Anemia

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Definition

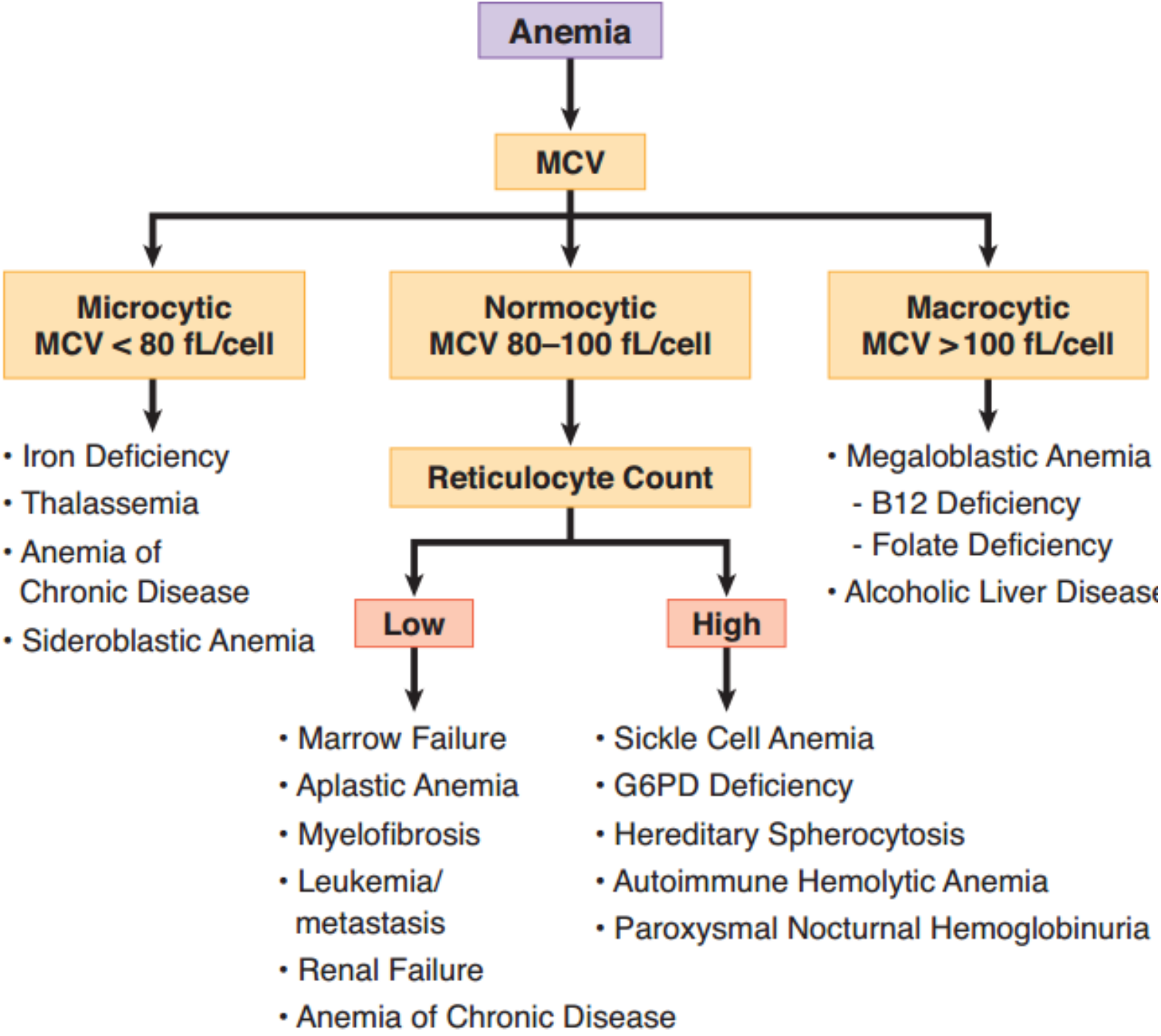
Anemia is defined as a reduction of the total circulating red cell mass below normal limits

In practice, anemia is usually diagnosed based on a reduction in the **hematocrit** (the ratio of packed red cells to total blood volume) and the **hemoglobin concentration** of the blood to levels that are below the normal range.

Classification of Anemia

Size and morphology and degree of hemoglobinization

- Microcytic hypochromic
- Normocytic normochromic
- Macrocytic


Anemia
MCV
Microcytic
MCV < 80 fL/cell
Normocytic
MCV 80–100 fL/cell
Macrocytic
MCV >100 fL/cell
• Iron Deficiency
• Thalassemia
• Anemia of Chronic Disease
• Sideroblastic Anemia
Reticulocyte Count
Low
High
• Marrow Failure
• Aplastic Anemia
• Myelofibrosis
• Leukemia/ metastasis
• Renal Failure
• Anemia of Chronic Disease
• Sickle Cell Anemia
• G6PD Deficiency
• Hereditary Spherocytosis
• Autoimmune Hemolytic Anemia
• Paroxysmal Nocturnal Hemoglobinuria
• Megaloblastic Anemia
- B12 Deficiency
- Folate Deficiency
• Alcoholic Liver Disease

Morphologic Classification of Anemia

Macrocytic
- Megaloblastic
- Alcohol use
- Liver disease
- Hypothyroidism
- Reticulocytosis
- Primary bone marrow disease

Microcytic
- Iron deficiency
- Anemia of chronic disease inflammation
- Thalassemias
- Sideroblastic anemias

Normocytic
- Anemia of chronic disease/inflammation
- Anemia of renal disease
- Acute blood loss

Classification of Anemia

Based on underlying pathophysiological mechanism

- Reduced production
- Ineffective production
- Increased destruction
- Blood loss

Classification of Anemia

Reduced production

- Aplastic anemia
- Pure red cell aplasia
- Iron deficiency anemia
- Anemia of renal disease
- Anemia of chronic disease
- Myelophthisic anemia
- Anemia of lead poisoning

Classification of Anemia

- Ineffective production
  - Megaloblastic anemia
  - β Thalassemias
    - Homozygous thalassemia (thalassemia major)
    - Heterozygous thalassemia (thalassemia minor)
  - αThalassemias
    - Silent carrier α thalassemia αThalassemia trait
    - Hemoglobin H disease
    - Homozygous α thalassemia (hydrops fetalis)

Classification of Anemia

Increased destruction

- Extravascular hemolysis
  - Membranopathy: spherocytosis and elliptocytosis, acanthocytosis
  - Hemoglobinopathy: Sickle cell disease, Hemoglobin C and Hemoglobin E disease
  - Warm antibody induced immune hemolytic anemia
- Extravascular or intravascular hemolysis
  - Cold antibody induced immune hemolytic anemia
  - G6PD deficiency
- Intravascular hemolysis
  - Cold hemolysin disease
  - Mechanical red cell fragmentation syndrome
  - Paroxysmal nocturnal hemoglobinuria

Clinical manifestations of anemia

Pallor, Weakness, malaise, easy fatigability and dyspnea on mild exertion.

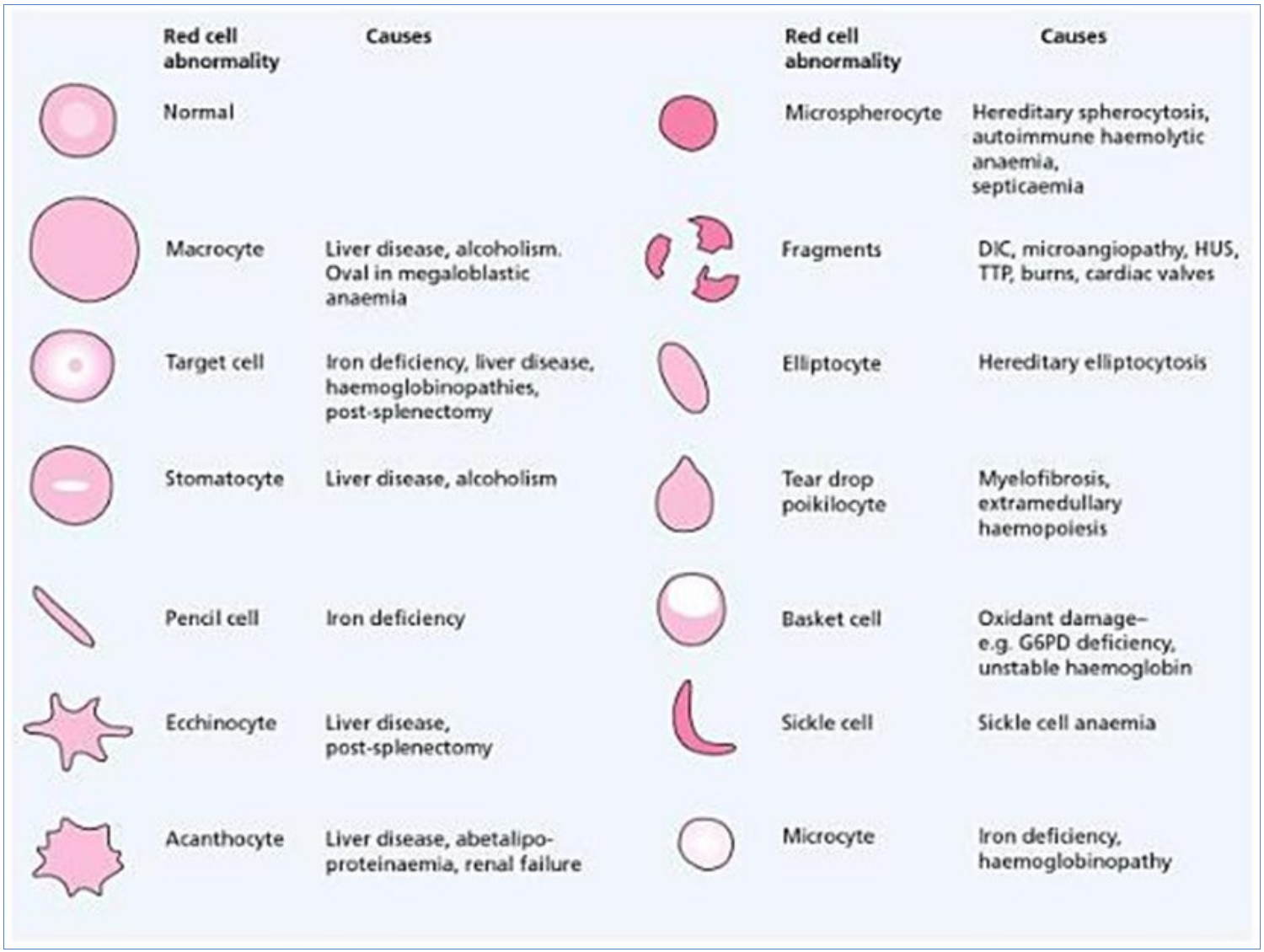
Hypoxia can cause fatty change in the liver, myocardium, and kidney.

Progression of fatty changes in the myocardium can lead to heart failure further aggravating tissue hypoxia

Central nervous system hypoxia can cause headache, dimness of vision, and faintness.

Characteristics of human red cells

Morphologic Features of Various Anemias



Red cell abnormality	Causes	Red cell abnormality	Causes
Normal		Microspherocyte	Hereditary spherocytosis, autoimmune haemolytic anaemia, septicaemia
Macrocyte	Liver disease, alcoholism. Oval in megaloblastic anaemia	Fragments	DIC, microangiopathy, HUS, TTP, burns, cardiac valves
Target cell	Iron deficiency, liver disease, haemoglobinopathies, post-splenectomy	Elliptocyte	Hereditary elliptocytosis
Stomatocyte	Liver disease, alcoholism	Tear drop poikilocyte	Myelofibrosis, extramedullary haemopoiesis
Pencil cell	Iron deficiency	Basket cell	Oxidant damage– e.g. G6PD deficiency, unstable haemoglobin
Ecchinocyte	Liver disease, post-splenectomy	Sickle cell	Sickle cell anaemia
Acanthocyte	Liver disease, abetalipo-proteinaemia, renal failure	Microcyte	Iron deficiency, haemoglobinopathy

Anemia Caused by Decreased Red Blood Cell Production

Iron deficiency anemia is the most common and will be discussed in a separate lecture

Aplastic Anemia

A disorder of pluripotential stem cells that leads to bone marrow failure, with a reduction of all precursor elements within the bone marrow

Bone marrow demonstrates variably reduced cellularity, increase in the amount of fat

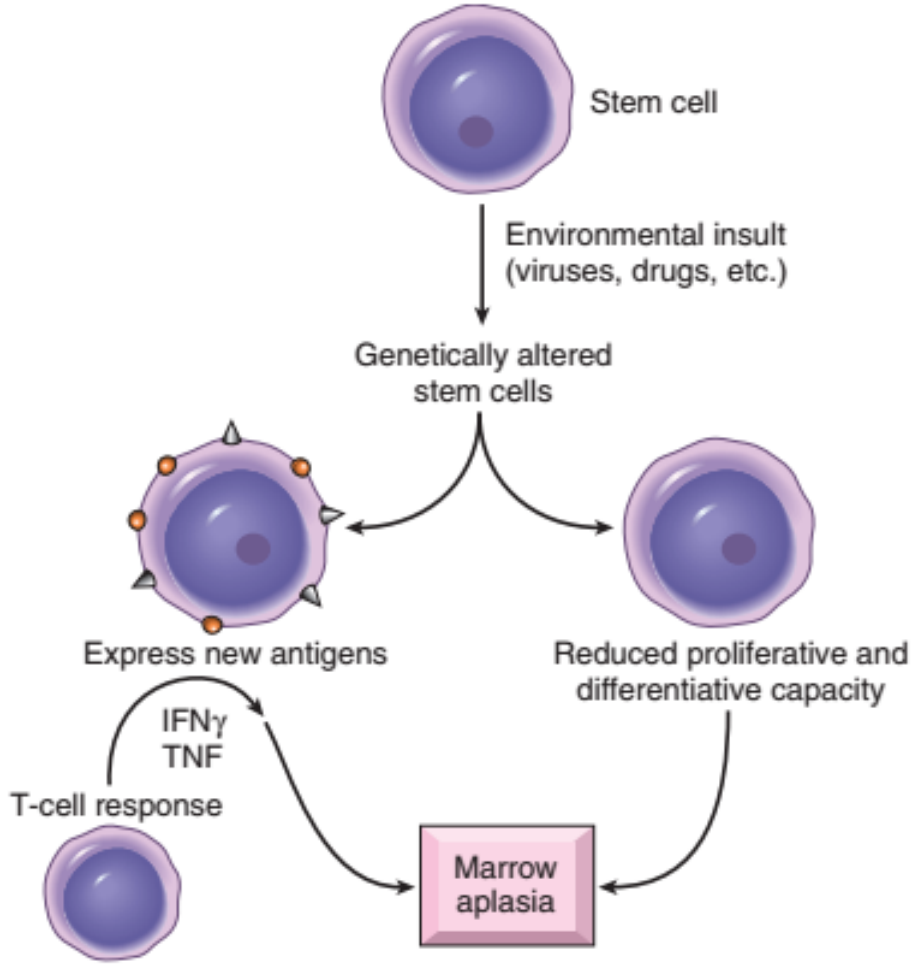
Etiology of Aplastic Anemia

- Idiopathic (two thirds of cases)
- Ionizing radiation
- Drugs
  - Chemotherapeutic agents
  - Chloramphenicol
  - Anticonvulsants
  - Nonsteroidal antiinflammatory agents
  - Gold
- Chemicals
  - Benzene
- Viruses
  - Hepatitis C virus (HCV)
  - Epstein-Barr virus (EBV)
  - HIV
  - Parvovirus B19
- Hereditary
  - Fanconi anemia (germline *FAC* mutation leading to chromosomal instability)

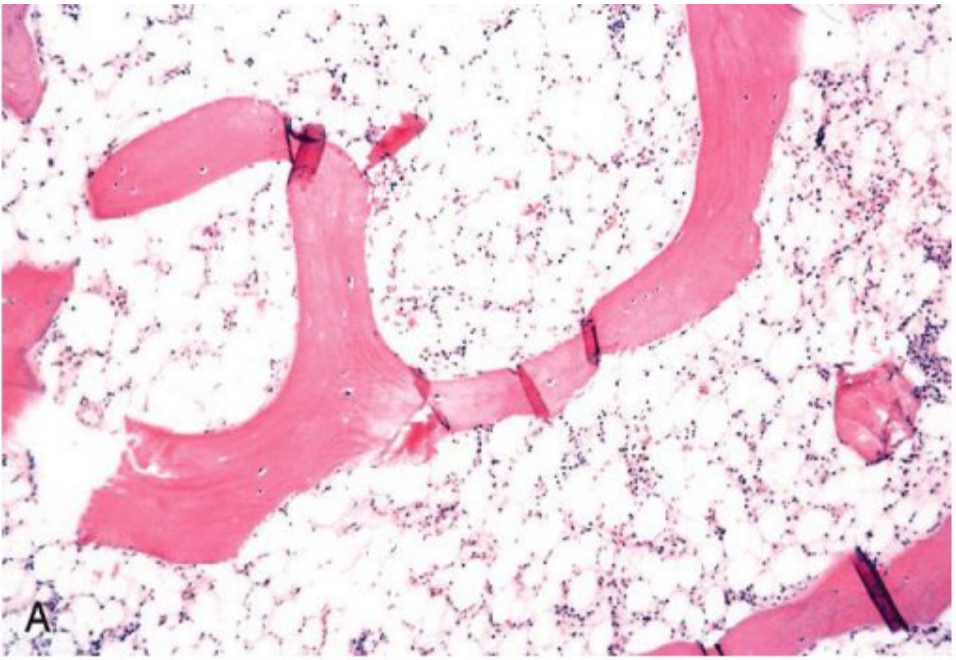
Aplastic Anemia

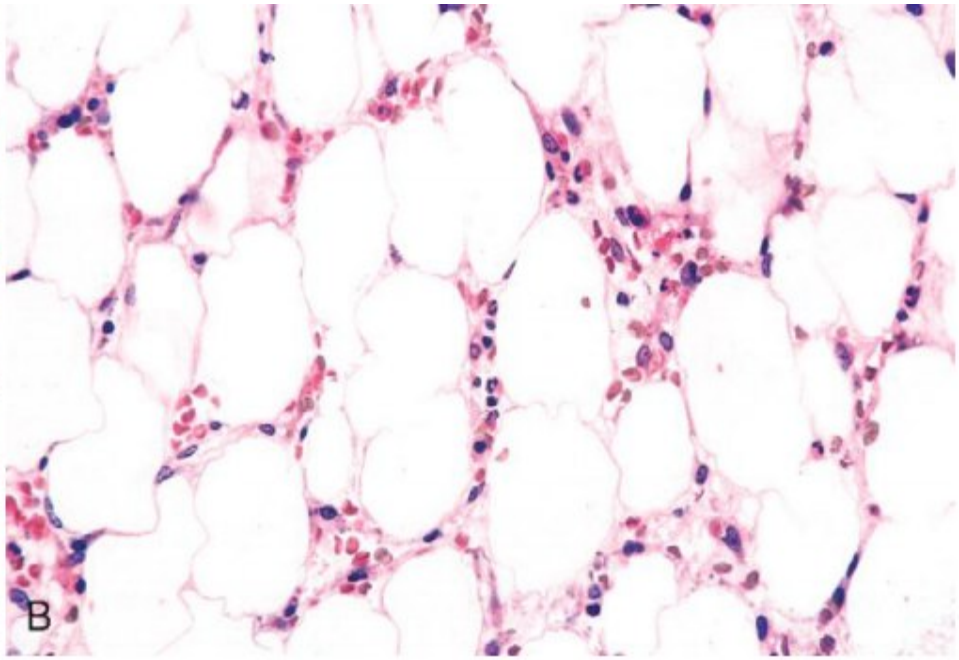
Pathogenesis

- Extrinsic, immune-mediated suppression of marrow progenitors
  - Upregulatred genes involved in apoptosis
  - Recovery in counts after T cell targeting therapy
- Intrinsic abnormality of stem cells
  - Karyotypic aberrations
  - Progression to cancerous and precancerous



Aplastic Anemia
biopsy under light microscopy





Aplastic Anemia

Clinically:

- weakness, fatigue, infections, and bleeding
- Fanconi anemia: first decade of life presentation, hypoplastic thumbs, absent radii, skin pigmentation, and renal anomalies

Peripheral blood

- Anemia, leukopenia (primarily granulocytopenia) and thrombocytopenia
- Red blood cells are often mildly macrocytic
- Reticulocytes very low or not identified

The treatment of aplastic anemia:

- Immunosuppressive therapy
- Bone marrow or stem cell transplantation.

Pure Red Cell Aplasia (PRCA)

Selective lack of erythroid precursor production in the bone marrow

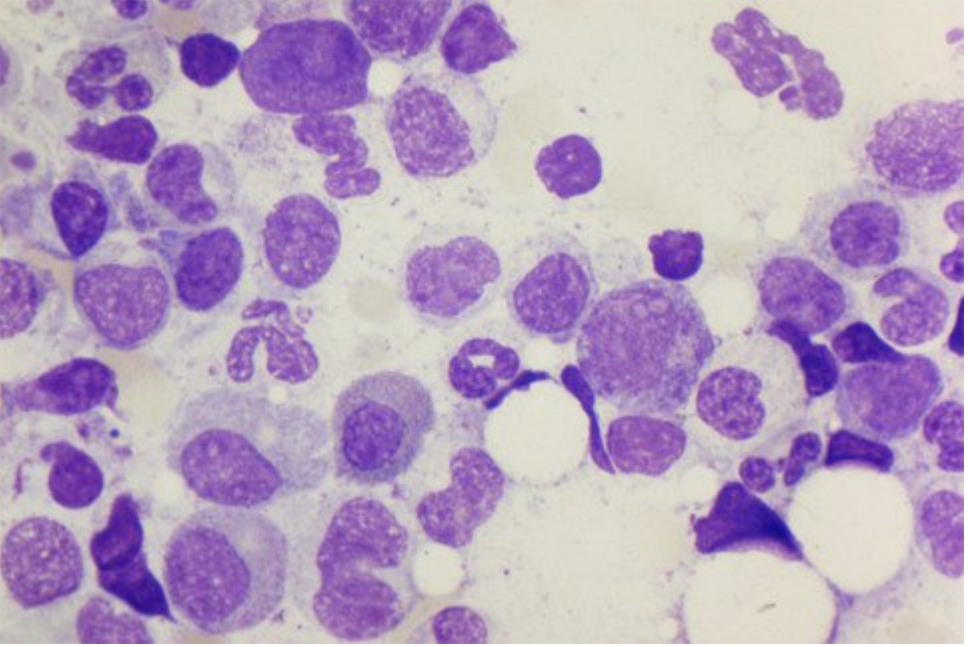
Etiology

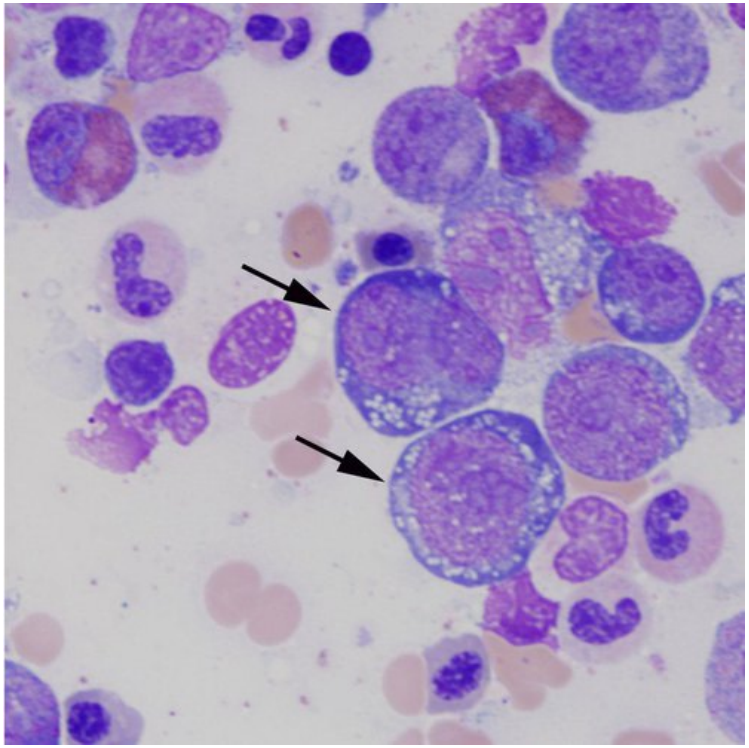
- Idiopathic
- Viral infections especially Parvovirus 19
- Thymic lesions i.e. thymoma
- Autoimmune/collagen vascular disorders
- Hereditary, Diamond-Blackfan anemia, infants less than 1 year

