the normal place of physiologic hemolysis of senescent erythrocytes.

**Examples:** hereditary spherocytosis, sickle cell anemia.

Damaged or abnormal RBC are removed in spleen, where hemoglobin is broken down intracellularly. **Free hemoglobin** is not released directly into the blood and urine, but hemoglobin breakdown products are increased (**hyperbilirubinemia**) and jaundice may result. **Spleen and liver may become enlarged** since these are sites of removal of RBC from the circulation. Chronically elevated levels of bilirubin can promote formation of **gallstones**.

**Intravascular hemolysis:** destruction of RBC within the circulation (less frequently).

**Examples:** mechanical trauma, hemolytic transfusion reaction.

Hemoglobin released from RBC into circulation (**hemoglobinemia**) is bound to haptoglobin, a binding protein, and cleared from the circulation by the liver. A **decrease in serum haptoglobin** is a key feature of intravascular hemolysis. When plasma hemoglobin levels exceed amount of available haptoglobin, free hemoglobin is excreted in the urine (**hemoglobinuria**); however hemoglobin is toxic to the kidney, and iron that accumulates in proximal tubular cells in the kidney as a breakdown product of hemoglobin is lost in the urine when these cells are shed (** hemosiderinuria**). Conversion of heme (derived from hemoglobin) to bilirubin leads to **hyperbilirubinemia and jaundice**. The degree of jaundice is dependent on the functional capacity of the liver and rate of hemolysis.

**Classification:**

According to the **mechanism responsible for red cell destruction**, hemolytic anemias are due to:

1. **Intrinsic hemolytic anemias** due to:
   1. Membrane abnormalities
2. Enzymes defects
3. Hemoglobin abnormalities (hemoglobinopathies)

II. Extrinsic hemolytic anemias due to:
1. Immune hemolysis (biosynthesis of antibodies)
2. Mechanical hemolysis.

I. Intrinsic hemolytic anemias due to:

1. Membrane abnormalities

**Hereditary spherocytosis**
This is an *autosomal dominant disorder* in which the principal abnormality appears to be a deficiency of spectrin & ankyrin, which are red cell membrane structural proteins of the cytoskeleton. The *erythrocyte envelope is abnormally permeable* and the *sodium pumps are overworked*. The exact nature of the red blood cell defect may vary from family to family. The *erythrocytes lose their biconcave shape* → become *spherical* & more *susceptible to osmotic lysis* → they are sequestered and destroyed almost exclusively in the spleen - *hemolysis is mainly extravascular*. Removal of spleen results in *normal red cell survival BUT NOT normal red cell morphology*. Production of spherocytes continues, but following splenectomy their destruction is decreased.

2. Enzymes defects

**Glucose-6-Phosphate Dehydrogenase (G-6-PD) deficiency**
G6PD deficiency is a hereditary enzyme defect that causes episodic hemolytic anemia because of the *decreased ability of red blood cells to deal with oxidative stresses*. G-6-PD enzyme = the major enzyme in the *hexoso-monophosphate shunt*, the function of this shunt is to service *the enzymes glutathione reductase* and *glutathione peroxidase*, which *protect the red cells against damage due to oxidation*.

The deficiency is inherited as an *X-linked disorder* with a high frequency among Black African males (female carriers are rarely affected — only when an unusually high percentage of cells producing the normal enzyme is inactivated). Many other G6PD variants have been described, including some Mediterranean variants with extremely low enzyme activity.

The *denaturation of oxidized hemoglobin* → *Hb precipitation* within the cell → *denaturated Hb attach to the RBC membrane* → *the RBC membrane’s flexibility is reduced* → *extravascular hemolysis*. These cells circulate with difficulty through the spleen and liver and are removed from the circulation, resulting in *hemolytic anemia*.