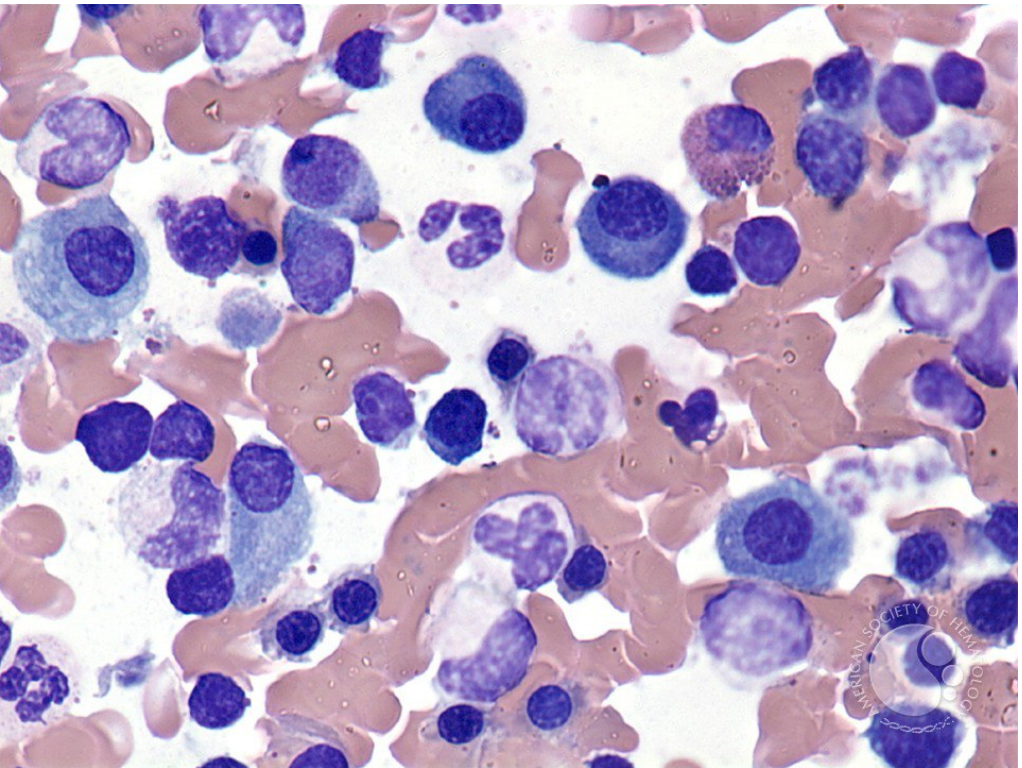
Microscopically

- Peripheral blood: moderate to severe macrocytic anemia, increased erythropoietin, reticulocyte count very low
- Bone marrow is normocellular, selective absence of erythroid precursors
- In cases of parvovirus B19 infection, nuclear inclusions may be found

Treatment involves transfusion, in cases of thymic lesion, thymectomy, Parvovirus B19 associated are often self limiting







Anemia of Renal Disease

Anemia of chronic renal insufficiency is multifactorial

- Decreased erythropoietin production
- A uremic toxin suppression of erythropoiesis
- Malnutrition
- Blood loss e.g. dialysis, bleeding from uremic PLT dysfunction
- Reduced RBCs survival

Examination of the blood reveals

- Normocytic, normochromic anemia
- Cells containing scalloped membranes (echinocytes, burr cells)
- If hypertension red cell fragments and schistocytes

Treatment involves administration of recombinant erythropoietin

Anemia of Chronic Disease

Chronic inflammatory conditions, malignancies

Pathophysiology

- Ineffective use of iron stored within bone marrow macrophages (↑ hepcidin).
- Effects from inflammatory cytokines.

The bone marrow: increased amounts of stored iron by Prussian blue staining.

Examination of the blood: mild to moderate micro/ normocytic anemia, decreased serum iron levels.

The total iron binding capacity is decreased

Treatment: control of underlying disease.

Anemias of Blood Loss
Acute blood loss

Massive hemorrhage can lead to cardiovascular collapse, shock, and death

After bleeding the blood volume is rapidly restored by the intravascular shift of water from the interstitial fluid compartment resulting in hemodilution

Increased secretion of erythropoietin from the kidney to stimulate more erythropoiesis

Bleeding into tissues: the iron in hemoglobin will be recaptured

Bleeding external: iron loss leads to iron deficiency, which can hamper the restoration of normal red cell counts.

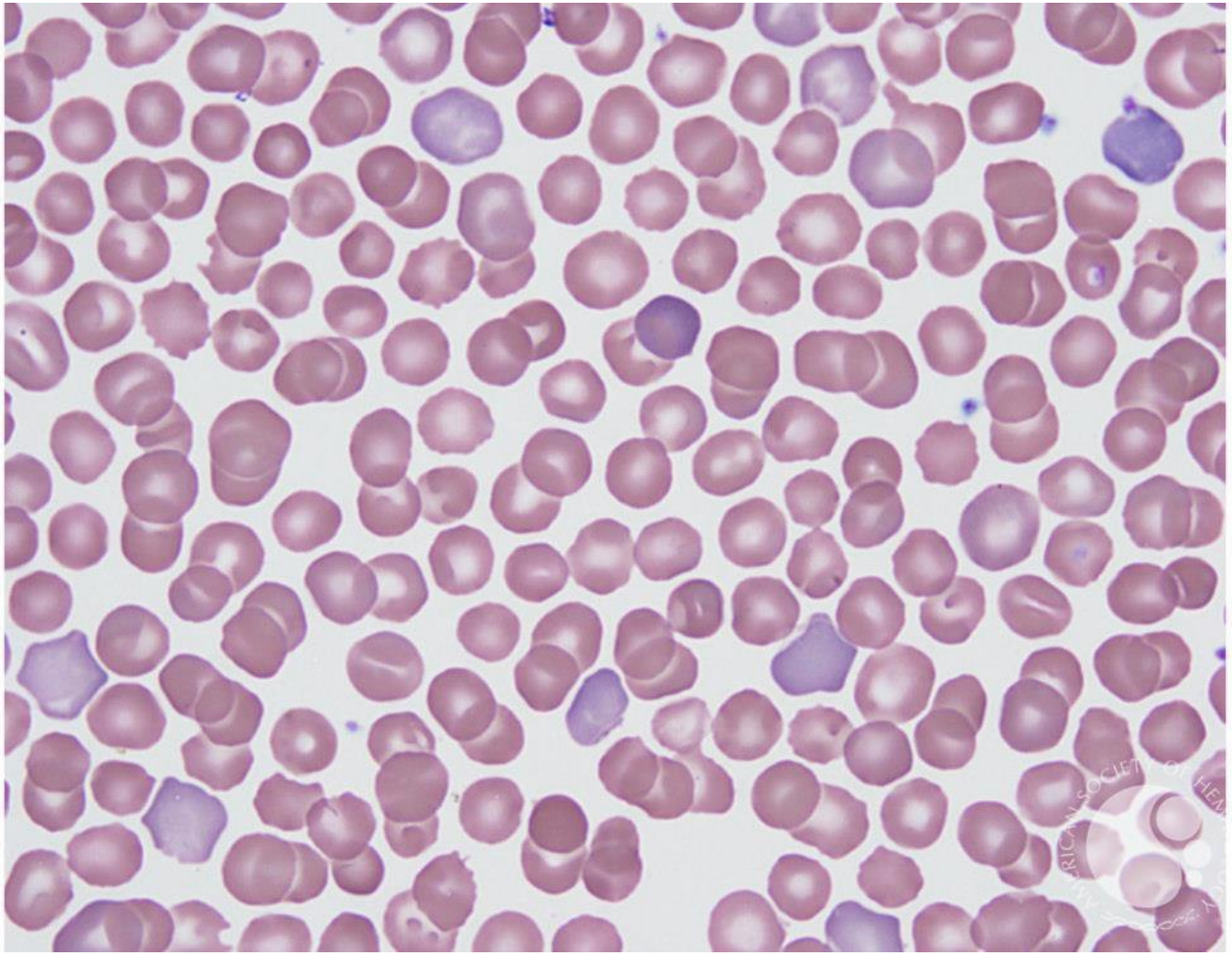
Anemias of Blood Loss
Acute blood loss

Significant bleeding results in predictable changes in other blood cells

- Leukocytosis,
- Thrombocytosis
- Reticulocytosis

  10% to 15% after 7 days,

  Reticulocytes are larger in size than normal red cells (macrocytes) and have a blue-red polychromatophilic cytoplasm, on the blood smear, this is called polychromasia



Anemias of Blood Loss
chronic blood loss

When the rate of loss exceeds the regenerative capacity of the marrow

Depletion of iron reserves leads to iron deficiency anemia

Hemolytic Anemias

Shortened RBCs life span, increase in erythropoiesis and accumulation of hemoglobin degradation products

If uncomplicated, the excess serum bilirubin is **unconjugated**. When the liver is normal, jaundice is rarely severe.

Excessive bilirubin excreted by the liver

- Gastrointestinal tract leads to increased formation and fecal excretion of urobilin,
- Gallstones derived from heme pigments.

In the great majority of hemolytic anemias, hemolysis occur in phagocytes, referred to as extravascular, less commonly intravascular hemolysis predominate

Extravascular (EV) hemolysis

Reduced deformability of RBCs make it difficult to navigate splenic sinusoids leading to their sequestration and phagocytosis by macrophages mainly in the spleen and to lesser extent in the liver leading to either **splenomegaly** or hepatomegaly respectively

There is **increased bilirubin** and decreased haptoglobin, but not to the degree seen with intravascular hemolysis.

Bilirubin-rich gallstones (pigment stones) on the long term

No hemoglobinemia, hemoglobinuria, and methemoglobin formation.

EV hemolysis may occur in membranopathies as hereditary spherocytosis, hemoglobinopahties as sickle cell disease and in warm antibody induced hemolysis

Intravascular (IV) hemolysis

Release of hemoglobin into the blood causes **hemoglobinemia** and **hemoglobinuria**

Markedly decreased hemoglobin-binding proteins in the blood, such as haptoglobin and hemosiderinuria (**characteristic feature**) results

The hemoglobin may be oxidized to methemoglobin, which causes methemoglobinemia and methemoglobinuria.

Increased bilirubin causes jaundice and an increased risk of pigment (bilirubin) gallstones.

No splenomegaly is seen.

IV hemolysis may be caused by mechanical injury like prosthetic cardiac valves, thrombotic narrowing of the microcirculation, glucose 6 phosphate dehydrogenate deficiency, Complement fixation in cold antibody induced autoimmune hemolytic anemia.

Changes in Hemolytic Anemia

Increased numbers of erythroid precursors (normoblasts)

Prominent reticulocytosis and polychromasia in the peripheral blood.

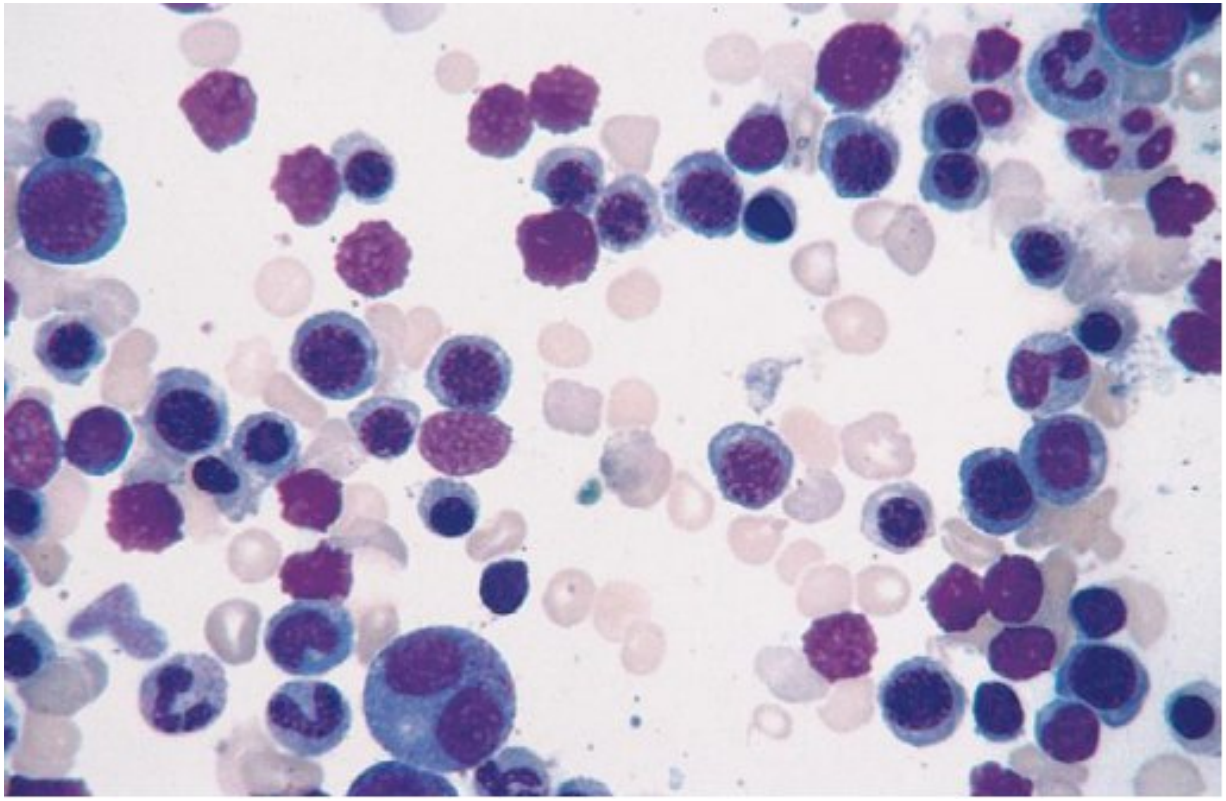
Accumulation of **hemosiderin**, a condition referred to as **hemosiderosis**

Renal hemosiderosis is characteristic of IV hemolysis

Pigment gallstones

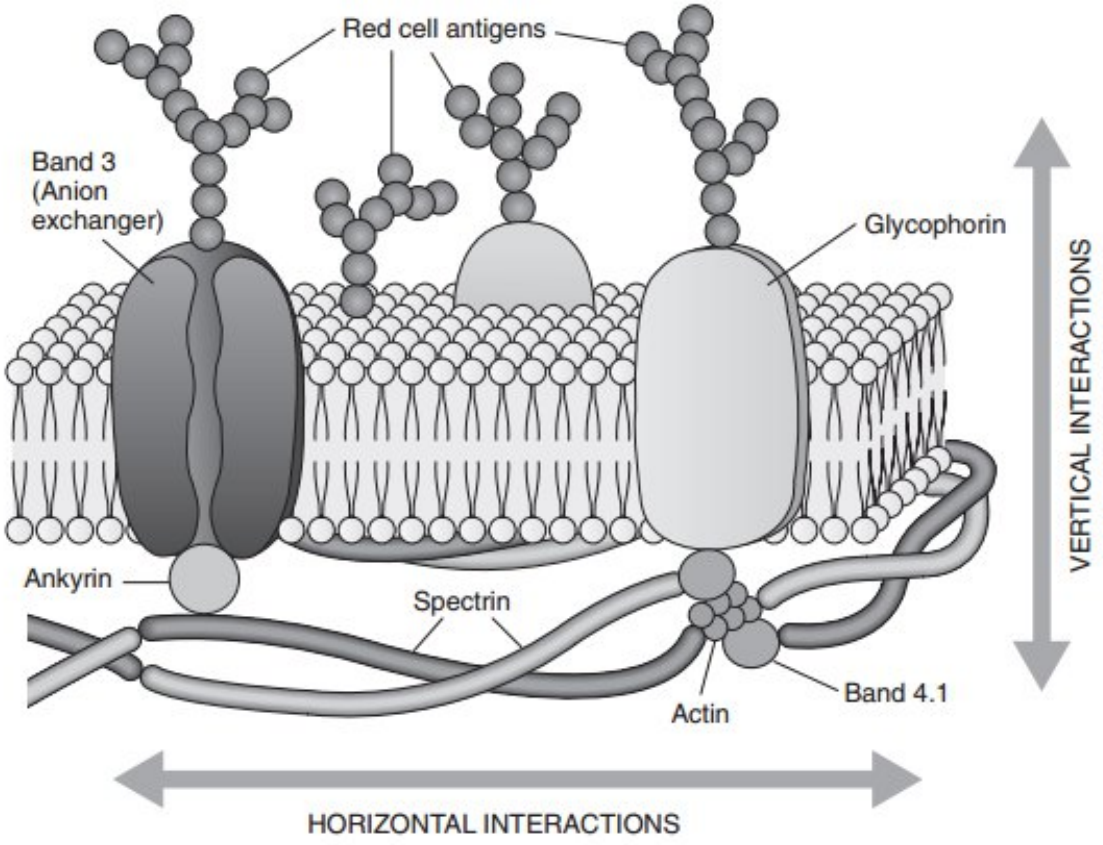
Extramedullary hematopoiesis can appear in the liver, spleen, and lymph nodes.

Marrow changes in hemolytic anemia



Hereditary Spherocytosis (HS)

Structure of the erythrocyte plasma membrane



Hereditary Spherocytosis (HS)

Inherited disorder caused by intrinsic defects in the red cell membrane skeleton

An autosomal dominant inheritance pattern is seen in about 75% of cases

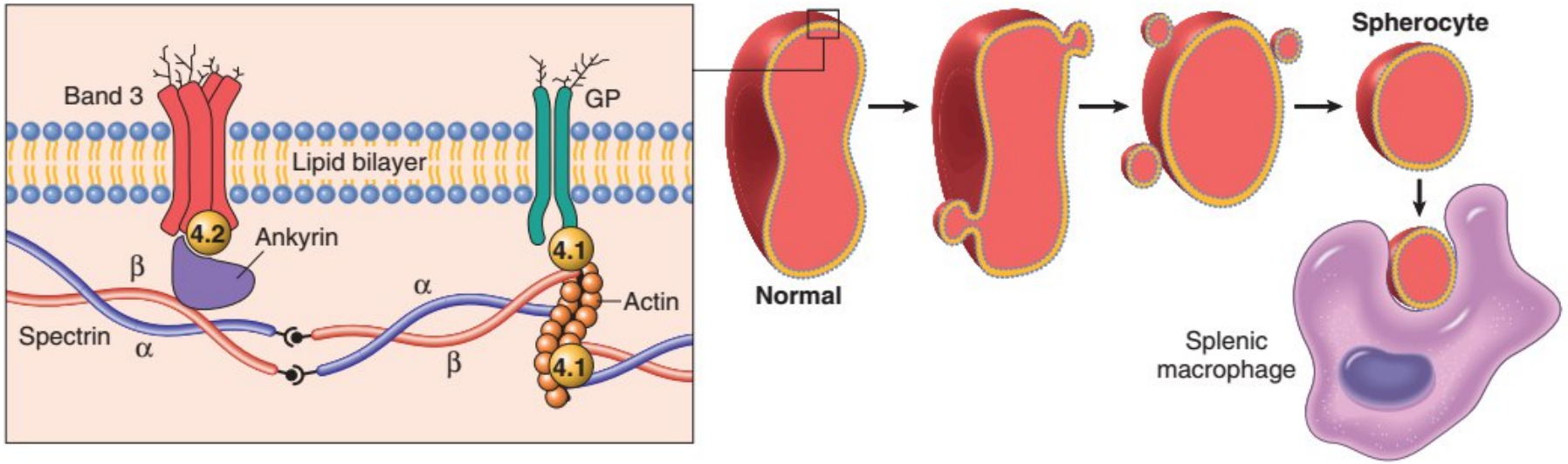
The pathogenic mutations most commonly affect ankyrin, band 3, spectrin, or band 4.2, the mutated allele fails to produce any protein.

Disturbed assembly of the skeleton as a whole, destabilizing the overlying plasma membrane

Red cells become spheroid, less deformable, and vulnerable to splenic sequestration and destruction.

Hereditary Spherocytosis (HS)

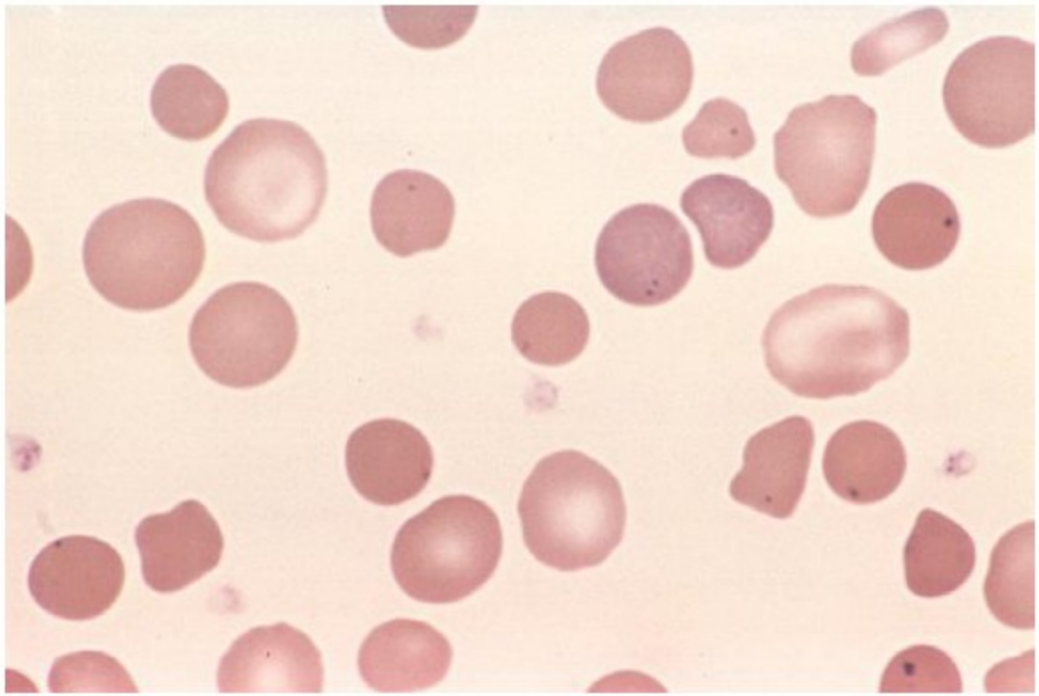
Young HS red cells are normal in shape, but the destabilized lipid bilayer sheds membrane fragments as red cells age in the circulation. The loss of membrane relative to cytoplasm “forces” the cells to assume the smallest possible diameter for a given volume, namely, a sphere



Spherocytes are less deformable, trapped in the splenic cords, where they are engulfed macrophages

The most specific morphologic finding is **spherocytosis**, apparent on smears as small, dark-staining (hyperchromic) red cells lacking the central zone of pallor

Spherocytes are also seen in other disorders associated with membrane loss, such as in autoimmune hemolytic anemia



Hereditary Spherocytosis (HS)
Clinical Features

Asymptomatic In 20% to 30% of patients mild disease, here the decreased red cell survival is readily compensated for by increased erythropoiesis.

More likely chronic hemolytic anemia of mild to moderate severity.

- **Anemia, splenomegaly, and jaundice** with greatly variable severity
- Jaundice at birth In minority (mainly compound heterozygotes).

The diagnosis is based on family history, hematologic findings, and laboratory evidence.

HS red cells have an increased mean cell hemoglobin concentration

In two thirds of the patients the red cells are abnormally sensitive to osmotic lysis when incubated in hypotonic salt solutions, which causes the influx of water into spherocytes with little margin for expansion.

Incubated Osmotic Fragility

Hereditary Spherocytosis (HS) complications

Aplastic crises

- Usually triggered by an acute parvovirus infection which kills red cell progenitors causing red cell production to cease until immune response commences in 1-2 weeks

Hemolytic crises

- Produced by intercurrent events leading to increased splenic destruction of red cells (e.g., infectious mononucleosis); these are clinically less significant than aplastic crises.

Cholelithiasis with bilirubin gallstones

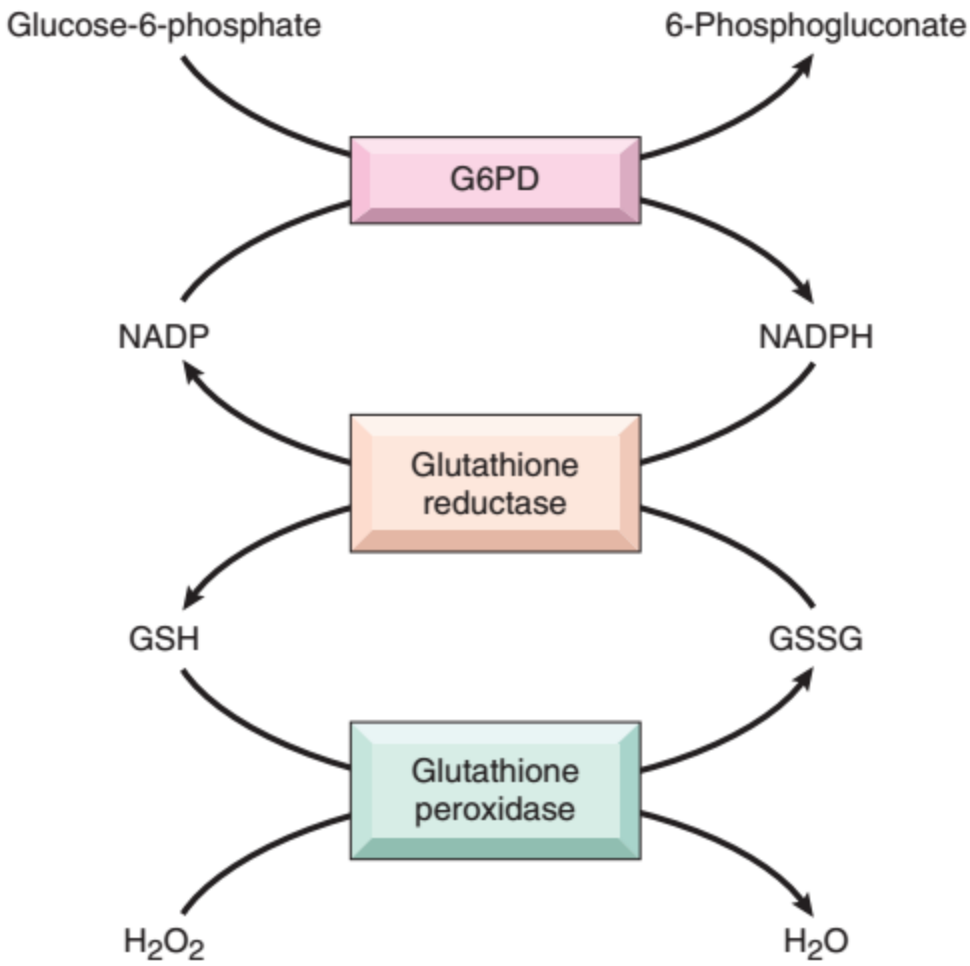
Glucose-6-phosphate dehydrogenase (G6PD) deficiency

G6PD deficiency is a genetic disorder affecting the hexose monophosphate shunt pathway leading to decreased levels of the antioxidant glutathione (GSH)

G6PD reduces nicotinamide adenine dinucleotide phosphate (NADP) to NADPH while oxidizing glucose-6-phosphate

NADPH then provides reducing equivalents needed for conversion of oxidized glutathione to reduced glutathione, which protects against oxidant injury by participating as a cofactor in reactions that neutralize compounds such as H2O2

G6PD deficiency is a recessive X-linked trait, placing males at higher risk for symptomatic disease.


Glucose-6-phosphate
6-Phosphogluconate
G6PD
NADP
NADPH
Glutathione reductase
GSH
GSSG
Glutathione peroxidase
H_2O_2
H_2O

G6PD deficiency

G6PD variants associated with hemolysis result in misfolding of the protein, making it more susceptible to proteolytic degradation. Two variants cause most of the clinically significant hemolytic anemias.

- G6PD **(A– type):** the half-life of G6PD − is moderately reduced, The hemolysis is intermittent because only older erythrocytes have decreased levels of glucose-6 phosphate dehydrogenase.
- G6PD **Mediterranean** : the half-life of G6PD is more markedly abnormal, the hemolysis is greater in individuals with the highly unstable G6PD Mediterranean variant, in addition to decreased stability there is decreased synthesis

G6PD deficiency

The episodic hemolysis that is characteristic of G6PD deficiency is caused by exposures that generate oxidant stress

- **Infections**; most common, viral hepatitis, pneumonia, and typhoid fever are most likely.
- The oxidant drugs including antimalarials (e.g., primaquine and chloroquine), sulfonamides, nitrofurantoins, and others.
- **Fava bean**, which generates oxidants when metabolized. "**Favism**" is prevalent in the Mediterranean, Middle East, and parts of Africa where consumption is prevalent

G6PD deficiency

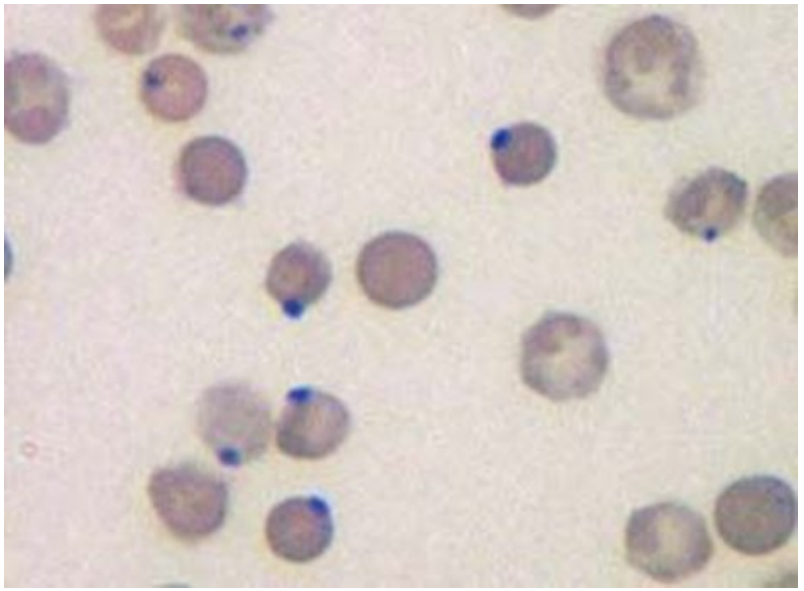
Uncommonly, G6PD deficiency presents as neonatal jaundice or a chronic low-grade hemolytic anemia in the absence of infection or known environmental triggers.

Acute intravascular hemolysis, marked by anemia, hemoglobinemia, and hemoglobinuria, usually begins 2 to 3 days following exposure of G6PD-deficient individuals to oxidants and is greater in individuals with the highly unstable G6PD Mediterranean variant

G6PD deficiency

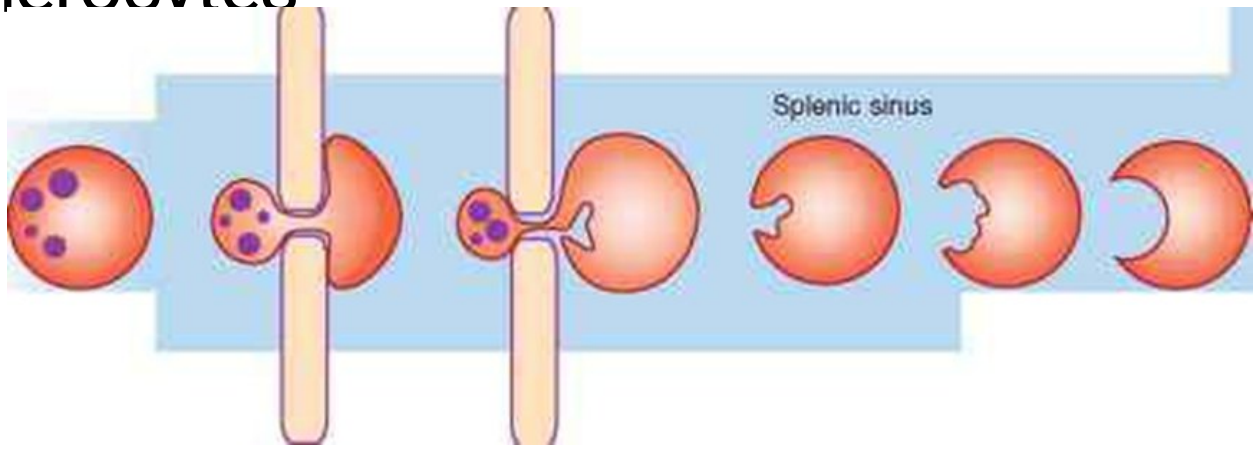
Oxidants cause both intravascular and extravascular hemolysis in G6PD-deficient individuals.

High levels of oxidants causes denaturation of hemoglobin and form membrane bound precipitates known as **Heinz bodies** which can damage the membrane sufficiently to cause intravascular hemolysis

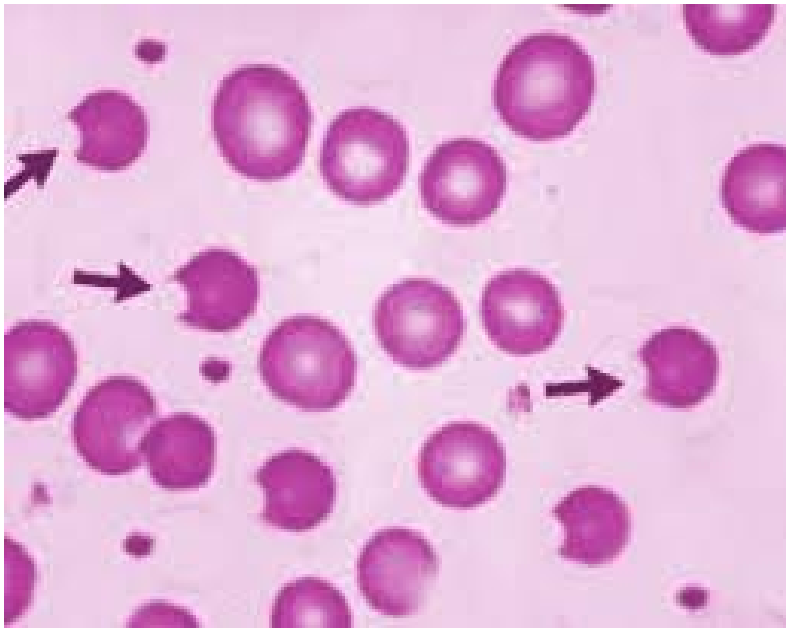


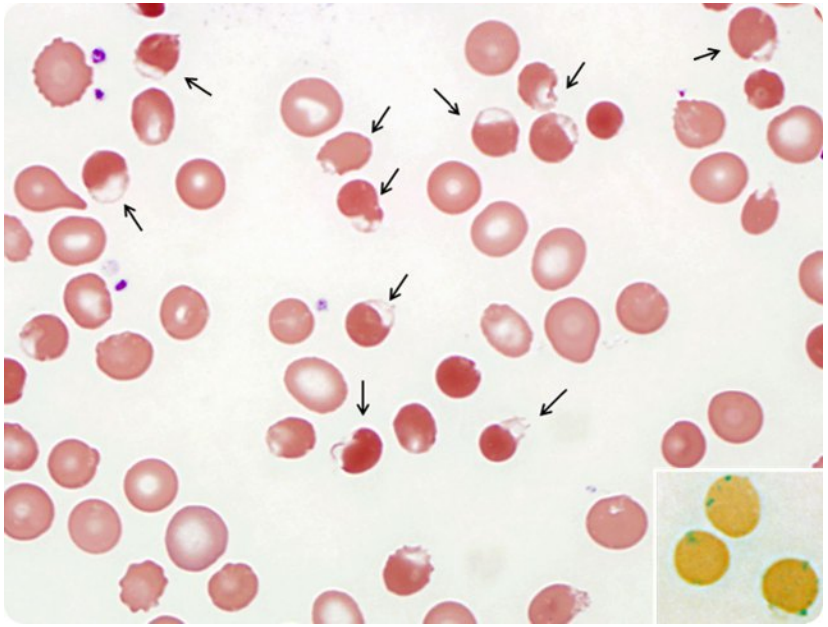
As inclusion-bearing red cells pass through the splenic cords, macrophages pluck out the Heinz bodies giving the RBCs the characteristic appearance of bite cells

Less severe membrane damage results in decreased red cell deformability i.e spherocytes



G6PD deficiency





G6PD deficiency

Both bite cells and spherocytes are trapped in splenic cords and removed rapidly by phagocytes

Because hemolytic episodes related to G6PD deficiency occur intermittently, features related to chronic hemolysis (e.g., splenomegaly, cholelithiasis) are absent.

Only older red cells are at risk for lysis, as younger RBCs has near normal levels of G6PD which is unstable and has shorter half life, the RBCs become more G6PD deficient as they mature, hemolysis ceases when only

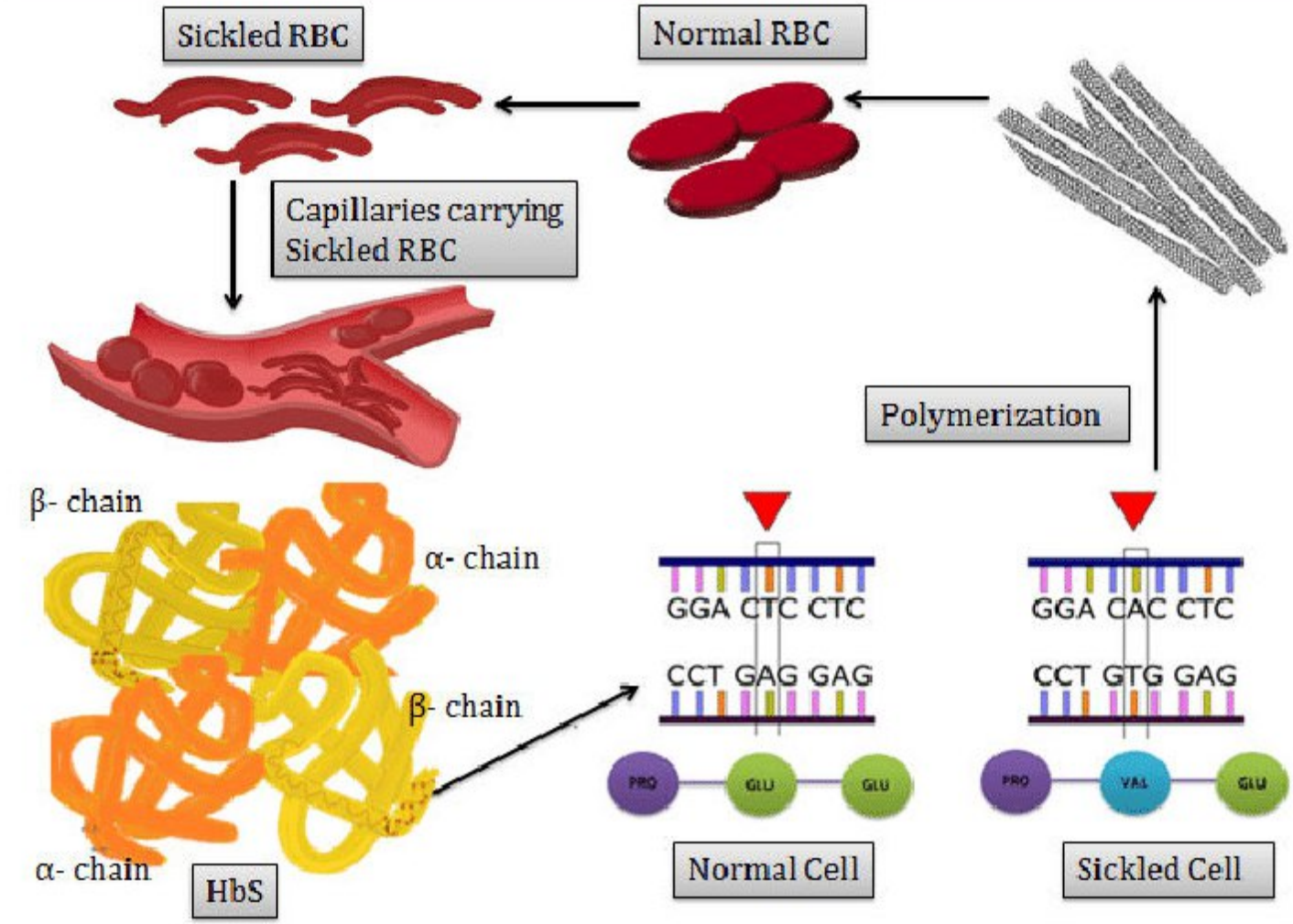
younger G6PD-replete red cells remain

Sickle Cell Disease

Sickle cell disease is caused by a point mutation in the sixth codon of β-globin gene that leads to the replacement of a glutamate amino acid with a valine amino acid.

Happens in patients who are homozygous (has 2 mutated allele) for HbS, a condition called **HbSS** disease, one mutated allele leads to a carrier state called **HbAS**

Resultant hemoglobin undergoes polymerization, leading to red cell distortion, hemolytic anemia, microvascular obstruction, and ischemic tissue damage.


Sickled RBC
Normal RBC
Capillaries carrying Sickled RBC
Polymerization
β- chain
α- chain
β- chain
α- chain
HbS
GGA CTC CTC
CCT GAG GAG
PRO
GLU
GLU
Normal Cell
GGA CAC CTC
CCT GTG GAG
PRO
VAL
GLU
Sickled Cell

Sickle Cell Disease Pathogenesis

The RBCs is sickle or holly-leaf in shape, non deformable.

The major pathologic manifestations are **chronic hemolysis, microvascular occlusions, and tissue damage**

Sickled red cells are also mechanically fragile, leading to **some** intravascular hemolysis as well.

Variables affecting the degree of sickling

Interaction of HbS with the other types of hemoglobin in the cell:

- Greatest sickling in HbSS
- In Sickle cell trait (HbAS), HbA interferes with HbS polymerization,
- HbF inhibits the polymerization of HbS, infants do not become symptomatic until they reach 5 or 6 months

Mean cell hemoglobin concentration (MCHC).

- Higher HbS concentrations increase the probability of sickling
- Lower MCHC e.g. α thalassemia decrease sickling

variables affecting the degree of sickling

A decrease in Intracellular pH. augments the tendency for sickling.

High Transit time of red cells through microvascular beds e.g. inflamed tissues

Sickle Cell Disease Pathogenesis

Microvascular occlusions, are responsible for the most serious clinical features, these are dependent on

- Subtle red cell membrane damage
- Local factors, such as inflammation or vasoconstriction
- Increased adhesion molecules on sickle red cells

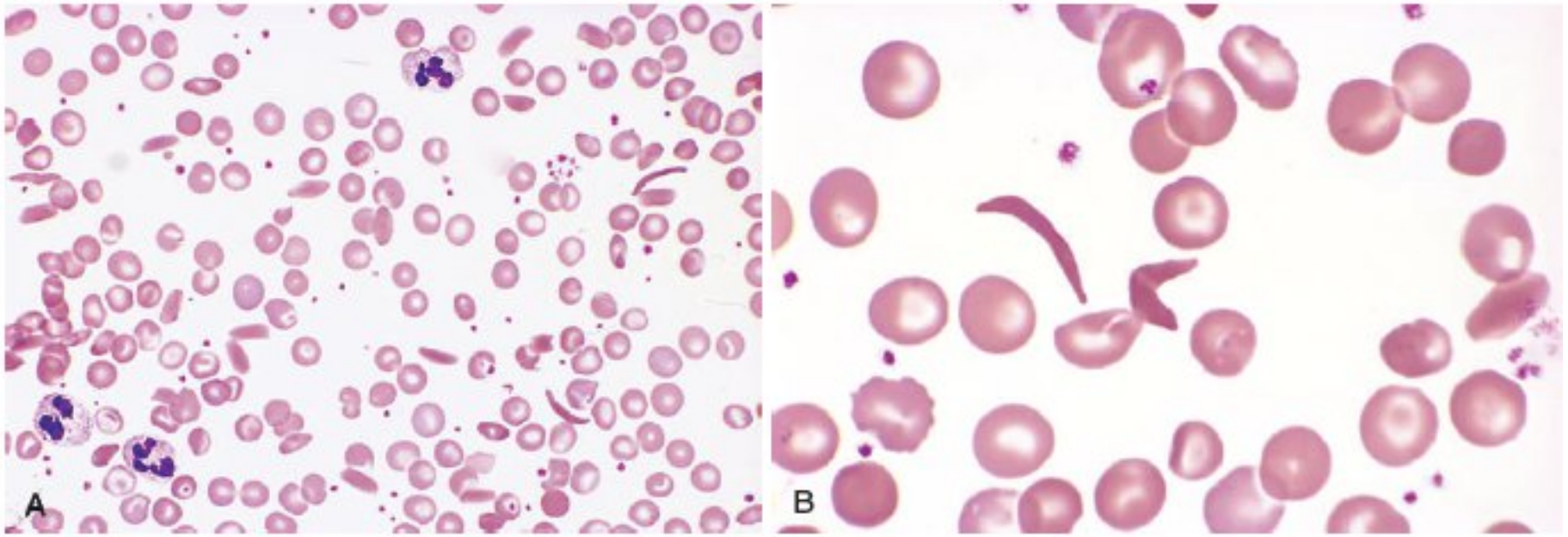
Stagnation of RBCs lead to prolonged exposure to low oxygen that augments sickling that in turn worsens occlusion and generate a vicious circle of occlusion and further sickling

Sickle Cell Disease Pathogenesis

In early childhood, trapping of sickled red cells occurs in the spleen leading to its enlargement

With time, splenic infarction, fibrosis, and progressive shrinkage, so that by adolescence or early adulthood only a fibrous splenic tissue is left; this process is called **autosplenectomy,** leading to significant risk of infection by encapsulated organisms in these patients

Sickle Cell Disease
Morphology

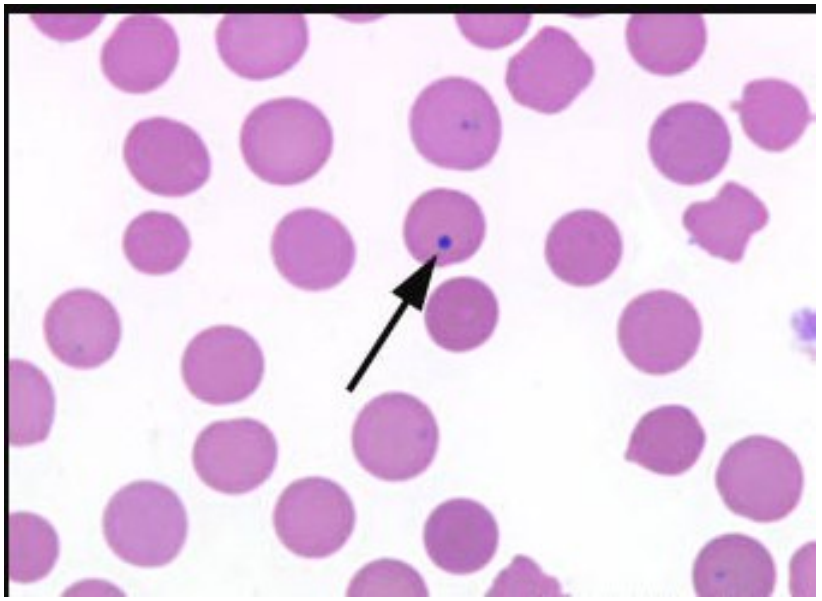


Sickle Cell Disease
Morphology

The peripheral blood demonstrates variable numbers of **sickled cells and** target cells and polychromasia from reticulocytosis

Howell-Jolly bodies (small nuclear remnants) are present in some red cells due to the asplenia

The bone marrow is hyperplastic as a result of a compensatory erythroid hyperplasia.



Sickle Cell Disease
Clinical Features

Manifestations of anemia and jaundice from hyperbilirubinemia

Course is characterized by a variety of acute complications called “crises”

Vaso-occlusive crises or pain crises

- hypoxic injury and infarction that cause severe pain
- Most commonly involved sites are the bones, lungs, and splanchnic vessels
- Infection, dehydration, acidosis

Sequestration crises

- Entrapment of sickle red cells leads to rapid splenic enlargement, hypovolemia, and sometimes shock
- In children

Sickle Cell Disease
Clinical Features

Aplastic crises, infection of red cell progenitors by parvovirus B19

Acute chest syndrome

- Vaso-occlusive crisis involving the lungs
- Fever, cough, chest pain, and pulmonary infiltrates

Priapism, stroke and **retinopathy**

Sickle Cell Disease
Clinical Features

Chronic complications

- Impairment of growth and development,
- organ damage affecting the spleen, heart, kidneys, and lungs and loss of concentrating ability of the kidney

Increased susceptibility to **infection** with encapsulated organisms, **Pneumococcus pneumoniae and Haemophilus influenza septicemia and meningitis**

Diagnosis based on Hb electrophoresis and DNA analysis

Mainstay of treatment is the disease modifying agent hydroxyurea

- Increase red cell Hb F levels, which occurs by unknown mechanisms
- Antiinflammatory effect, stems from an inhibition of leukocyte production.