The condition is asymptomatic in the absence of the oxidative stress. *Oxidant damage* of RBC followed by intravascular hemolysis is *induced by*:
- Infections
- Ingestion of Fava beans
- Oxidant drugs (antimalarial, chloramphenicol)
- Surgery

**Piruvatkinase deficiency** – major enzyme of the anaerobic glycolysis pathway
Pathogenesis: **↓ ATP synthesis** → functional deficit of Na⁺/K⁺ pump → rigid erythrocytes → are rapidly destroyed in the spleen → *extravascular hemolysis*. 
3. Hemoglobin abnormalities

**Sickle cell anemia**
Sickle cell anemia is an *autosomal recessive disorder* due to an *inherited defect in the structure of globin chain* that causes hemoglobin to gel upon deoxygenation. The specific defect is a *single base pair substitution in DNA that causes a single amino acid substitution* (valine for glutamic acid) *at position 6 in the beta chain of globin* to produce sickle hemoglobin (HbS). Under low oxygen conditions the abnormal hemoglobin polymerizes, causing the RBC to assume a "sickle" shape. The sickled cells are rigid and *vulnerable to splenic sequestration* (decreased survival), and can also block the microcirculation → ischemia and/or infarction.

Sickle cell disease occurs in homozygotes (HbSS) *at a PO$_2$ of 80 mmHg* and is characterized by **severe anemia** and vaso-occlusive crises. Complications may include *painful crises*, leg ulcers, retinal and renal thromboses and in long term, *autosplenectomy* (fibrosis due several infarctions).

If the patient is heterozygous (Hb AS), the **sickling phenomenon occurs at a PO$_2$ of 40 mmHg**; the heterozygote defect is essentially asymptomatic because less than half of the hemoglobin is abnormal and the concentration of HbS within the RBC is insufficient to cause sickling.

Prenatal diagnosis is now available for couples at risk of producing a child with sickle cell anemia. DNA from fetal cells can be directly examined, and the presence of the sickle cell mutation can be accurately and definitively diagnosed.

**Thalassemia**
Is an inherited defect (*autosomal codominant*) that results in *diminished or absent synthesis of either the alpha or beta globin chains of hemoglobin*. The type of thalassemia is named for the globin chain produced in reduced amounts.

Decreased globin production leads to *decreased hemoglobin production* ⇒ **anemia**, as major manifestation. In addition, precipitation of the relative excess of the other globin chain within RBC is responsible for *membrane damage* and *premature destruction of RBC precursors in the marrow (ineffective erythropoiesis)* and *spleen* (extravascular hemolysis).

Clinical manifestations vary from severe transfusion-dependent anemia and iron overload to mild anemia. In almost all cases there is a **moderate to marked microcytosis** with *target cells* and *basophilic stippling* of the red cells present on the blood smear.

**Alpha – thalassemia**
Mechanism: deletion of the genes encoding for α chains synthesis (located on chromosome 16)

Normal: 2 genes on each chromosome 16 (α α / α α)

Pathological: deletion of:
- **1 gene**: - α / α α - sickle cell trait/silent α 2 thalassemia
  - **asymptomatics** carriers
- **2 genes**: - α / - α - specific α 1 thalassemia 1
  - - / α α - mild anemia, hypochrome microcytic
- **3 genes**: - - / - α - hemoglobin H (β4), instable
  ⇒ precipitated Hb = Heinz bodies
  - **severe anemia** hypochrome microcytic
  - ± splenomegaly – extravascular hemolysis
- **4 genes**: - - / - - - hemoglobin BARTS (γ4), with highly increased O$_2$ affinity
  ⇒ **hidrops fetalis**
Beta – thalassemia

Mecanism: deletion of the genes encoding for β chains (located on chromosome 11)

Normal: 1 gene on each chr. 11 (β / β)