Thank you and good Luck

Anemia

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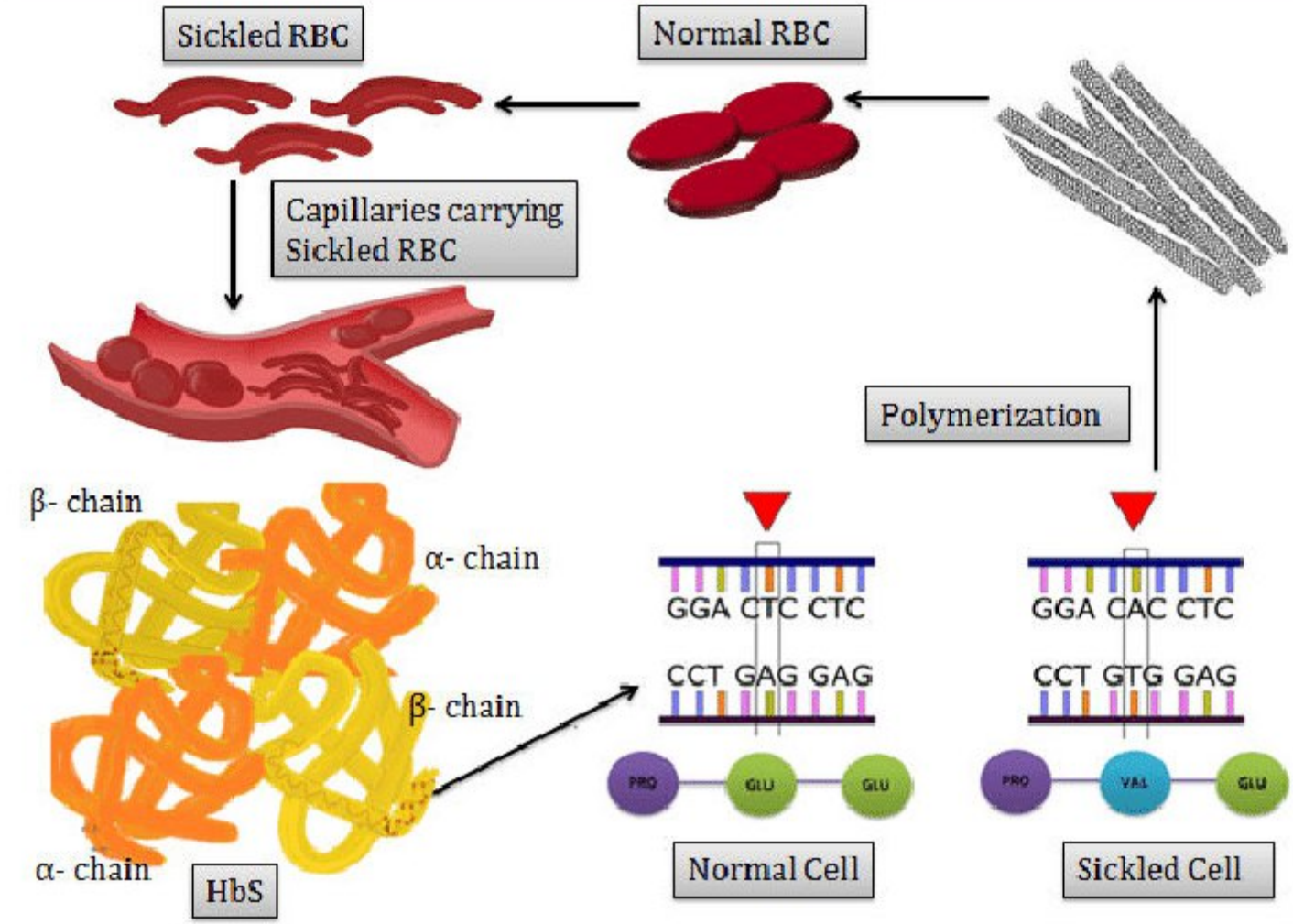
Sickle Cell Disease

Sickle cell disease is caused by a point mutation in the sixth codon of β-globin gene that leads to the replacement of a glutamate amino acid with a valine amino acid.

Happens in patients who are homozygous (has 2 mutated allele) for HbS, a condition called **HbSS** disease, one mutated allele leads to a carrier state called **HbAS**

Resultant hemoglobin undergoes polymerization, leading to red cell distortion, hemolytic anemia, microvascular obstruction, and ischemic tissue damage.


Sickled RBC
Normal RBC
Capillaries carrying Sickled RBC
Polymerization
β- chain
α- chain
β- chain
α- chain
HbS
GGA CTC CTC
CCT GAG GAG
PRO
GLU
GLU
Normal Cell
GGA CAC CTC
CCT GTG GAG
PRO
VAL
GLU
Sickled Cell

Sickle Cell Disease Pathogenesis

The RBCs is sickle or holly-leaf in shape, non deformable.

The major pathologic manifestations are **chronic hemolysis, microvascular occlusions, and tissue damage**

Sickled red cells are also mechanically fragile, leading to **some** intravascular hemolysis as well.

Variables affecting the degree of sickling

Interaction of HbS with the other types of hemoglobin in the cell:

- Greatest sickling in HbSS
- In Sickle cell trait (HbAS), HbA interferes with HbS polymerization,
- HbF inhibits the polymerization of HbS, infants do not become symptomatic until they reach 5 or 6 months

Mean cell hemoglobin concentration (MCHC).

- Higher HbS concentrations increase the probability of sickling
- Lower MCHC e.g. α thalassemia decrease sickling

variables affecting the degree of sickling

A decrease in Intracellular pH. augments the tendency for sickling.

High Transit time of red cells through microvascular beds e.g. inflamed tissues

Sickle Cell Disease Pathogenesis

Microvascular occlusions, are responsible for the most serious clinical features, these are dependent on

- Subtle red cell membrane damage
- Local factors, such as inflammation or vasoconstriction
- Increased adhesion molecules on sickle red cells

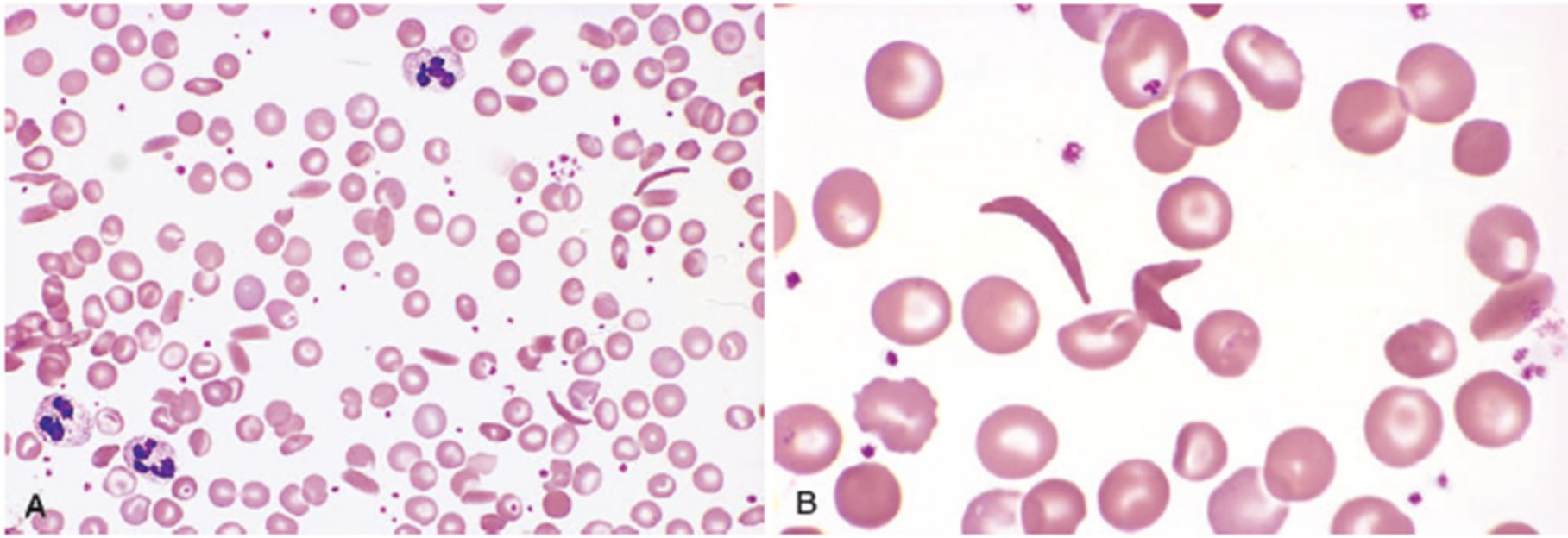
Stagnation of RBCs lead to prolonged exposure to low oxygen that augments sickling that in turn worsens occlusion and generate a vicious circle of occlusion and further sickling

Sickle Cell Disease Pathogenesis

In early childhood, trapping of sickled red cells occurs in the spleen leading to its enlargement

With time, splenic infarction, fibrosis, and progressive shrinkage, so that by adolescence or early adulthood only a fibrous splenic tissue is left; this process is called **autosplenectomy,** leading to significant risk of infection by encapsulated organisms in these patients

Sickle Cell Disease
Morphology

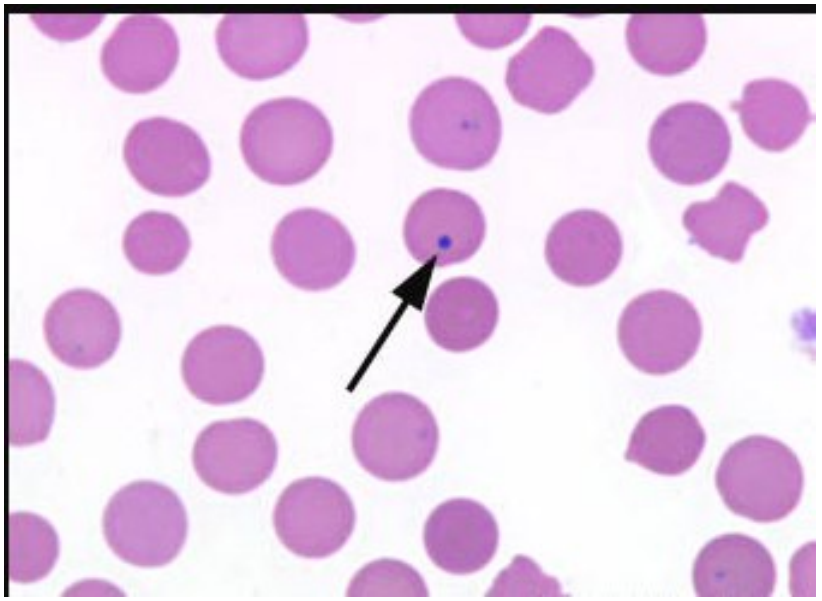


Sickle Cell Disease
Morphology

The peripheral blood demonstrates variable numbers of **sickled cells and** target cells and polychromasia from reticulocytosis

Howell-Jolly bodies (small nuclear remnants) are present in some red cells due to the asplenia

The bone marrow is hyperplastic as a result of a compensatory erythroid hyperplasia.



Sickle Cell Disease
Clinical Features

Manifestations of anemia and jaundice from hyperbilirubinemia

Course is characterized by a variety of acute complications called “crises”

Vaso-occlusive crises or pain crises

- hypoxic injury and infarction that cause severe pain
- Most commonly involved sites are the bones, lungs, and splanchnic vessels

Sequestration crises

- Entrapment of sickle red cells leads to rapid splenic enlargement, hypovolemia, and sometimes shock
- In children

Sickle Cell Disease
Clinical Features

Aplastic crises, infection of red cell progenitors by parvovirus B19

Acute chest syndrome

- Vaso-occlusive crisis involving the lungs
- Fever, cough, chest pain, and pulmonary infiltrates

Priapism, stroke and **retinopathy**

Sickle Cell Disease
Clinical Features

Chronic complications

- Impairment of growth and development,
- organ damage affecting the spleen, heart, kidneys, and lungs and loss of concentrating ability of the kidney

Increased susceptibility to **infection** with encapsulated organisms, **Pneumococcus pneumoniae and Haemophilus influenza septicemia and meningitis**

Diagnosis based on Hb electrophoresis and DNA analysis

Thalassemia Syndromes

Inherited mutations that decrease the synthesis of either the **α-globin (α-thalassemia)** or **β-globin chains (β-thalassemia**) leading to anemia, tissue hypoxia, and red cell hemolysis.

The two α chains in HbA are encoded by 4 α-globin genes on **chromosome 16**, while the two β chains are encoded by a 2 β-globin genes on chromosome 11.

Anemia occurs through two mechanisms: decreased red cell production, and decreased red cell lifespan

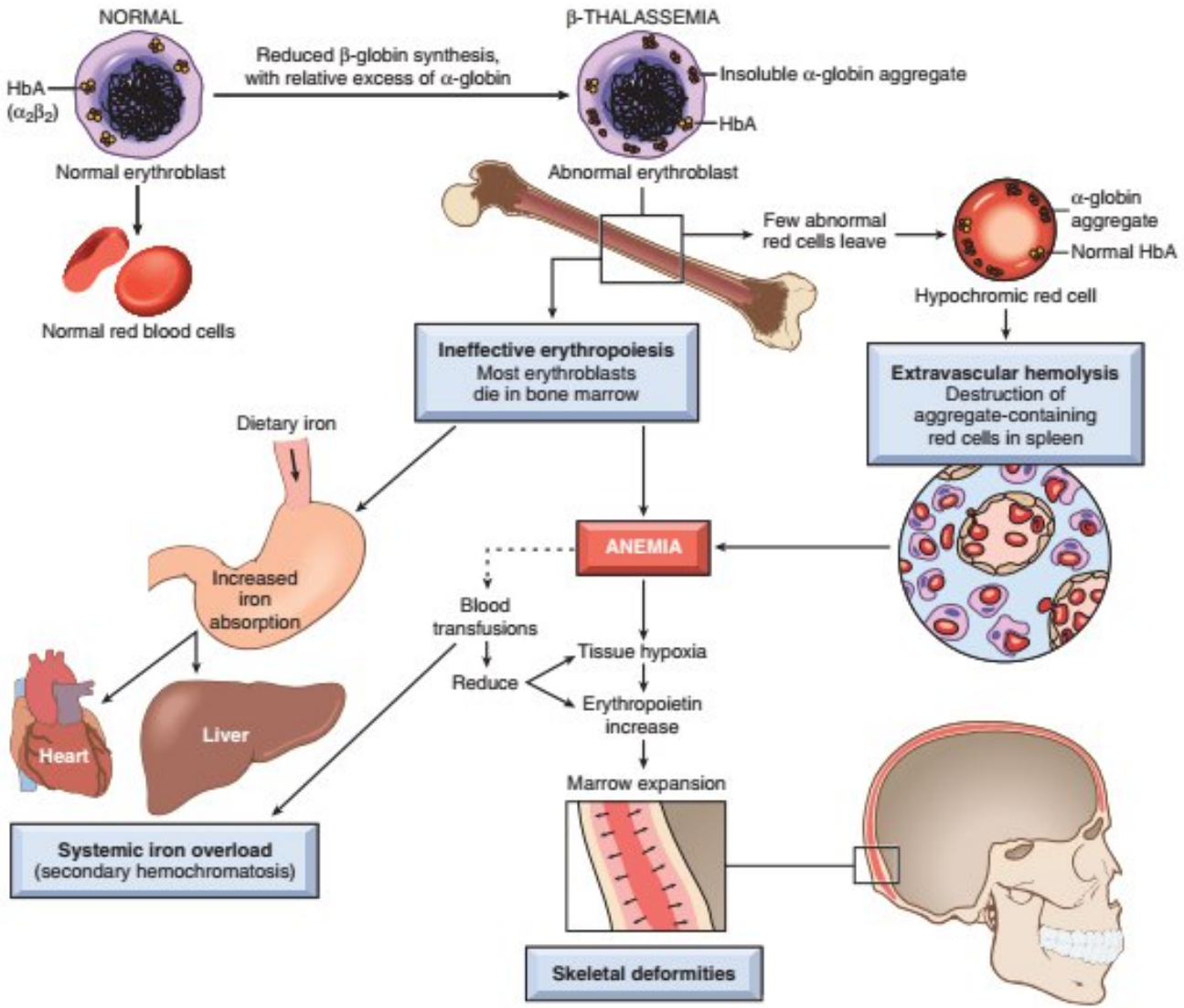
β-Thalassemia

The β- thalassemias are caused by mutations that diminish the synthesis of β-globin chains.

The causative mutations fall into two categories:

- β0 mutations, associated with absent β-globin synthesis
- β+ mutations, characterized by reduced (but detectable) β-globin synthesis.

β-Thalassemias Pathogenesis



β-Thalassemias
Pathological Consequences

Anemia, produced by different mechanisms

- **Ineffective erythropoiesis,** Unpaired α chains precipitate within red cell precursors, forming insoluble inclusions that lead to damage of its membrane, 70% to 85% of red cell precursors undergo apoptosis.
- **Extravascular hemolysis** red cells that are released from the marrow also contain inclusions and have membrane damage, leaving them prone to splenic sequestration and destruction

β-Thalassemia Pathological Consequences

Massive erythroid hyperplasia

- The expanding mass of red cell precursors erodes the bony cortex, impairs bone growth, and produces skeletal abnormalities

Extramedullary hematopoiesis

- Erythropoiesis will involve liver spleen, and lymph nodes
- The metabolically active erythroid progenitors steal nutrients from other tissues that are already oxygen-starved, causing severe cachexia in untreated patients.

Secondary hemochromatosis

β-Thalassemia Clinical Syndromes

β-thalassemia major

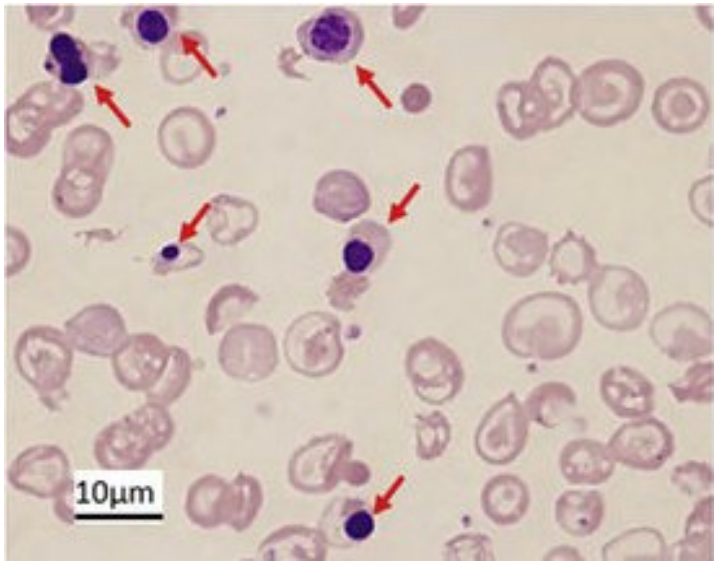
- Two βthalassemia alleles (Homozygous i.e. β+/β+, β+/β0, or β0/β0)
- Severe, transfusion-dependent anemia, Common in **Mediterranean countries** , parts of Africa, and Southeast Asia.
- Anemia manifests at 6 to 9 months age (Hb F $\rightarrow$HbA)
- Hemoglobin is very low, composed mainly of Hb F, HbA is either absent or very low, HbA2 may be high

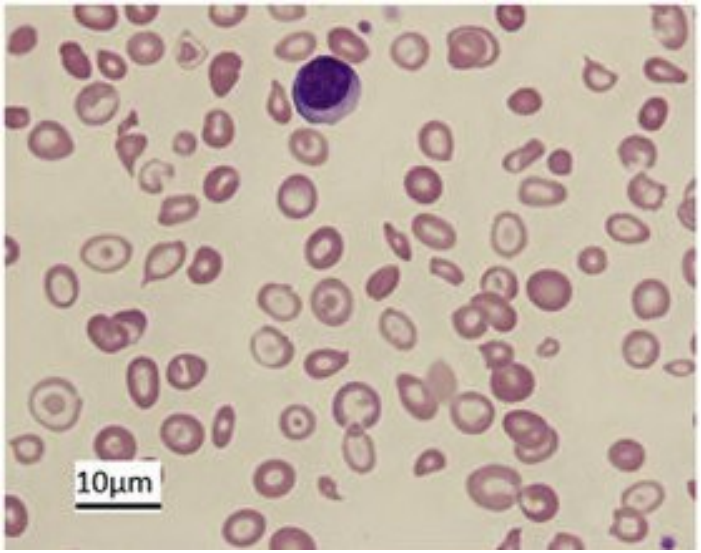
β-Thalassemia Clinical Syndromes

β-thalassemia major

- Severe anemia, growth retardation and death if untreated
- Pallor, jaundice, cheekbones and other bony prominences are enlarged and distorted.
- Hepatosplenomegaly due to extramedullary hematopoiesis
- Hemochromatosis leads to Cardiac disease, most important cause of death, affects liver, endocrine glands and gonads too

β-Thalassemia
MORPHOLOGY





β-Thalassemia MORPHOLOGY

Marked anisocytosis: variation in size

Poikilocytosis: variation in shape

Microcytosis and **hypochromia**

Target cells: hemoglobin collects in the center of the cell

The reticulocyte count is elevated, but it is lower than expected for the severity of anemia because of the ineffective erythropoiesis.

Variable numbers of normoblasts seen in the peripheral blood as a result of “stress” erythropoiesis

β-Thalassemia Clinical Syndromes

β-thalassemia minor or β-thalassemia trait

- Heterozygotes (one allele i.e. β+/β or β0/β)
- Affected patients are usually asymptomatic, anemia is mild and mild abnormalities on blood smear.
- Hemoglobin electrophoresis usually reveals an increase in HbA2 (α2δ2) to 4% to 8% of the total hemoglobin
- HbF levels are generally normal or occasionally slightly increased.

β-Thalassemia Clinical Syndromes

β-thalassemia intermedia.

- Milder variants of β+/β+ or β+/β0-thalassemia and unusual forms of heterozygous β-thalassemia
- The clinical and morphologic features as well as transfusion dependence of β-thalassemia intermedia are milder than β-thalassemia major

α-Thalassemia

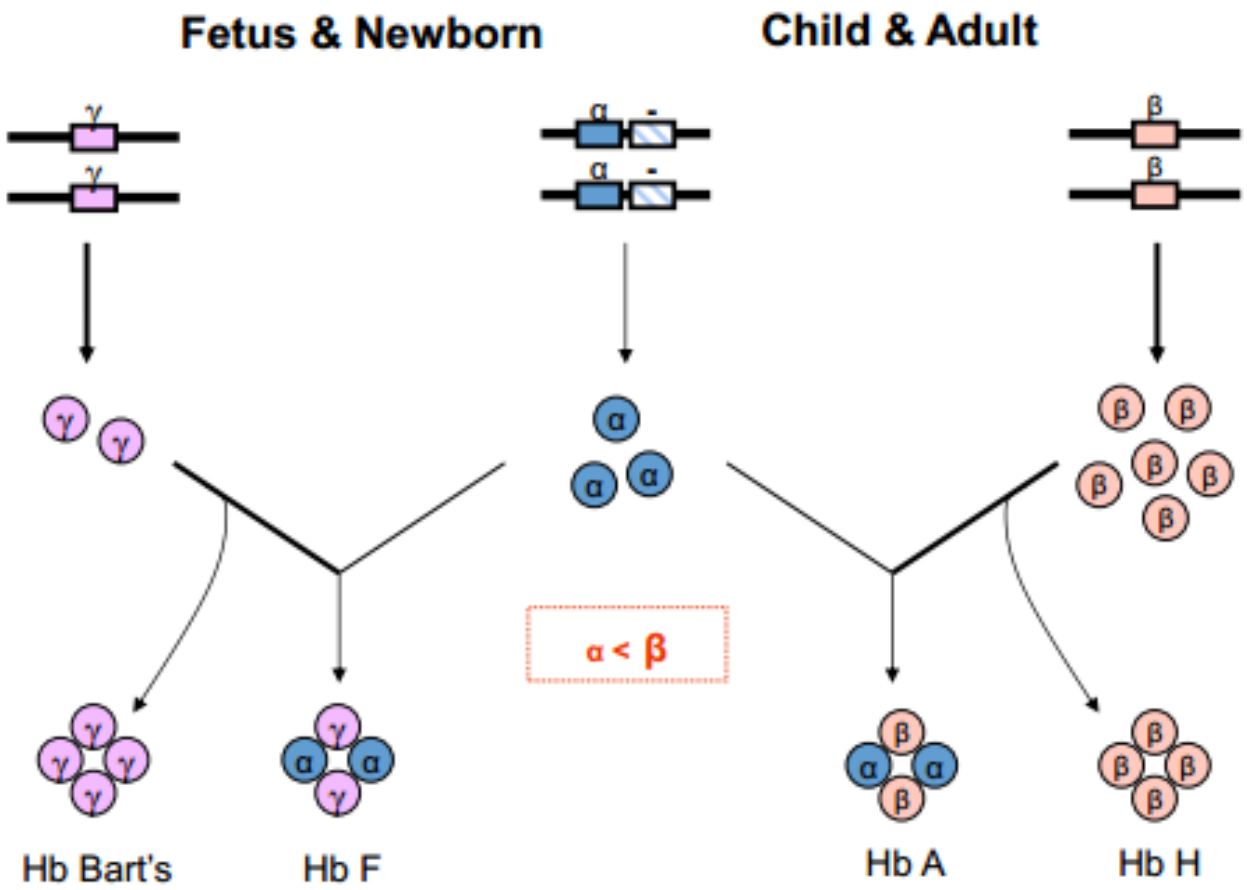
Inherited deletions that result in reduced or absent synthesis of α-globin chains.

There are four α-globin genes, and the severity of α-thalassemia depends on how many α-globin genes are affected.

Unpaired globin chains are either β, γ or δ based on patient's age, γ-globin In newborns form tetramers called hemoglobin Barts while β chain tetramers formed in adults are called HbH.

Hemolysis and ineffective erythropoiesis are less severe than in β-thalassemia because free β and γ chains are more soluble than free α chains and form fairly stable homotetramers

Alpha Thalassemia



α-Thalassemias
Clinical Syndromes

Silent Carrier State

- A single α-globin gene deletion
- No symptoms but have slight microcytosis

α-Thalassemia Trait

- Caused by the deletion of two α-globin genes
- The clinical picture in α-thalassemia trait is identical to β-thalassemia minor, minimal or no anemia, and no abnormal physical signs, **HbA2 levels are normal or low**

α-Thalassemias
Clinical Syndromes

Hemoglobin H Disease

- Deletion of three α-globin genes.
- The synthesis of α chains is markedly reduced and tetramers of β-globin forms, called HbH
- HbH precipitates and form intracellular inclusions that promote red cell sequestration and phagocytosis in the spleen. The result is a moderately severe anemia resembling β-thalassemia intermedia

α-Thalassemia Clinical Syndromes

Hydrops Fetalis

- Deletion of all four α-globin genes
- In the fetus, excess γ-globin chains form tetramers (hemoglobin Barts) that have a very poor oxygen delivery
- Fetal distress usually become evident by the third trimester of pregnancy, tissue anoxia lead to death in utero or shortly after birth, the fetus shows severe pallor, generalized edema, and massive hepatosplenomegaly

Risk of Hydrops Fetalis in Offspring Depends on Trait Genotype in Parents

Immunohemolytic Anemias

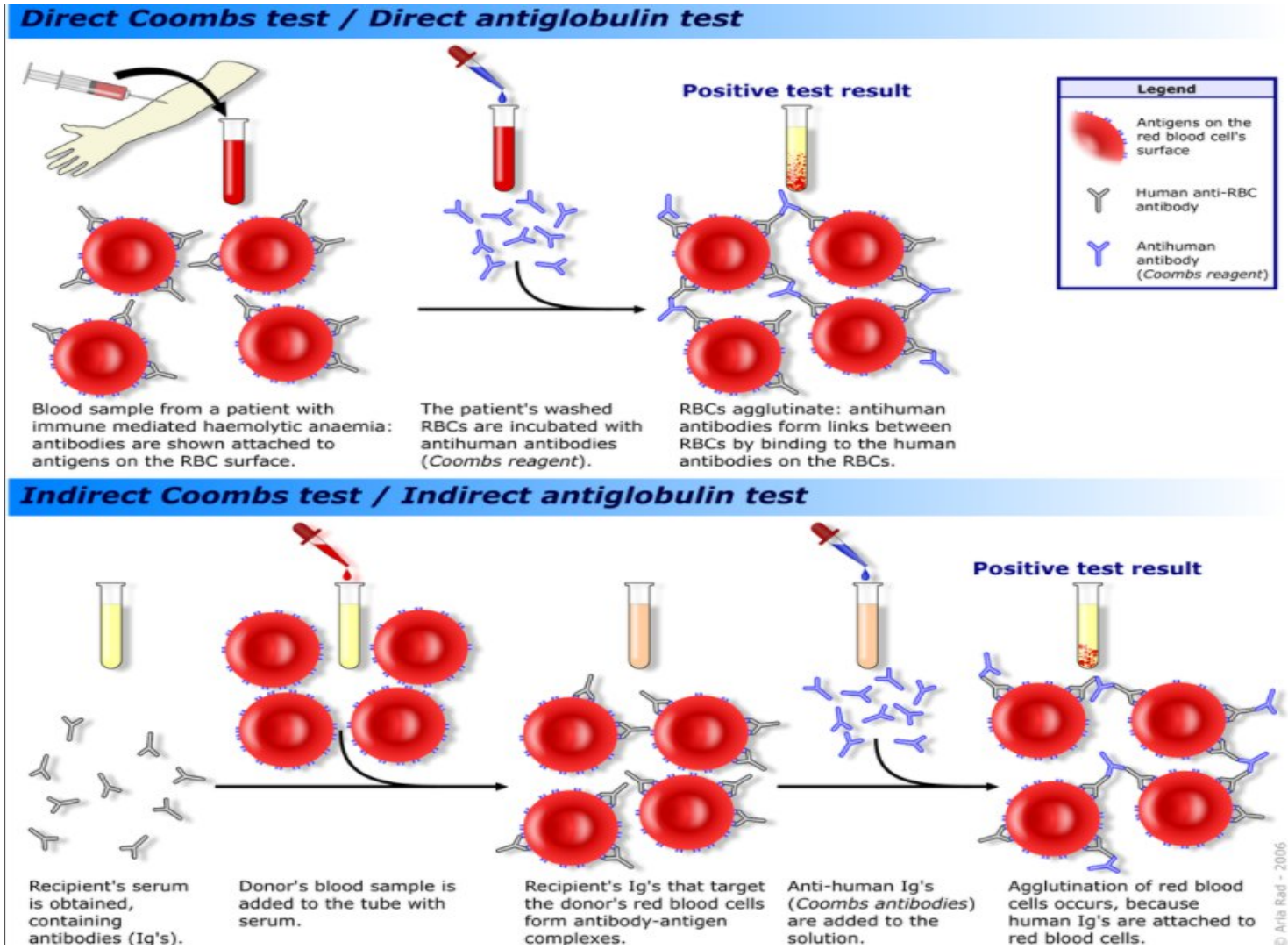
Caused by antibodies that bind to red cells, leading to their premature destruction

Divided into warm reacting antibody, clod agglutinin and cold hemolysin type

The diagnosis of immunohemolytic anemia requires the detection of antibodies and/or complement on red cells from the patient

This can be done using the direct and indirect coomb’s test

Coomb's test


Direct Coombs test / Direct antiglobulin test
Positive test result
Legend
Antigens on the red blood cell's surface
Human anti-RBC antibody
Antihuman antibody (Coombs reagent)
Blood sample from a patient with immune mediated haemolytic anaemia: antibodies are shown attached to antigens on the RBC surface.
The patient's washed RBCs are incubated with antihuman antibodies (Coombs reagent).
RBCs agglutinate: antihuman antibodies form links between RBCs by binding to the human antibodies on the RBCs.
Indirect Coombs test / Indirect antiglobulin test
Positive test result
Recipient's serum is obtained, containing antibodies (Ig's).
Donor's blood sample is added to the tube with serum.
Recipient's Ig's that target the donor's red blood cells form antibody-antigen complexes.
Anti-human Ig's (Coombs antibodies) are added to the solution.
Agglutination of red blood cells occurs, because human Ig's are attached to red blood cells.
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Immunohemolytic Anemias

Direct Coombs antiglobulin test

- Patient's red cells are mixed with sera containing antibodies that are specific for human immunoglobulin or complement
- If either immunoglobulin or complement is present on the surface of the red cells, the antibodies cause agglutination, which is easily appreciated visually as clumping.

Indirect Coombs antiglobulin test

- Patient's serum is tested for its ability to agglutinate commercially available red cells bearing particular defied antigens

Immunohemolytic Anemias

Warm Antibody Type (IgG Antibodies Active at 37°C)
Primary (idiopathic)
Secondary
Autoimmune disorders (particularly systemic lupus erythematosus)
Drugs
Lymphoid neoplasms
Cold Agglutinin Type (IgM Antibodies Active Below 37°C)
Acute (mycoplasmal infection, infectious mononucleosis)
Chronic
Idiopathic
Lymphoid neoplasms
Cold Hemolysin Type (IgG Antibodies Active Below 37°C)
Rare; occurs mainly in children following viral infections

Warm Antibody Type

The most common form

About 50% of cases are idiopathic, others are related to predisposing conditions or drugs e.g. penicillin, ceftriaxone and methyledopa

Antibodies are of the IgG class; less commonly, IgA

Partial phagocytosis of antibody covered RBCs leads to loss of there membrane converting them to **spherocytes**

The red cell hemolysis is mostly extravascular

Splenomegaly due to hyperplasia of splenic phagocytes is usually seen.

Cause of idiopathic immunohemolytic anemia is unknown, but antibodies are directed **to Rh antigens** in many cases

Drug-induced immunohemolytic anemia

Antigenic drugs

- e.g. penicillin and cephalosporins
- Hemolysis happens 1 to 2 weeks after large, intravenous doses of the offending drug
- The antibody may bind the drug only or complex of the drug and membrane protein

Tolerance-breaking drugs

- e.g. α-methyldopa
- Alteration of the immunogenicity of native epitopes and circumvention of T cell tolerance

Cold Agglutinin Type

Less common than warm antibody immunohemolytic anemia

Caused by IgM antibodies occasionally called Cold Agglutinins

Idiopathic chronic disease in adults over 50 years of age

Cold agglutinin antibodies in children and young adults appear transiently following certain infections, such as with **Mycoplasma pneumoniae,** Epstein Barr virus, cytomegalovirus, inflenza virus, and human immunodeficiency virus (HIV) leading to self-limited disease and rarely causing clinically important hemolysis

Cold Agglutinin Type

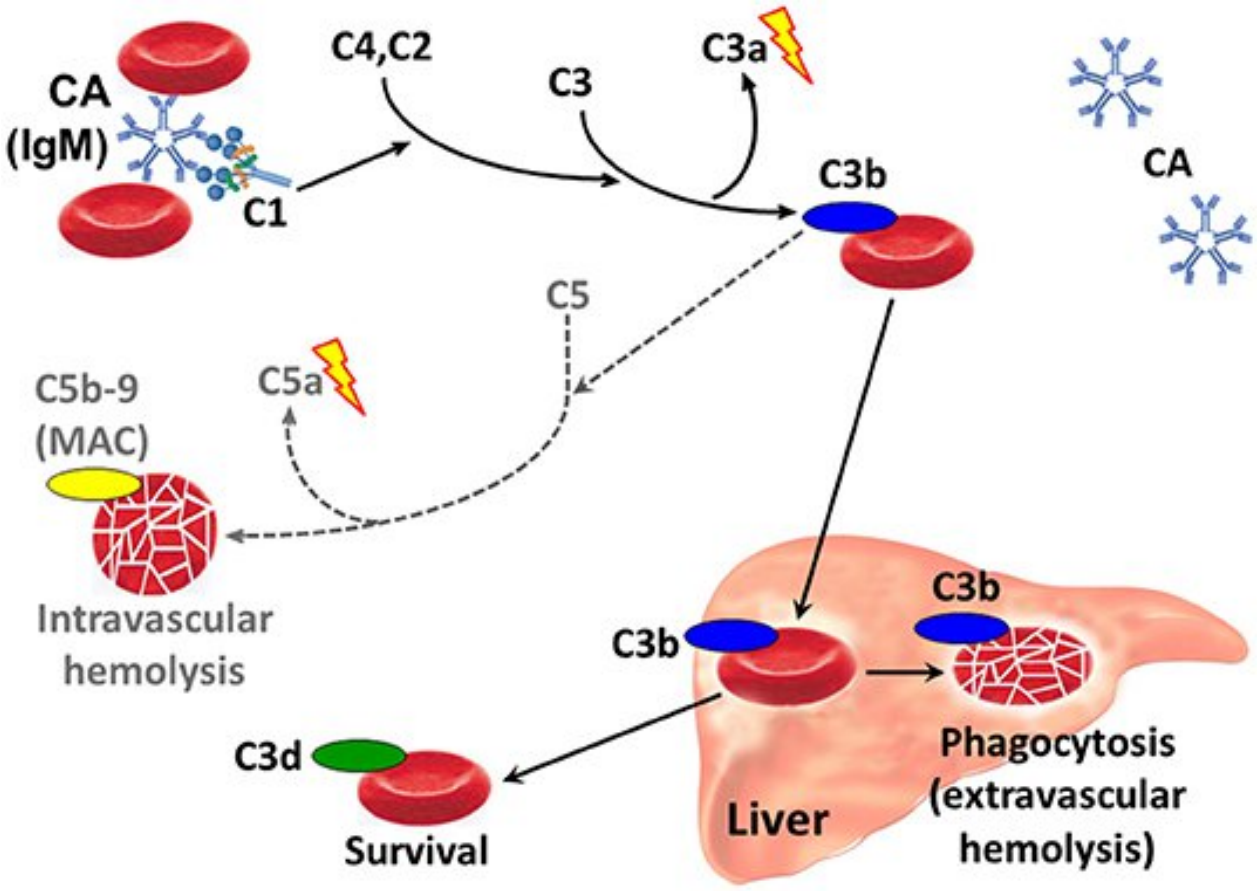
IgM binds red cells in vascular beds where the temperature may fall below 30°C, such as in exposed fingers, toes, and ears

IgM binding agglutinates red cells and fixes complement rapidly so C3b is deposited on the red cell membrane.

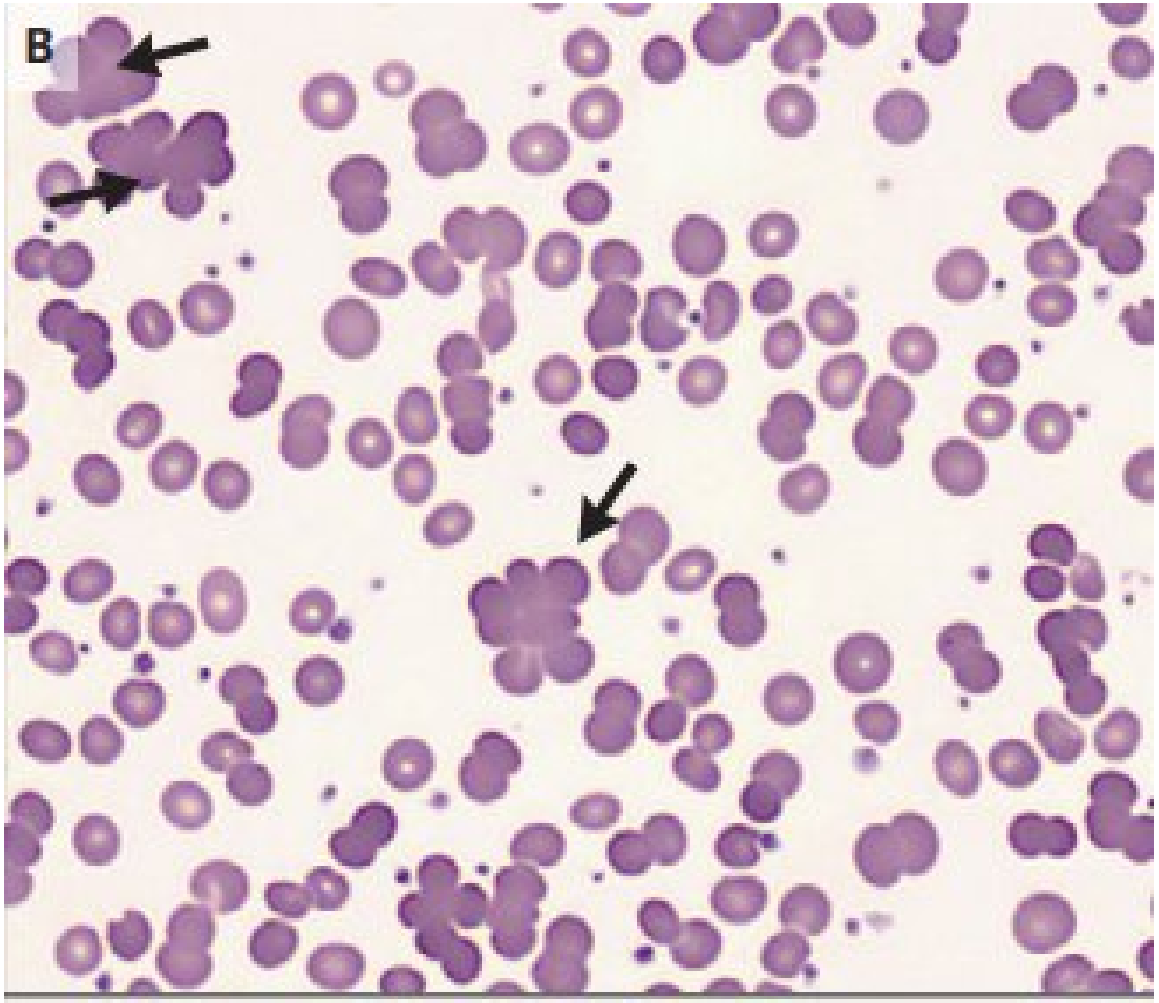
When blood warms, IgM dissociates and C3b mediate phagocytosis or occasionally the entire complement cascade is activated to the membrane attack complex

Vascular obstruction caused by agglutinated red cells results in pallor, cyanosis, and Raynaud phenomenon in body parts exposed to cold temperature

Cold Agglutinin Type



Cold Agglutinin Type



Cold Hemolysin Type

Rare , occasionally called paroxysmal cold hemoglobinuria

Primarily a disease of children

Cold reacting IgG against RBCs, initiates aggressive hemolysis through activation of the entire complement cascade

Patients experience a severe anemia, decreased haptoglobin levels, and hemoglobinuria secondary to **intravascular hemolysis**

Mechanical Red Cell Fragmentation Syndrome

Red cell are fragmented within the blood vessels (intravascular hemolysis)

Macroangiopathic hemolytic anemia ,occur in larger vessels

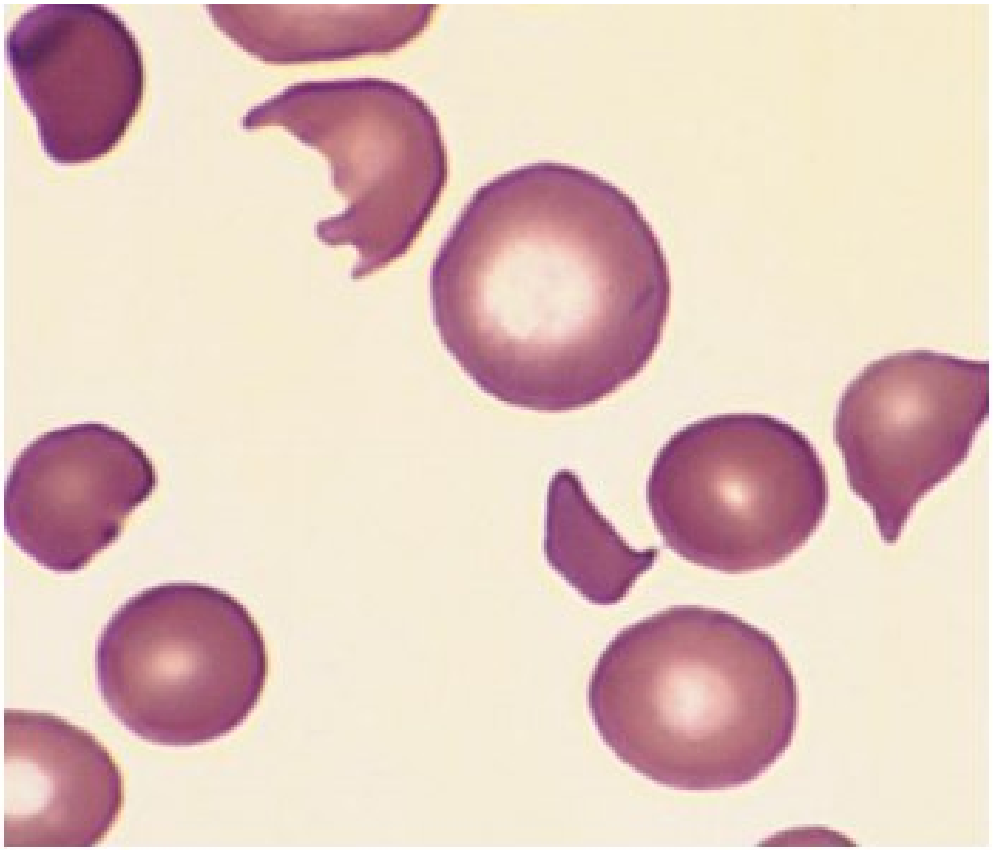
- Synthetic vascular graft
- Prosthetic valve

Microangiopathic hemolytic anemia occur in smaller vessels that become partially obstructed or narrowed

- Disseminated intravascular coagulation
- Malignant hypertension,
- Thrombocytopenic purpura (TTP)
- Hemolytic uremic syndrome (HUS)

Examination of the blood reveals a mild to moderate anemia, reticulocytosis, and fragmented red blood cells (schistocytes) and helmet cells.

Microangiopathic hemolytic anemia



Megaloblastic Anemia

Caused by impaired DNA synthesis, which often is secondary to vitamin B12 or folate deficiency.