Pathological:
- Beta–thalassemia minor (common condition in Mediterranean Basin, Africa, Asia):
  - heterozygot defect ⇒ mild anemia
  - spleen sometimes is palpable
  - asymptomatic
- Beta–thalassemia major (Cooley anemia): ineffective beta chain synthesis due to point mutation in the beta gene promoter or enhancer on chromosome 11, excess alpha chains relative to beta chain leading to ineffective erythropoiesis and hemolysis of RBC, compensatory increase in HbF
  - it is a homozygous defect, with the following clinical manifestations:
    - Onset at 3-6 months because of replacement of HbF by HbA
    - Severe anemia developing in the first year of life
    - Jaundice
    - Stunted growth and development (hypogonadal dwarf)
    - Gross hepatosplenomegaly (extramedullary hematopoiesis)
    - Skeletal changes (expanded marrow cavity)
    - Skull x-ray has “hair-on-end” appearance
    - Pathological fractures common
    - Evidence of increased Hb catabolism (e.g. gallstones)
    - Death from: untreated anemia, infection (early), iron overload (late, secondary to transfusions), usually at 20-30 years old

II. Extrinsic Hemolytic Anemias

1. Autoimmune hemolytic anemias

Patients make antibodies to their own RBCs ⇒ Antibody-coated cells can be lysted (complement activation) or removed by the reticuloendothelial system. Phagocytosis of antibody-coated RBC ⇒ partial loss of red cell membrane: spherocytes ⇒ are sequestered by the spleen, further contributing to the anemia.

Types:
- IgG Warm Antibody type
  - IgM Cold Antibody type

  a. Warm antibody type autoimmune anemia (most frequent form)

Pathophysiology:
- RBC are coated with IgG, or complement or both at the body temperature (37ºC) ⇒ erythrocytes change their shape (spherocytes) and become rigid ⇒ extravascular hemolysis in RES (mainly spleen).

Etiology:
- Idiopathic forms: 50% of the cases
- Secondary forms in:
  - Lymphoproliferative disorders (chronic lymphocytic leukemia, Hodgkin’s and non-Hodgkin’s lymphomas)
  - Autoimmune disorders (SLE)

Clinical Features: usually insidious, with anemia, jaundice and splenomegaly.

b. Cold antibody type autoimmune anemia

Pathophysiology:
- Either monoclonal or polyclonal IgM antibodies attach to RBC surface antigens in peripheral circulation where T decreases below 37ºC and trigger intravascular hemolysis.
Etiology:
- Idiopathic forms
- Secondary forms in:
  - Lymphoproliferative disorders (chronic lymphocytic leukemia, Hodgkin’s and non-Hodgkin’s lymphomas)
  - Infections (Mycoplasma pneumoniae, Epstein-Barr virus).
Clinical features: anemia, joint pain, vasculitic rash, Raynaud phenomena, and rarely splenomegaly.

c. **Drug induced autoimmune anemia** – can be caused by:
  - alpha methyldopa – which induces a warm antibody type autoimmune anemia
  - penicillin
    - act as an incomplete antigen, that becomes complete after it locates itself on the erythrocytes’ cellular membrane, causing antibody production.
    - the antibodies will then attack those red-cells on which the penicilin has bound itself.
    - due to the immune reaction, the erythrocytes suffer a transformation, becoming spherocytes, leading to extrinsic hemolysis (type II hypersensibility reaction).
  - quinine, quinidine, isoniazide
    - these drugs target serum proteins, inducing the synthesis of IgG or IgM
    - the result are immune complexes between the drug, the serum proteins and the antibody, that locate themselves on the erythrocytes’ or platelets’ cellular membrane and induce extrinsic or intrinsic hemolysis (type III hypersensibility reaction).

2. **Mechanical trauma induced hemolytic anemias**
Examples:
- **Cardiac valve prosthesis**: red cells are disrupted by physical trauma as they pass through areas of turbulence and abnormal pressure related to abnormal valve function.
- **DIC** (disseminated intravascular coagulation) where RBCs are lysed as they pass through fibrin clots/strands in the microcirculation. Loss of large portion of membrane produces schistocytes.

II. PATHOPHYSIOLOGY OF POLYCYTHEMIA

The opposite of anemia is polycythemia, which is characterized by:
- an **increase in the number of circulating red cells**
- an **increase of the hematocrit (Ht) (over 45% in females, over 48% in males)**

The pathophysiological consequences of the increasing hematocrit are:
- an **increase of blood viscosity**, which can lead to an increased risk of thrombosis
- a **decrease in the tissues’ blood and oxygen supply**, thus leading to hypoxia and an overload of the heart function.