B12 and folate are required for the formation of thymidine, one of the four bases found in DNA molecules

Megaloblastic Anemia Causes

Vitamin B_{12} Deficiency
Decreased Intake
Inadequate diet, vegetarianism
Impaired Absorption
Intrinsic factor deficiency
Pernicious anemia
Gastrectomy
Malabsorption states
Diffuse intestinal disease (e.g., lymphoma, systemic sclerosis)
Ileal resection, ileitis
Competitive parasitic uptake
Fish tapeworm infestation
Bacterial overgrowth in blind loops and diverticula of bowel

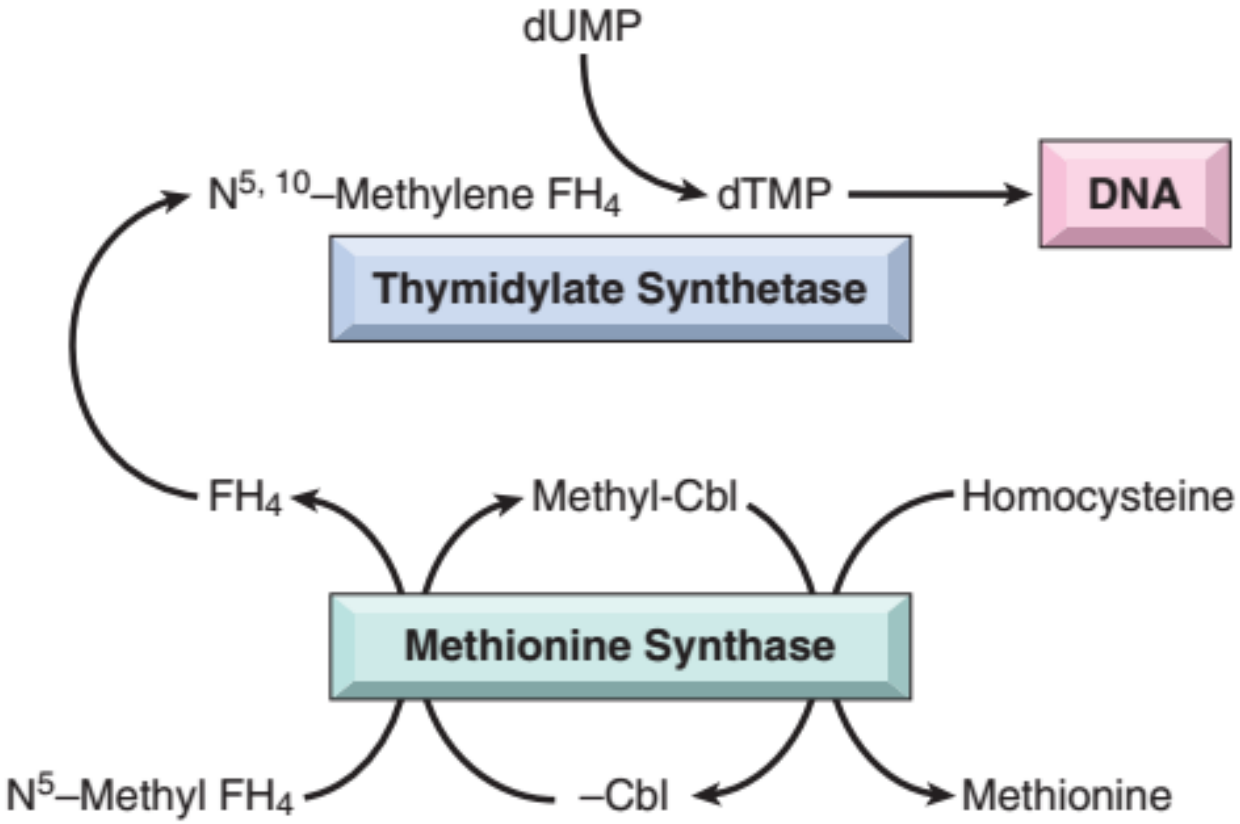
Anemias of Vitamin B12 Deficiency: Pernicious Anemia

A form of megaloblastic anemia caused by an autoimmune gastritis that impairs the production of intrinsic factor, which is required for vitamin B12 uptake from the gut.

Vitamin B12 is found in many animal food sources and is synthesized by intestinal organisms, plants and vegetables contain little cobalamin

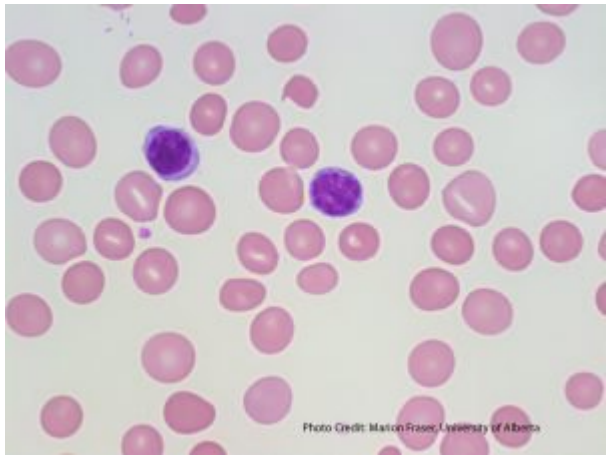
After ingestion, vitamin B12 binds to intrinsic factor (IF) secreted by gastric parietal cells and is ultimately absorbed in the distal ileum and transferred to the carrier
molecule transcobalamin II in the blood

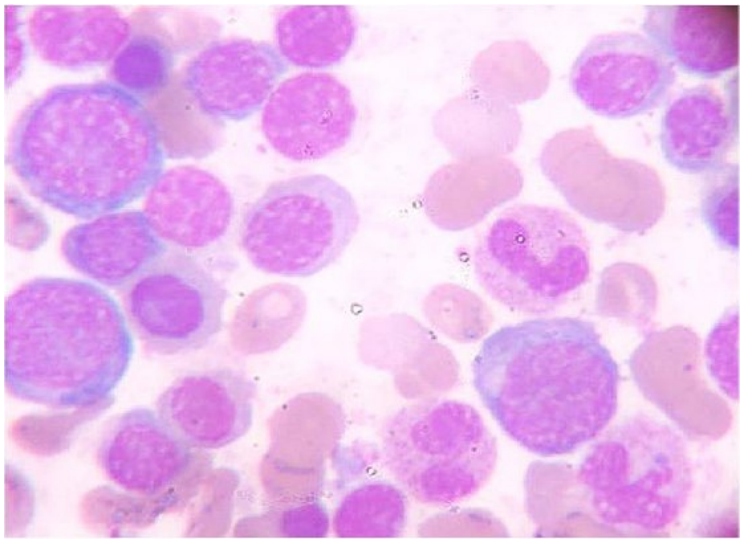
Biochemical Functions of Vitamin B12



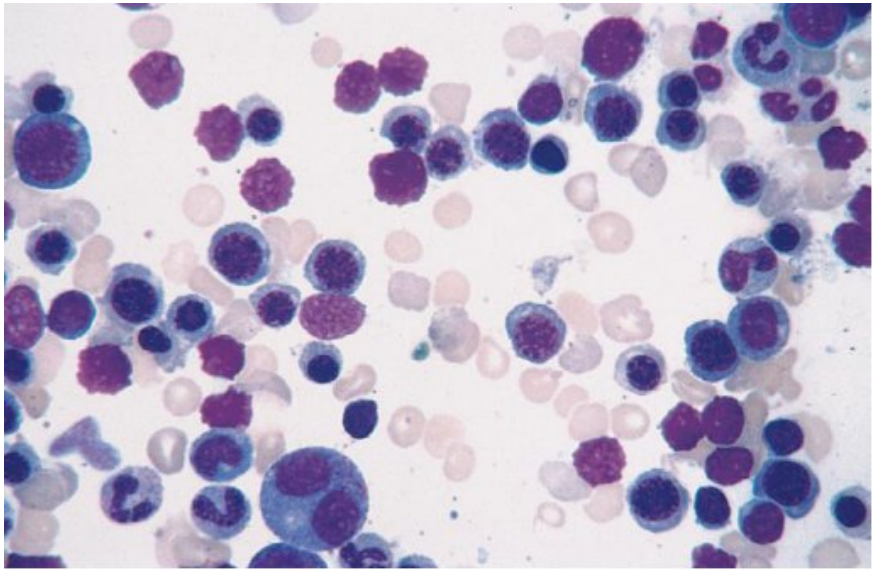
Megaloblastic Anemia

MORPHOLOGY





Erythroid hyperplasia with normal maturation



MORPHOLOGY

Peripheral blood features shared by all megaloblastic anemias

- **Macro-ovalocytes** mostly lacking the normal central pallor anisocytosis and poikilocytosis
- The reticulocyte count is low
- **Nuclear hypersegmentation** (five or more nuclear lobules)

MORPHOLOGY

The marrow is usually markedly hypercellular and **Megaloblastic changes** are detected at all stages of erythroid development**.**

- Most primitive cells are large, with a deeply basophilic cytoplasm, prominent nucleoli, and a distinctive, fine nuclear chromatin pattern
- Cytoplasmic maturation and hemoglobin accumulation proceed
  at a normal pace but the nucleus retains its finely distributed
  chromatin and fails to develop the clumped pyknotic chromatin
  typical of normoblasts, leading to nuclear-to-cytoplasmic asynchrony.
- DNA synthesis is impaired in all lines

  giant metamyelocytes and band forms

  Megakaryocytes can be abnormally large and have bizarre, multilobate nuclei

Pernicious Anemia Pathogenesis

Pernicious anemia is believed to result from an autoimmune attack on the gastric mucosa. Histologically, there is a chronic atrophic gastritis marked by a loss of parietal cells, a prominent infiltrate of lymphocytes and plasma cells

Autoantibodies are present either preventing the binding of B12 to intrinsic factor or prevent its internalization

Other Causes of B12 deficiency

- Achlorhydria
- Gastrectomy
- Loss of exocrine pancreatic function
- Ileal resection or diffuse ileal disease
- Certain tapeworms

Pernicious Anemia
Clinical Features

It is a disease of older adults; the median age at diagnosis is 60 years, and it is rare in people younger than 30.

Insidious in onset

The diagnosis is based on

- a moderate to severe megaloblastic anemia
- leukopenia with hypersegmented granulocytes
- low serum vitamin B12
- Elevated serum levels of homocysteine and methylmalonic acid

Response to parenteral administration of vitamin B12 confirms the diagnosis

B12 deficiency can also lead to neurologic complications postulated to be due to myelin sheath break down and degeneration of the posterior and

Pernicious Anemia Complications

Gastric carcinoma

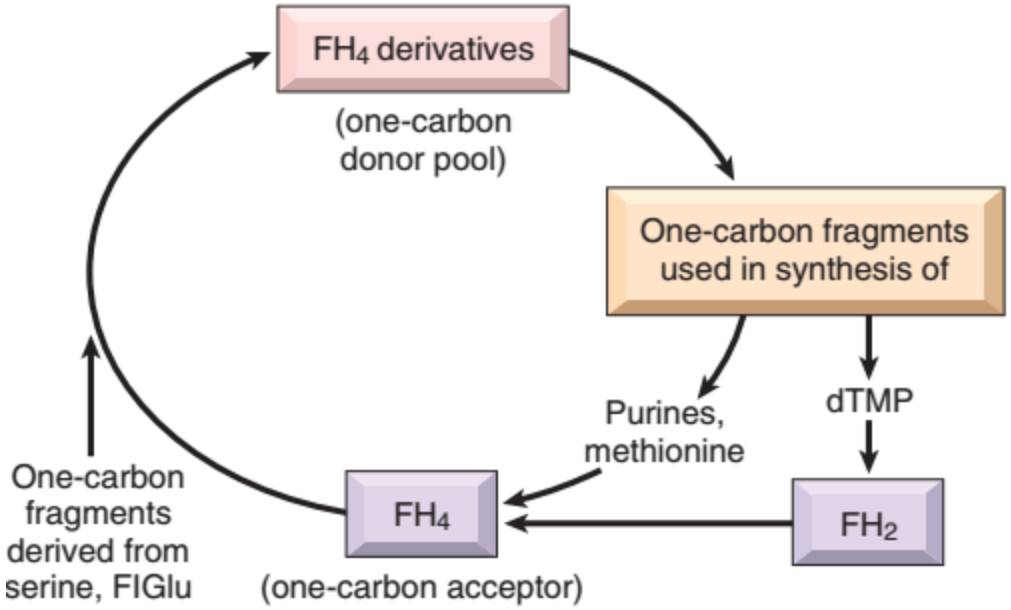
Atherosclerosis

With parenteral or high-dose oral vitamin B12, the anemia is cured and the progression of the peripheral neurologic disease can be reversed or at least halted, changes in the gastric mucosa and the risk of carcinoma are unaffected

Anemia of Folate Deficiency

A deficiency of folic acid results in a megaloblastic anemia having the same pathologic features as that caused by vitamin B12 deficiency

FH4 derivatives act as intermediates in the transfer of one-carbon units such as formyl and methyl groups to various compounds



Anemia of Folate Deficiency

Suppressed synthesis of DNA, the common denominator of folic acid and vitamin B12 deficiency, is the immediate cause of megaloblastosis

Folate Deficiency Causes

Folic Acid Deficiency
Decreased Intake
Inadequate diet, alcoholism, infancy Impaired Absorption Malabsorption states Intrinsic intestinal disease Anticonvulsants, oral contraceptives Increased Loss Hemodialysis
Increased Requirement
Pregnancy, infancy, disseminated cancer, markedly increased hematopoiesis
Impaired Utilization
Folic acid antagonists
Unresponsive to Vitamin B_{12} or Folic Acid Therapy
Metabolic Inhibitors of DNA Synthesis and/or Folate Metabolism (e.g., Methotrexate)

Folate Deficiency

Megaloblastic anemia that results from a deficiency of folic acid and vitamin B12 are identical, but neurological pathology is not found in folate deficiency

The diagnosis of folate deficiency can be made only by demonstration of decreased folate levels in the serum or red cells

Homocysteine levels are increased, methylmalonate concentrations are normal

Iron Deficiency Anemia

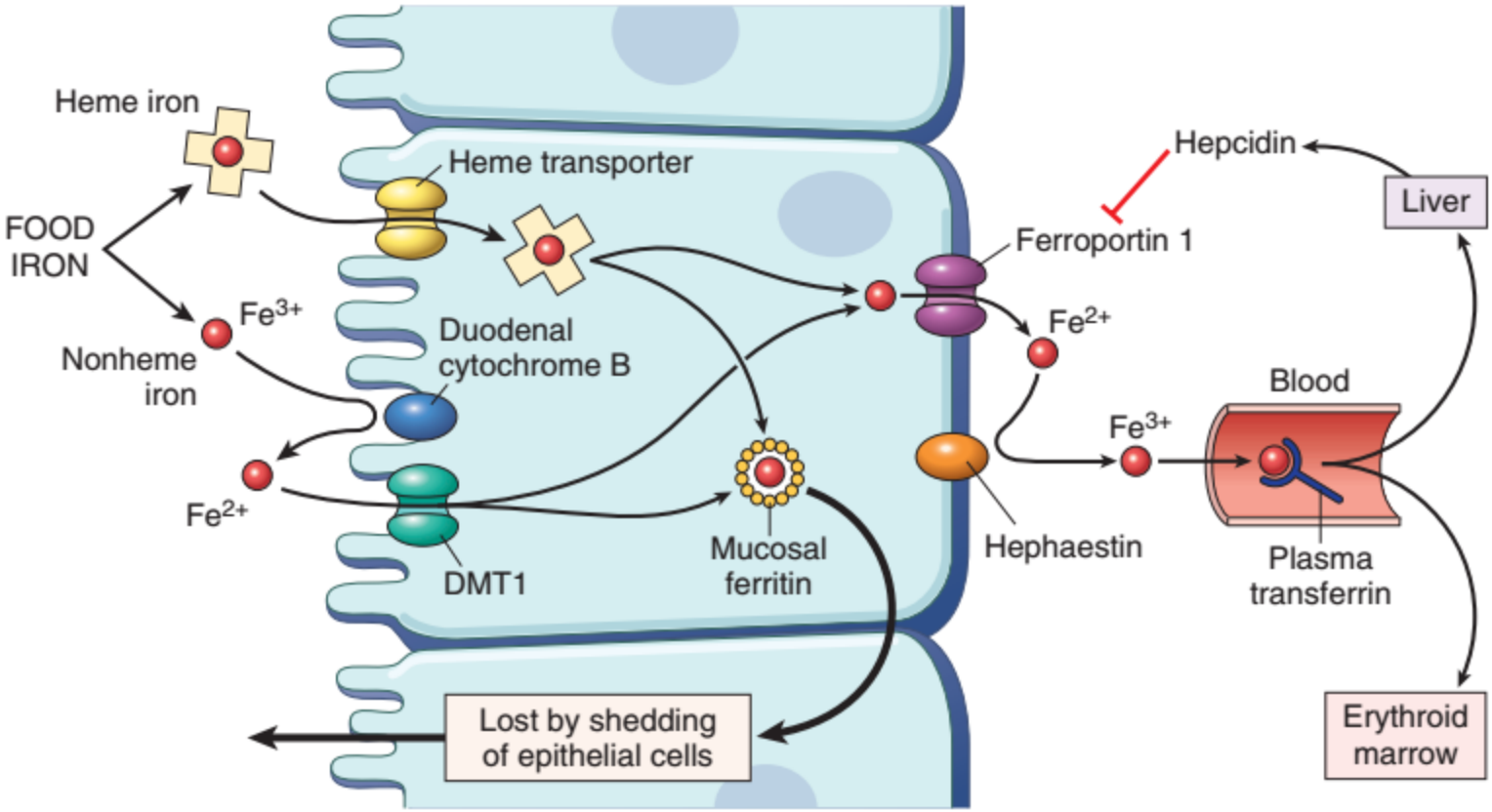
The most common nutritional disorder in the world

Dietary iron presents in 2 forms

- Heme contained in animal products, in the Fe2 + (ferrous) state About 20% of it is absorbable
- Inorganic nonheme iron in vegetables, is mostly in the Fe3 + (ferric) 1% to 2% is absorbable after reduced to Fe2 +

Body iron is either

- Functional, 80% of total body iron, found in hemoglobin; myoglobin and iron-containing enzymes
- Storage pool, 15% to 20% of total body iron represented by hemosiderin and ferritin


Heme iron
FOOD IRON
Nonheme iron
Fe3+
Fe2+
Heme transporter
Duodenal cytochrome B
DMT1
Mucosal ferritin
Lost by shedding of epithelial cells
Hepcidin
Liver
Ferroportin 1
Fe2+
Fe3+
Hephaestin
Blood
Plasma transferrin
Erythroid marrow

Iron Deficiency Anemia

Iron in the body is recycled between the functional and storage pools. It is transported in plasma by an iron-binding glycoprotein called **transferrin** , which is synthesized in the liver.

Free iron is highly toxic, and it is therefore important that storage iron be sequestered. This is achieved by binding of iron in the storage pool to either **ferritin** or hemosiderin.

Plasma ferritin levels correlate well with body iron stores, in iron deficiency, serum ferritin is below 12 µg/L, whereas in iron overload values approaching 5000 µg/L may be seen.

Luminal nonheme iron is mostly in the Fe3 + (ferric) state and must first be reduced to Fe2 + (ferrous) to be absorbed

Iron Deficiency Anemia

Iron that enters the duodenal cells can either be transported to the blood or stored in the mucosa if body iron stores are adequate, to be shed with mucosal cell later

Iron absorption is regulated by **hepcidin**, a small circulating peptide that is synthesized and released from the liver in response to increases in intrahepatic iron levels.

when the body is replete with iron, high hepcidin levels inhibit its absorption into the blood. Conversely, with low body stores of iron, hepcidin synthesis falls and
this in turn facilitates iron absorption.

Iron Deficiency Anemia Etiology

Dietary lack

- Common in developing countries
- The absorption of inorganic iron is enhanced by ascorbic acid, citric acid, amino acids, and sugars in the diet, and inhibited by tannates (found in tea), carbonates, oxalates, and phosphates.
- Groups at risk are infants, the impoverished, older adults and teenagers

Iron Deficiency Anemia Etiology

Impaired absorption

- Celiac disease, other causes of malabsorption and chronic diarrhea
- Gastrectomy decreasing the acidity of the proximal duodenum (which enhances uptake), and also by increasing the speed with which gut contents pass through the duodenum.
- Certain dietary items e.g. tannates and phosphate

Increased requirement

- Growing infants, children, and adolescents
- Premenopausal women, particularly during pregnancy

Iron Deficiency Anemia Etiology

Chronic blood loss

- The most common cause of iron deficiency in the Western world
- External hemorrhage
- Bleeding into the gastrointestinal, urinary, or genital tracts
- Iron deficiency in adult men and postmenopausal women in the Western world must be attributed to gastrointestinal blood loss until proven otherwise, not to miss an occult gastrointestinal cancer or other bleeding lesion

Iron Deficiency Anemia Pathogenesis

Whatever the cause, the following consequences happen in a raw

- Depletion of stores, i.e. ferritin and hemosiderin
- Lowered serum iron and transferrin saturation
- Anemia appears only when iron stores are completely depleted

Whatever its basis, iron deficiency produces a hypochromic microcytic anemia

Iron Deficiency Anemia
Clinical Features

Features of underlying cause e.g. gastrointestinal or gynecologic disease, malnutrition, pregnancy, and malabsorption

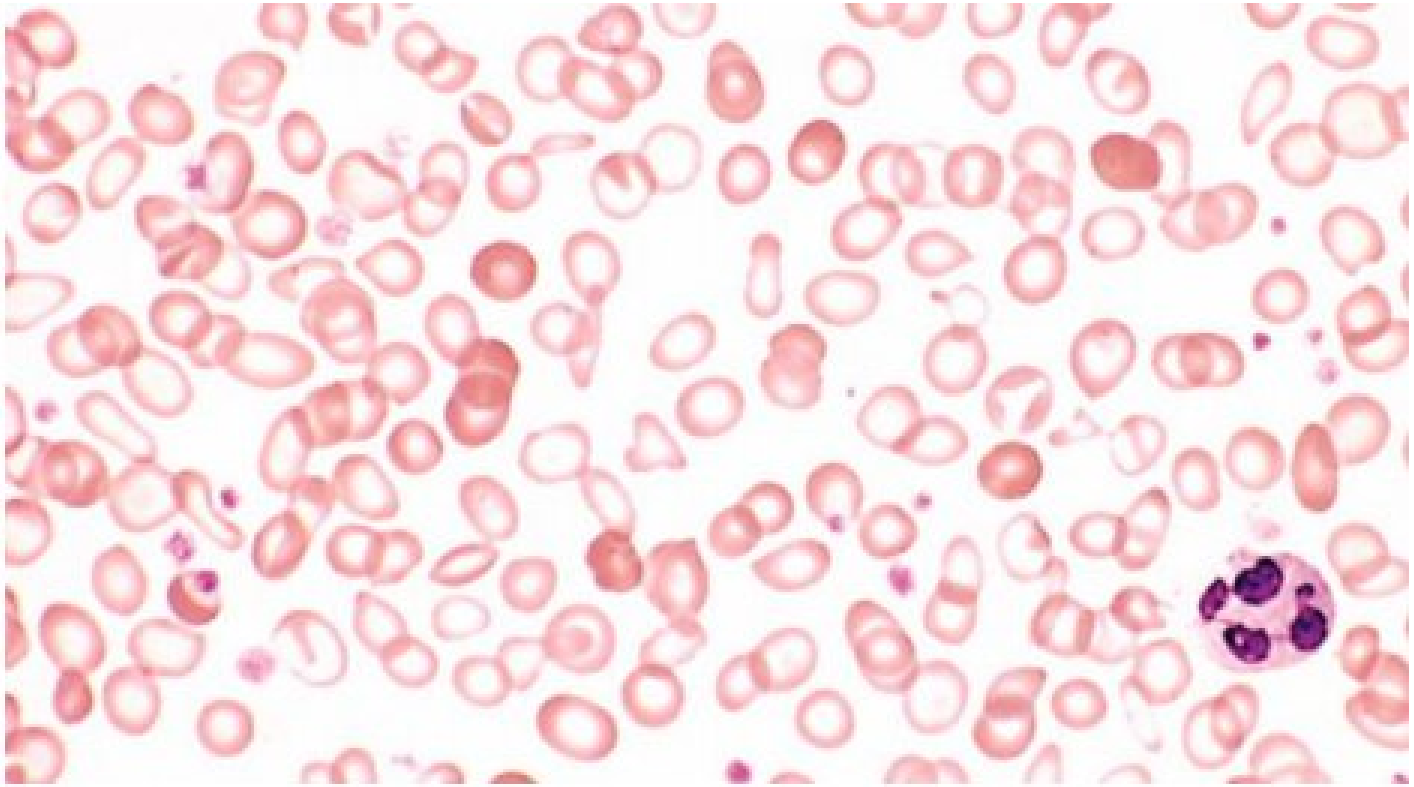
Plummer-Vinson syndrome rare triad of Esophageal webs, microcytic hypochromic anemia and atrophic glossitis

Long standing depletion affect enzymes causing other changes e.g. **koilonychia, alopecia, atrophic changes in the tongue and gastric mucosa, and intestinal malabsorption**

Pica

- Depletion of iron from the central nervous system
- Consume non-food stuffs such as clay

Iron Deficiency Anemia Morphology



Iron Deficiency Anemia Diagnosis

Based on laboratory studies

- Both the hemoglobin and hematocrit are depressed
- Hypochromia, microcytosis, anisocytosis and modest poikilocytosis
- The serum iron and ferritin are low
- Total plasma iron-binding capacity (TIBC) is high
- Transferrin saturation (serum iron divided by TIBC, expressed as percentage) decreased below 15%

In uncomplicated iron deficiency, oral iron supplementation produces an increase in reticulocytes in about 5 to 7 days that is followed by a steady increase in blood counts and the normalization of red cell indices

Paroxysmal nocturnal hemoglobinuria (PNH)

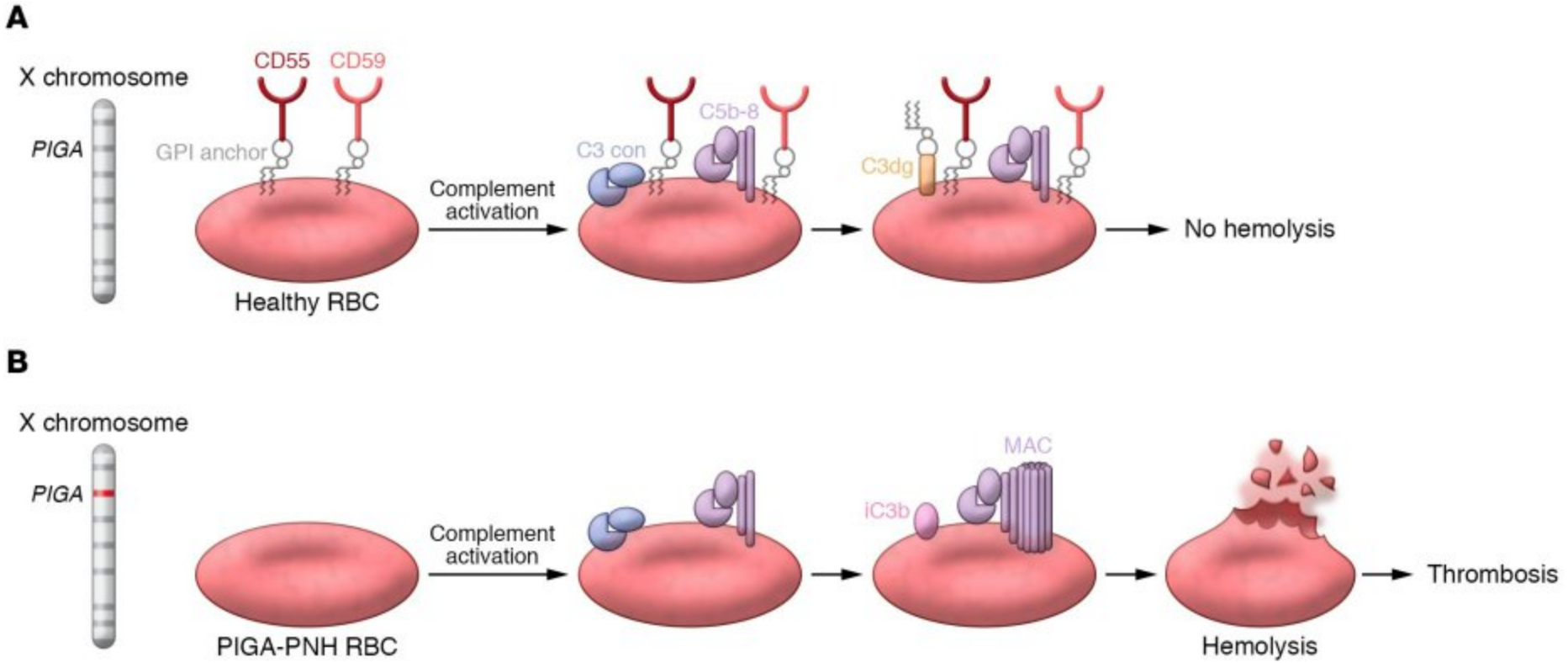
(PNH) is a hemolytic anemia caused by an **acquired somatic mutation** of a gene (PIGA) that encodes an anchor called GPI for proteins (CD55 and CD59) in the cell membrane,

Deficiency in CD 55 and CD59 **cause complement-mediated lysis in red cells** , white cells, and platelets.

The condition causes episodes (paroxysms) of **IV hemolysis** at night.

Acidosis in vivo occurs during sleep (breathing slowly retains CO2) and exercise (lactic acidosis), and the acidosis in turn causes activation of complement.

Hemolysis mechanism of paroxysmal nocturnal hemoglobinuria (PNH) via the complement system



Paroxysmal nocturnal hemoglobinuria (PNH)

PNH is a clonal stem cell disorder that affects all blood cell lines, leading to pancytopenia (**anemia, leukopenia, and thrombocytopenia**) that is apparent in the peripheral blood.

Venous thrombosis may ensue, the leading cause of death.

Susceptibility to Myelodysplastic syndrome and subsequent acute myeloid leukemia

Most PNH patients have mild disease and don’t require treatment, but a monoclonal antibody against complement protein C5 can be therapeutic. Bone marrow tranplantation is another therapeutic option

Good Luck

Bleeding Disorders

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Hemostasis Physiology

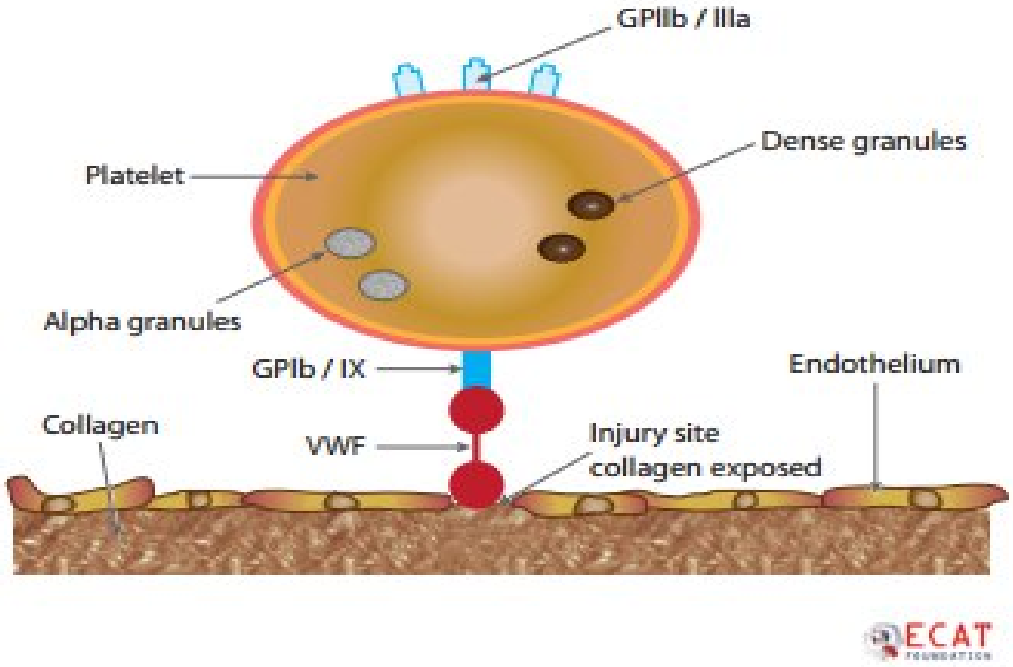
Hemostasis is a complex process in which multiple components of the blood clotting system are activated in response to vessel injury to control bleeding

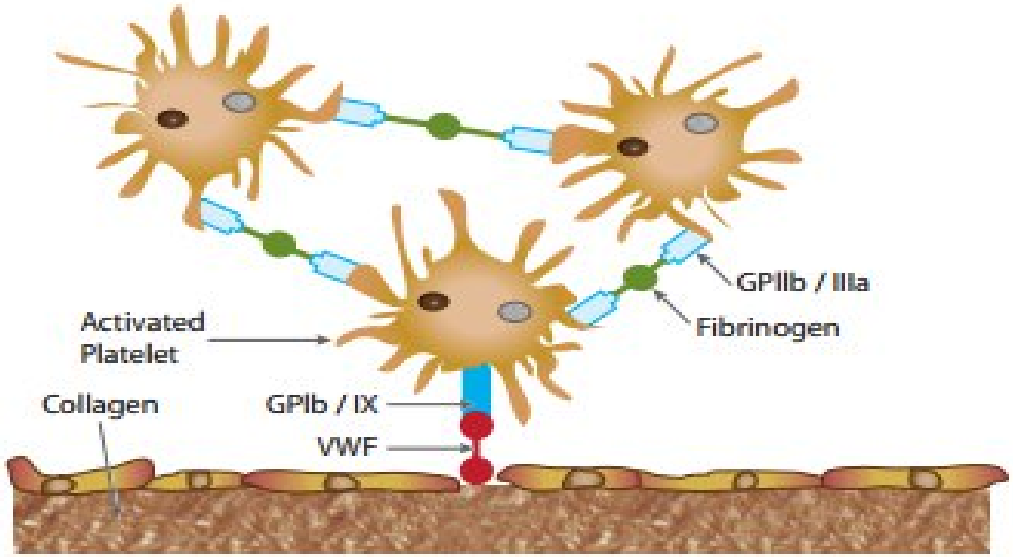
It is composed of

1 . Primary hemostasis
2. Secondary hemostasis
3. Fibrin clot formation and stabilization
4. Inhibition of coagulation

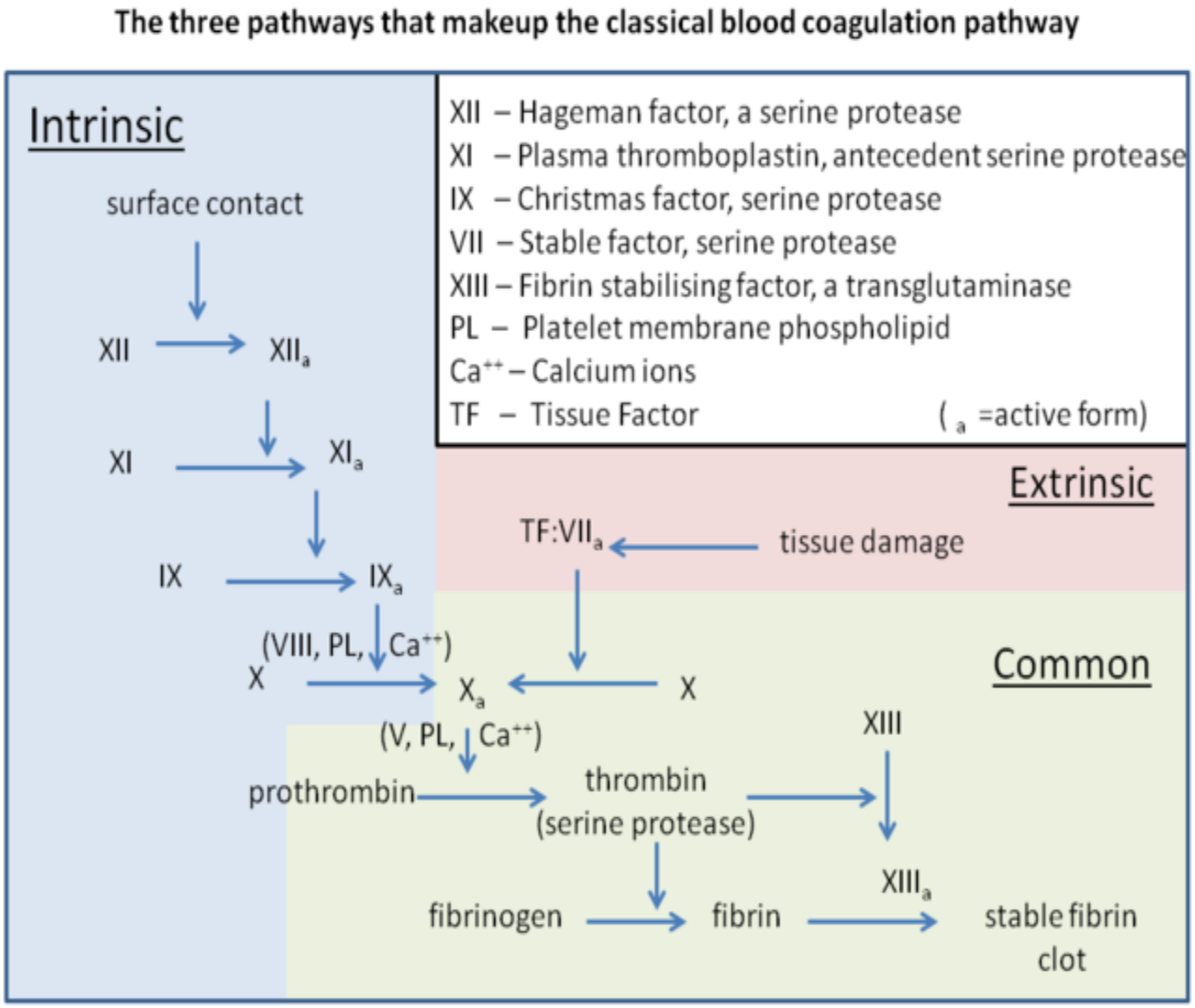
Primary Hemostasis

- Vasoconstriction
- PLT adhesion
- PLT aggregatio





Secondary Hemostasis
fibrin clot stabilization



Hemostasis Physiology

Inhibition of coagulation

- Thrombin binds to the membrane receptor thrombomodulin and activates Protein C that binds protein S and inhibit factor V and VIII

Fibrinolysis

- Tissue plasminogen activator (t-PA) converts plasminogen to plasmin which breaks down cross-linked fibrin to several fibrin degradation products, the smallest of which is D-dimer

Tests of Hemostasis

Platelet counts
- Electronic particle counter and confirmed by blood smear

Bleeding time
- Older less specific test for PLT function, sometimes prolonged in vessel wall abnormalities

Tests of platelet function
- The ability of PLTs to aggregate in response to different agonists

Prothrombin time (PT)
- Assesses the extrinsic and common coagulation pathways

Partial thromboplastin time (PTT)
- This test assesses the intrinsic and common clotting pathways

Excessive bleeding can result from

1. Increased fragility of vessels
2. Platelet deficiency or dysfunction
3. Derangement of coagulation

Primary hemostatic defect

Bleeding Disorders Caused by Vessel Wall Abnormalities

Relatively common, do not usually cause serious bleeding,

Platelet count, function and tests of coagulation (PT, PTT) are normal (unless affected by underlying disease e.g meningococcemia)

Bleeding time may be increased

Most often, they present with small hemorrhages (petechiae and purpura), occasionally cause joints, muscles, menorrhagia,

- Infections e.g. meningococcemia and infective endocarditis
- Henoch-Schönlein purpura
- Scurvy and the Ehlers-Danlos syndrome
- Hereditary hemorrhagic telangiectasia, autosomal dominant characterized by dilated, tortuous blood vessels with thin walls that bleed readily, bleeding can be serious

Bleeding due to Thrombocytopenia

- Important cause of generalized bleeding
- Normal PT and PTT that test coagulation
- Involves small vessels involving he skin and the mucous membranes
- Intracranial bleeding is serious complication

Causes of Thrombocytopenia

Decreased Production of Platelets

- Drug-induced: alcohol, thiazides, cytotoxic drugs
- Infections: measles, human immunodeficiency virus (HIV)
- Nutritional deficiencies: B12, folate deficiency
- Bone marrow failure
- Bone marrow replacement
- Ineffective hematopoiesis: Myelodysplastic syndromes

Causes of Thrombocytopenia

Decreased Platelet Survival
Immunologic destruction
Primary autoimmune
Chromic immune thrombocytopenic purpura
Acute immune thrombocytopenic purpura
Secondary autoimmune
Systemic lupus erythematosus, B-cell lymphoid neoplasms
Alloimmune: posttransfusion and neonatal
Drug-associated: quinidine, heparin, sulfa compounds
Infections: HIV, infectious mononucleosis (transient, mild), dengue fever
Nonimmunologic destruction
Disseminated intravascular coagulation
Thrombotic microangiopathies
Giant hemangiomas
Sequestration
Hypersplenism
Dilution
Transfusions

Neonatal Thrombocytopenia

Inherited forms include

- Wiskott-Aldrich syndrome: X linked recessive; affected boys have small platelets, eczema, and immunodeficiency
- Amegakaryocytic thrombocytopenia
- Thrombocytopenia-absent radius syndrome
- Fanconi anemia

Neonatal Thrombocytopenia

Acquired forms include:

- Neonatal alloimmune thrombocytopenia (NAIT):

  Caused by alloimmunization to platelet antigens such as HPA-1a during pregnancy.

  NAIT leads to destruction of fetal platelets, predisposing the fetus or neonate to intracranial hemorrhage.

- Additional causes: birth asphyxia, necrotizing enterocolitis, and thrombosis

Chronic Immune Thrombocytopenic Purpura (ITP)

Autoantibody mediated destruction of platelets

Either

- Primary (idiopathic)

  Secondary immune thrombocytopenia causes has to be excluded

- Secondary: systemic lupus erythematosus, HIV infection, and B-cell neoplasms such as chronic lymphocytic leukemia

Chronic ITP Pathogenesis

The autoantibodies, mostly of the IgG class, most often against platelet membrane glycoproteins IIb-IIIa or Ib-IX

Antiplatelet antibodies act as opsonins that are recognized by IgG Fc receptors expressed on phagocytes, leading to increased platelet destruction.

Autoantibodies may also bind to and damage megakaryocytes, leading to decreases in platelet production that further exacerbate the thrombocytopenia.

ITP MORPHOLOGY

The spleen is

- Enlargement of the splenic follicles, with prominent reactive germinal centers.
- **Congestion of the sinusoids and Foamy histiocytes** are seen in the red pulp. The foamy appearance is secondary to accumulation of phospholipids from **phagocytosed platelets** .

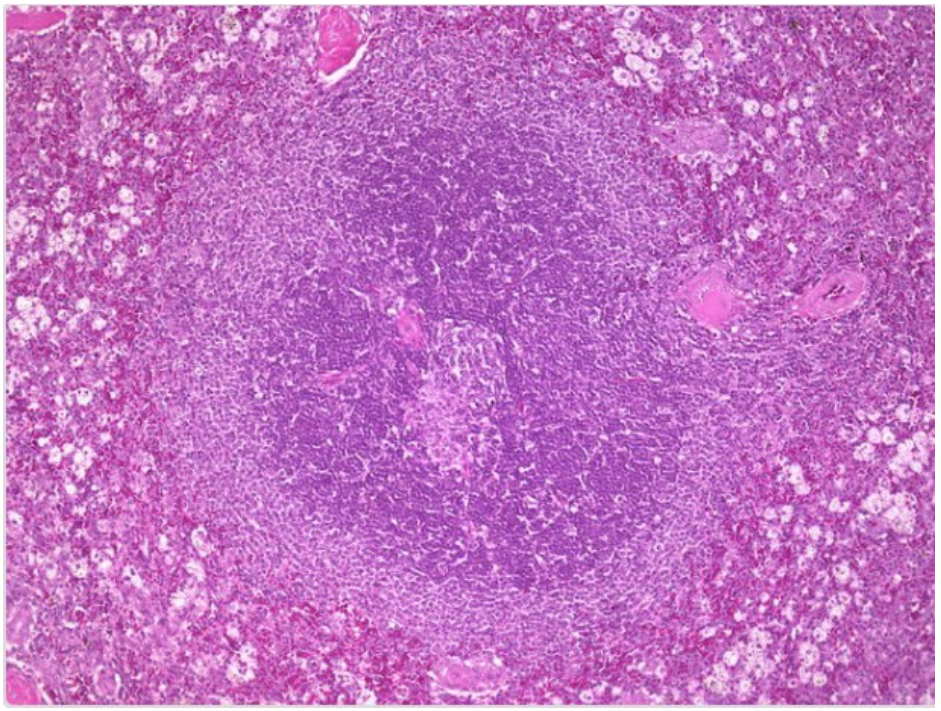
The bone marrow

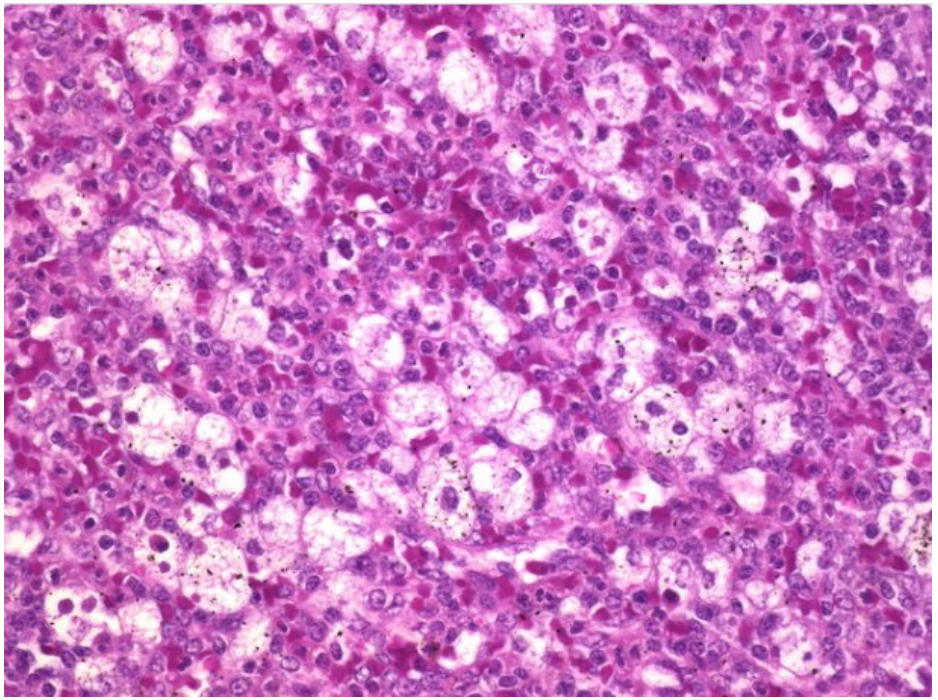
- **A modestly increased number of megakaryocytes,** some are apparently immature, with large, nonlobulated, single nuclei.
- The importance of bone marrow examination is to rule out thrombocytopenia resulting from bone marrow failure or other primary bone marrow disorders.

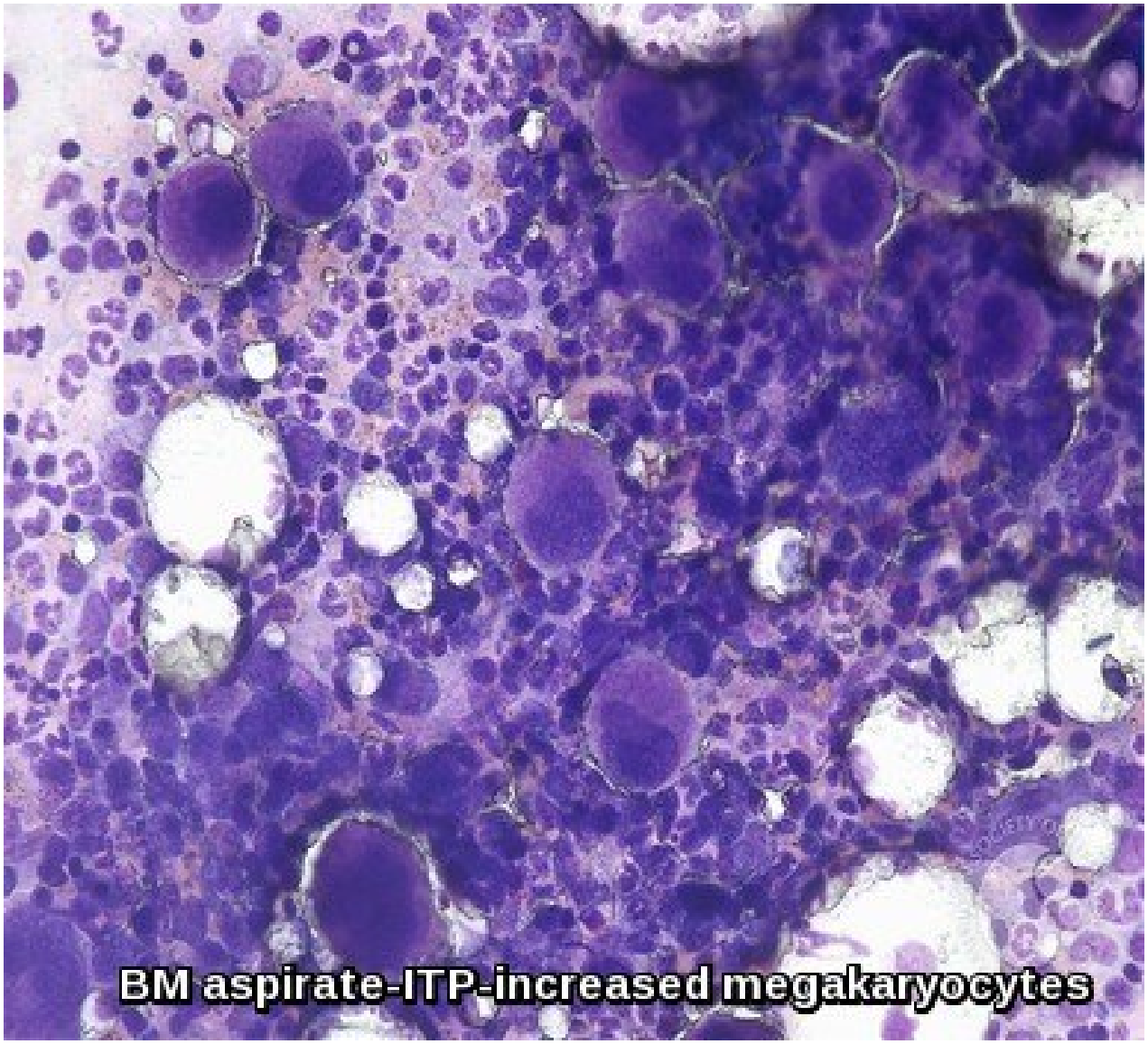
The peripheral blood smear **reveals abnormally large platelets** (marco or megathrombocytes), which is a sign of accelerated thrombopoiesis.