The increased medulary activity will be accompanied by an increased cellular distuction, which can lead to a high level of uric acid (risk for developing gout).

Polycythemia is divided into:
A. **Relative polycythemia (erythrocytosis)** (the **red cell mass is normal**, but the **plasma volume is reduced**)
B. **Absolute polycythemia (erythrocytosis)** (a **true increase in red-cell mass**).
A. Relative polycythemia is characterized by an increase of the haematocrit, due to a decrease of the circulating volume, but without any changes in the number of red blood cells (normal level of red blood cells).

- causes:
  - pathological states associated with a decreased circulating volume:
    - hemoconcentration from dehydration
    - vomiting
    - diarrhea
    - excessive use of diuretics
  - stress-induced polycythemia (Gainsbrock syndrome)
    - affects middle-aged males, with an anxious personality
    - frequently associated with obesity and high blood pressure
    - asymptomatic (does not require treatment)

B. Absolute polycythemia is characterized by a true increase of the red blood cell level. It can be a primary or secondary phenomenon.

- Primary absolute polycythemia or polycythemia vera = a neoplastic proliferation of red cells and myeloid cells precursors and is associated with normal or low levels of erythropoietin.
- Stimuli which increase erythropoietin (a growth and differentiation factor for red cell precursors) can produce secondary absolute polycythemia.

Erythropoietin levels are helpful in distinguishing primary from secondary cases of absolute polycythemia. Cases of secondary absolute polycythemia have increased levels of erythropoietin, while primary absolute polycythemia has normal or suppressed levels of erythropoietin.

Primary absolute polycythemia (polycythemia vera)

Polycythemia vera (PV) is an acquired myeloproliferative disorder that causes overproduction of all three hematopoietic cell lines, most prominently the red blood cells; it usually begins after 50 years of age.

The etiology of PV is unknown; however, it is likely a result of acquired genetic changes in the stem cell leading to disturbances of normal cellular growth.

The number of erythrocytes increases up to 6-12 million/mm³, with a Hb concentration of 18-24 g/dL. Increased blood viscosity and thrombosis in the later stages of the disease.

Hyperuricemia and hyperuricosuria are seen in 40% of the cases at diagnosis.

Serum iron and ferritin are low owing to excessive iron use, and the leukocyte alkaline phosphatase is elevated.

Erythropoietin levels are normal or decreased.

Vitamin B₁₂ transcobalamin II and alkaline phosphatase serum levels are decreased.

PV may progress to myelofibrosis or acute myeloid leukemia in 5-50% of cases, with a median survival of 10 to 16 years.

A characteristic clinical picture of ruddy cyanosis is seen on the face and extremities, owing to the presence of deoxygenated blood in cutaneous vessels. Patients complain of headache, dizziness, fullness of the head and face, and pruritus. Splenomegaly is a common finding on physical examination.
**Secondary polycythemia (reactive)**

Secondary polycythemia means a reactive increase in the number of red-blood cells due to increased medullary activity (erythropoietin levels are always increased).

Secondary polycythemia is due to:
- a **physiological increase in erythropoietin production to compensate for hypoxia**. This reactive erythrocytosis has been described in people who live at **high altitudes with low atmospheric pressure**, **smokers** and in people with **chronic pulmonary disease**, **congenital heart disease (right-to-left shunt)**, **congestive heart failure and renal disease (hydronephrosis)**. Methaemoglobin and carboxyhaemoglobin can be found. **Pheochromocytoma** and other endocrine disorders also have been described as possible causes of erythrocytosis.
- a **pathological increase in erythropoietin production** which may occur with some **tumors**, particularly brain, renal, lung, hepatic and ovarian carcinomas, **that produce an erythropoietin-like substance**.

The **increased in blood viscosity** leads to **thrombosis or coagulation defects**. When the elevated erythrocyte volume becomes dangerously high, it should be treated by phlebotomy to reduce viscosity.