ITP

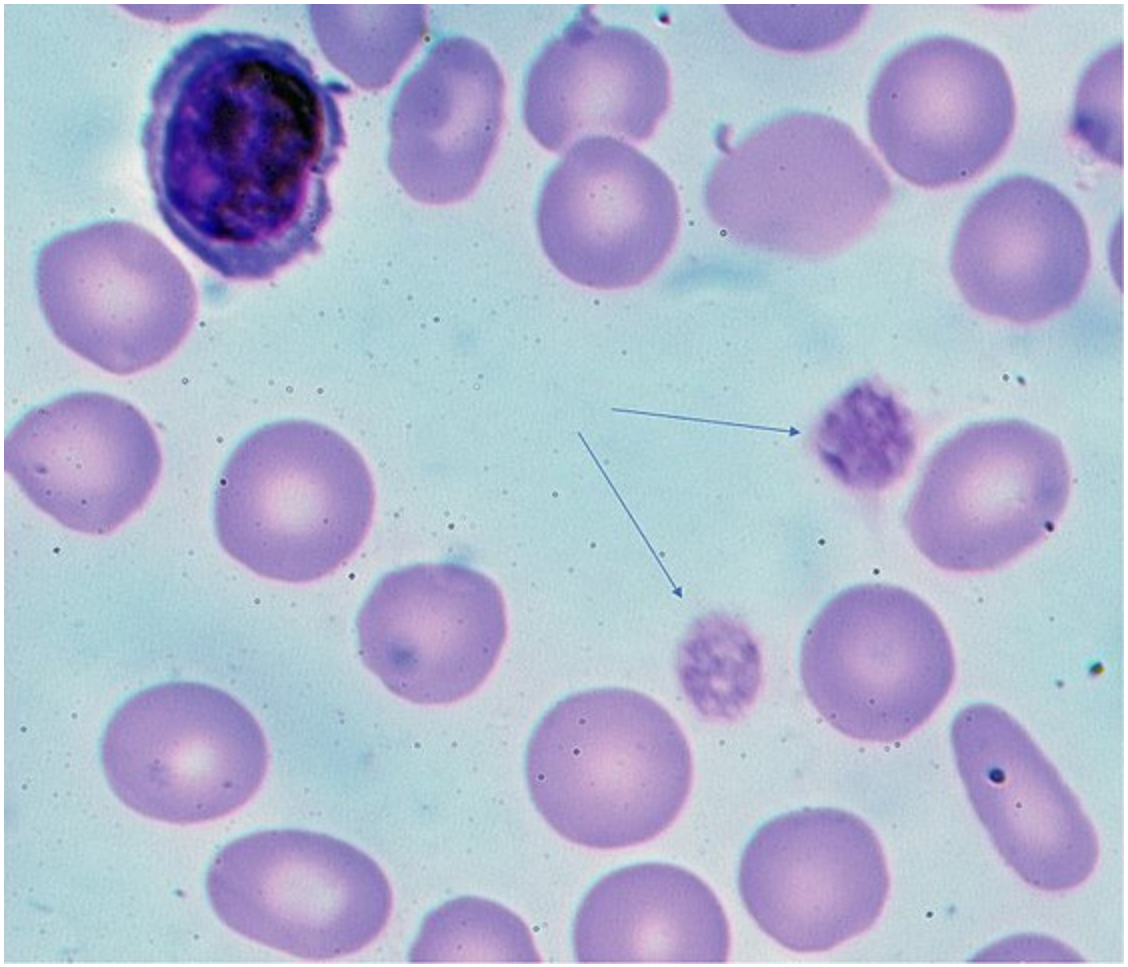
Spleen, germinal center and foamy histiocytes






BM aspirate-ITP-increased megakaryocytes

ITP- macrothrombocyte



Chronic ITP Clinical features

Adult women younger than 40 years, F:M 3:1

Insidious onset, mucocutaneous bleeding

Petechiae and ecchymoses, easy bruising, nose bleeds, bleeding from the gums, and hemorrhages into soft tissues from relatively minor trauma.

May manifest first with melena, hematuria, or excessive menstrual flow

Intracranial hemorrhage, serious but rare in treated patients.

Chronic ITP Clinical features

Splenomegaly and lymphadenopathy are uncommon in primary disease

low platelet count, normal or increased megakaryocytes in the bone marrow, and large platelets in the peripheral blood are presumptive evidence

Treatment include glucocorticoids, intravenous immunoglobulin, anti-CD20 antibody (rituximab) and splenectomy , drugs that mimic the effects of thrombopoietin (so-called TPO-mimetics), splenectomy.

Acute Immune Thrombocytopenic Purpura

Childhood illness with equal frequency in both sexes

Abrupt onset, often 1 to 2 weeks after a self-limited viral illness triggering the development of autoantibodies

Self-limited, usually resolving spontaneously within 6 months.

Glucocorticoids or intravenous immunoglobulin treatment for severe thrombocytopenia.

Chronicity in 20% of children following a coarse similar to adult chronic ITP

Drug-Induced Thrombocytopenia

Drugs can induce thrombocytopenia through direct effects on platelets and secondary to immunologically mediated platelet destruction

The drugs most commonly implicated are quinine, quinidine, and vancomycin

Heparin-induced thrombocytopenia (HIT) has a distinctive pathogenesis and occurs in 5% of persons receiving heparin and is of two types

Heparin-induced thrombocytopenia (HIT)

Type I thrombocytopenia

- Rapidly after heparin therapy
- Little clinical importance

Type II thrombocytopenia,

- Less common
- Greater clinical significance and severity,
- Five to 14 days after heparin therapy begins
- Caused by antibodies that recognize complexes of heparin and platelet factor 4
- Often leads to life threatening venous and arterial thrombosis

HIV-Associated Thrombocytopenia

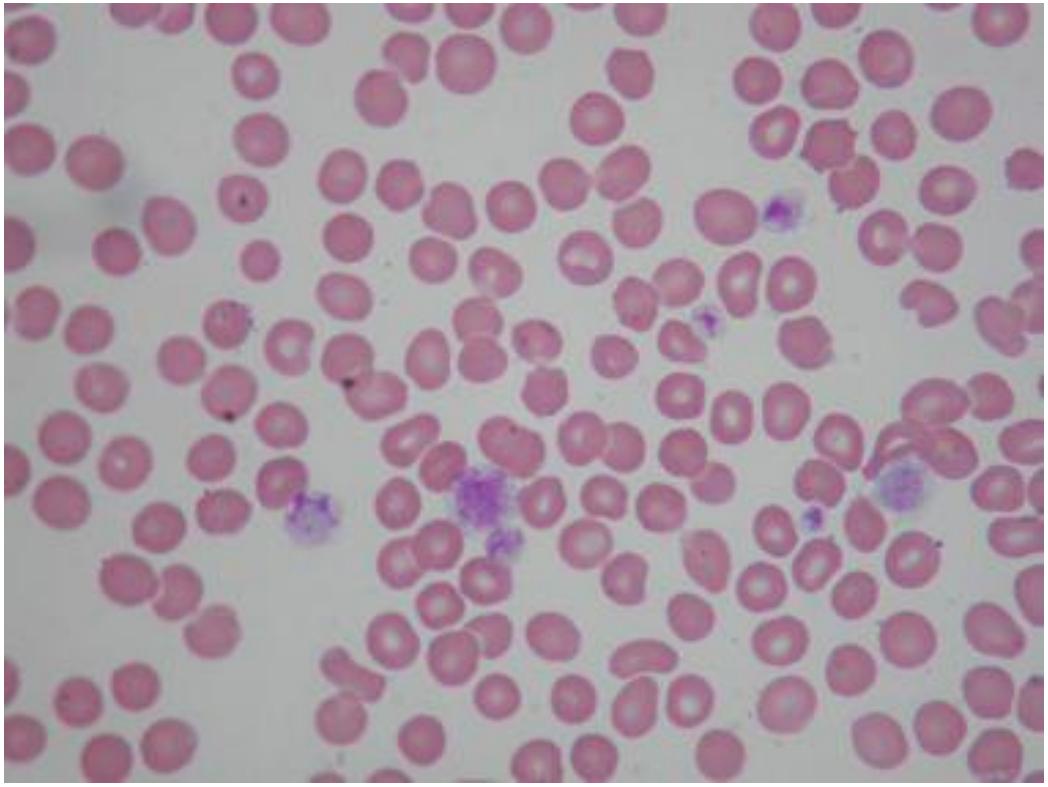
Thrombocytopenia is one of the most common hematologic manifestations of HIV infection

- Impaired platelet production: CD4 receptors are found on megakaryocytes
- Increased destruction: B-cell hyperplasia and dysregulation

Bleeding Disorders Related to Defective Platelet Functions

Bernard-Soulier syndrome

- Also known as giant platelet syndrome
- Is an autosomal recessive disorder, defect in the membrane glycoprotein complex GPIb/IX or GPV that
  binds vWF
- Examination of the blood reveals thrombocytopenia and circulating giant platelets.
- The disease becomes symptomatic during childhood with ecchymoses, epistaxis, and gingival bleeding. During later ages, the disease is manifested by menorrhagia and gastrointestinal bleeding



Bleeding Disorders Related to Defective Platelet Functions

Glanzmann thrombasthenia

- Is an autosomal recessive disorder
- Defect in the glycoprotein complex IIb/IIIa, which binds fibrinogen
- Platelets fail to aggregate or retract, leading to impaired hemostasis.
- Patients present shortly after birth with mucocutaneous or gingival hemorrhage, epistaxis, or bleeding after circumcision.

Bleeding Disorders Related to Defective Platelet Functions

Acquired PLT dysfunction

- Aspirin and NSAID: inhibit cyclooxygenase that synthesizes thromboxane A2 and prostaglandins, both mediators play an important roles in platelet aggregation and subsequent degranulation
- Uremia: pathogenesis of platelet dysfunction involves defects in adhesion, granule secretion, and aggregation.

Bleeding Disorders

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Thrombotic Microangiopathies

Excessive activation of platelets, which deposit as thrombi in small blood vessels.

A spectrum of clinical syndromes that includes **Thrombotic Thrombocytopenic Purpura (TTP) and Hemolytic-Uremic Syndrome (HUS)**

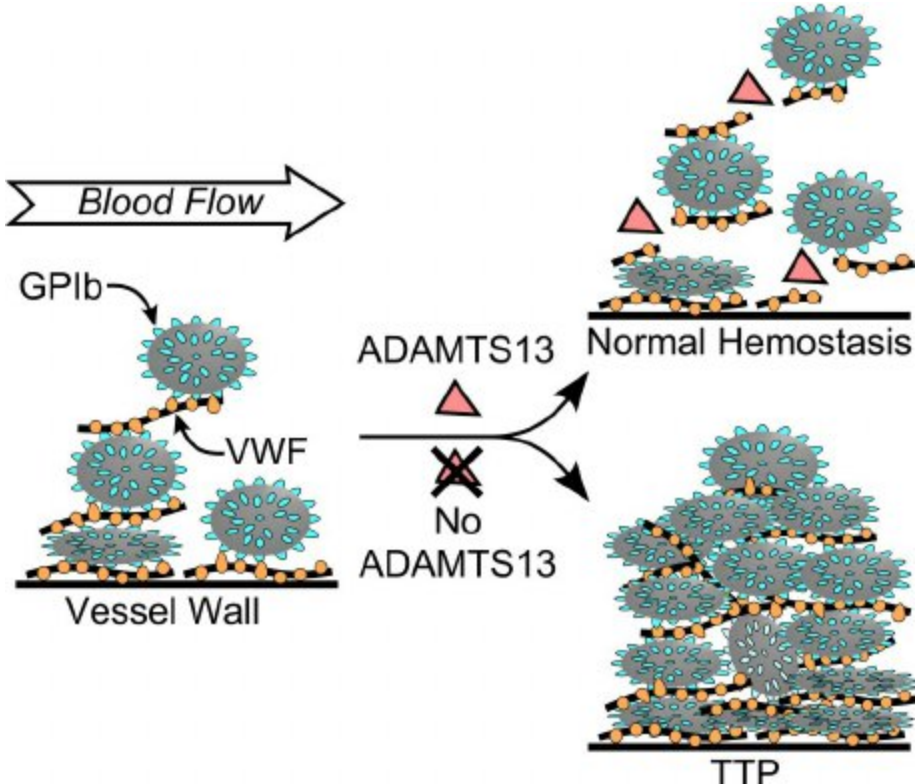
Laboratory tests of coagulation are usually normal

Thrombotic Thrombocytopenic Purpura (TTP)

Deficiency in a plasma ADAMTS13 enzyme which degrades very high-molecular-weight multimers of von Willebrand factor (vWF), leading to their accumulation and promoting platelet activation and aggregation.

Intravascular thrombi cause a microangiopathic hemolytic anemia and widespread organ dysfunction, in addition to thrombocytopenia

The deficiency of ADAMTS13 can be acquired (inhibitory autoantibodies) or less commonly inherited



Manifestation of TTP

Pentad of fever, thrombocytopenia, microangiopathic hemolytic anemia, transient neurologic deficits, and renal failure

Seriously fatal disease, high index of suspicion is important

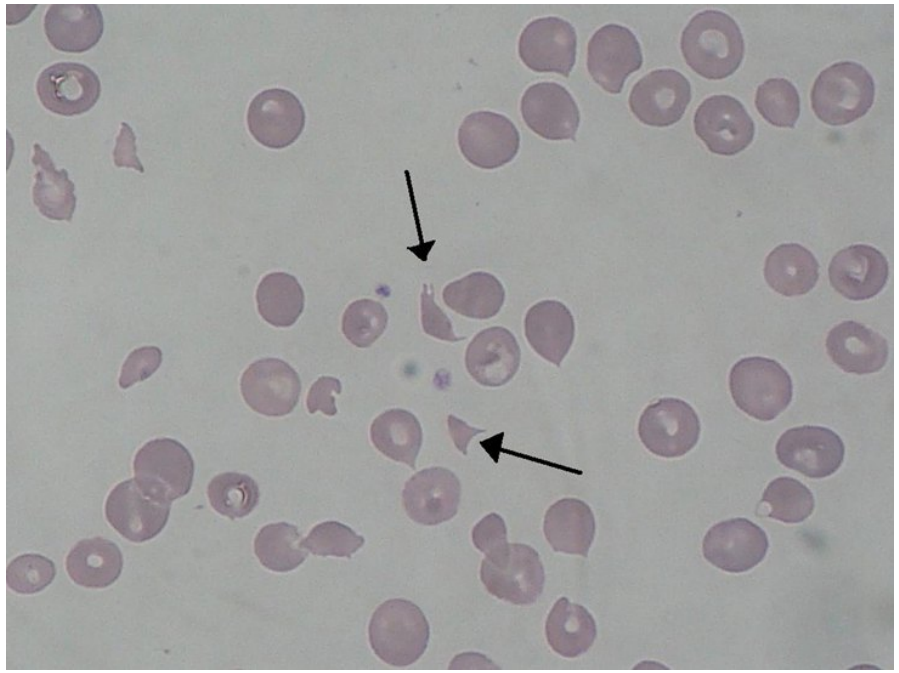
In hereditary form, onset is often delayed until adolescence and symptoms are episodic, factors other than ADAMTS13 (e.g., some superimposed vascular injury or prothrombotic state) must be involved in triggering full-blown TTP

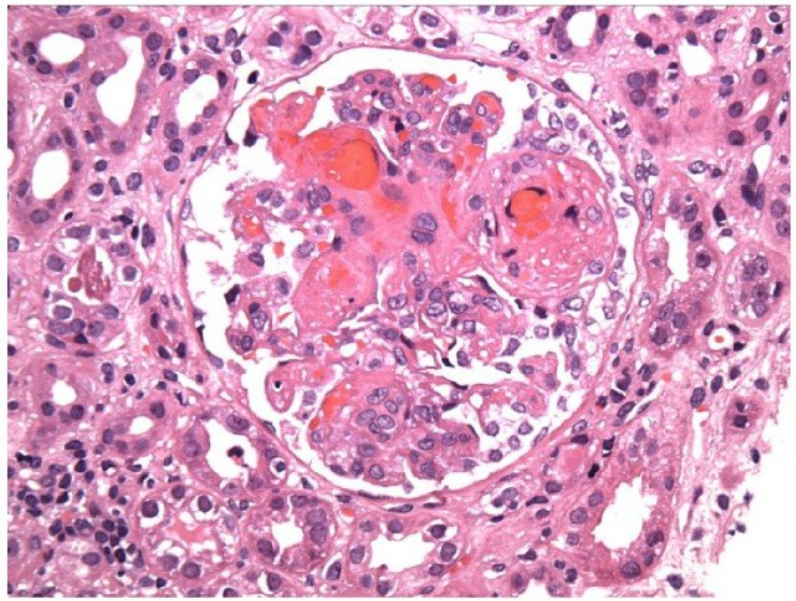
Treatment with plasma exchange

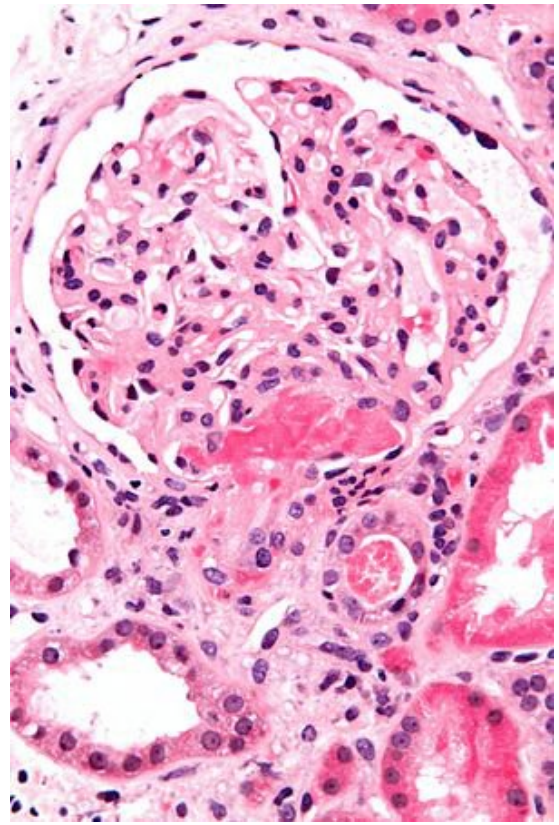
Thrombotic Thrombocytopenic Purpura (TTP)

Morphology

- Hyaline microthrombi are present in the arterioles and capillaries of end organs, such as the kidney, brain, and heart.
- Blood film reveals schistocytes (fragmented RBCs)







Hemolytic-Uremic Syndrome (HUS)

Another form of thrombotic microangiopathhy.

Differs from TTP

- Absence of neurologic symptoms
- Prominence of acute renal failure
- Frequent occurrence in children

HUS occurs in either typical or atypical form.

Unclear basis of platelet activation.

Hemolytic-Uremic Syndrome (HUS)

Typical HUS

- Strongly associated with infectious gastroenteritis caused by Escherichia coli strain O157:H7, which elaborates a Shiga-like toxin
- Shiga-like toxin alters endothelial cell function and results in platelet activation and aggregation.
- Children and older adults are at highest risk.
- Bloody diarrhea, precedes HUS appearance by a few days
- With appropriate supportive care complete recovery is possible, but irreversible renal damage and death can occur in more severe cases

Hemolytic-Uremic Syndrome (HUS)

Atypical HUS

- Defects (inherited or acquired inhibitory autoantibodies) in complement regulatory proteins
- Excessive alternative complement pathway activation underlies the pathogenesis of this form of HUS that shows a remitting, relapsing clinical course
- Therapeutic antibodies that inhibit the activation of complement factor C5 are effective in preventing thrombosis in patients with inherited deficiencies

Other Thrombotic microangiopathies

Drugs (cyclosporine, chemotherapeutic agents)

Radiation, bone marrow transplantation

Other infections (HIV, pneumococcal sepsis)

Conditions associated with autoimmunity (systemic lupus erythematosus, HIV infection, lymphoid neoplasms)

Hemorrhagic Diatheses Related to Abnormalities in Clotting Factors

Bleeding due to coagulation factor deficiencies often occurs into

- The gastrointestinal and urinary tracts
- Weight-bearing joints (hemarthrosis)
- Deep muscular tissue

Hereditary deficiencies affect a single clotting factor

- vWF (von Willebrand disease)
- Factor VIII (Hemophilia A)
- Factor IX (hemophilia B)

Acquired deficiencies involve multiple coagulation factors

- Vitamin K deficiency (factors II, VII, IX, X and protein C)
- Disseminated intravascular coagulation

Von Willebrand Disease

Defective vWF

vWF function

- Platelet binding (via a PLT binding domain)
- Carrier molecule for FVIII (via a F VIII binding domain)

After synthesis, vWF undergoes multimerization to high and intermediate molecular weight multimers which are very much potent in adhering PLTs

Von Willebrand Disease

The most common inherited bleeding disorder of humans

- 1% by lab testing
- 1 per 500 symptomatic
- Wide variability in clinical expression is common

Most common symptoms related to defects in PLTs function

- Mostly mucocutaneous bleeding
- Epistaxis, easy bruising, oral bleeding, post-surgical (oropharyngeal) bleeding
- Menorrhagia and post-partum bleeding in females of childbearing age
- Bleeding in Joints in type 3

Three broad categories of von Willebrand disease are present

Von Willebrand Disease

Type 1 von Willebrand disease

- Autosomal dominant disorder
- Most common form accounting for 70% of cases
- Mild to moderate vWF deficiency i.e. quantitative
- Variable clinical expression but generally is associated with mild disease

Von Willebrand Disease
Type 2 von Willebrand disease

Qualitative defects in vWF with autosomal dominant inheritance, vWF is expressed in normal/near normal amounts

Associated with mild to moderate bleeding

Has 4 subtypes,

- Type 2A is the most common,

  vWF is expressed in normal/near normal amounts but there is defective multimerization of the vWF molecules (multimers are absent from the plasma)
- Type 2B, Gain of function mutation in PLT binding domain of VWF
- Type 2M, Loss of function mutation in PLT binding domain (opposite of 2B)
- Type 2N, Loss of vWF binding function to FVIII

Von Willebrand Disease

Type 3 von Willebrand disease

- Autosomal recessive disorder
- very low levels of vWF and severe clinical manifestations.
- Markedly affects factor VIII half-life, so the bleeding characteristics resemble those seen in hemophilia.

Level of active vWF is reduced

Factor VIII may be secondarily affected resulting in PTT prolongation

The plasma level of active vWF, measured as the ristocetin cofactor activity

Hemophilia A (Factor VIII Deficiency)

Caused by mutations in factor VIII, inherited as an X-linked recessive trait

30% of patients have no family history; their disease is caused by new mutations

- Heterozygous females are affected by bleeding if the random inactivation of X chromosome involve the normal factor VIII allele

Three degrees of severity

- Severe HA: FVIII concentration < 1% of normal
- Moderate HA: FVIII concentration 2-5% of normal
- Mild HA: FVIII concentration 6-50% of normal

The marked heterogeneity in genetic lesions causes the variability in factor deficiency

The most severe deficiencies result from mutations that completely abolishes the synthesis of factor VIII.

Hemophilia A (Factor VIII Deficiency) Clinical Features and Diagnosis

Easy bruising and massive hemorrhage after trauma or operative procedures

Spontaneous Joint hemorrhages (hemarthrosis)

Recurrent hemarthrosis leads to hemophilic arthropathy

Petechiae are characteristically absent

Prolonged PTT, normal PT

Factor VIII-specific assays are needed for diagnosis

Treatment involves recombinant factor VIII replacement, inhibitors is a challenging complication

Hemophilia B (Christmas Disease, Factor IX Deficiency)

X-linked recessive trait

A wide spectrum of mutations involving the gene that encodes factor IX produces disease with variable severity

A clinically indistinguishable disorder from hemophilia A

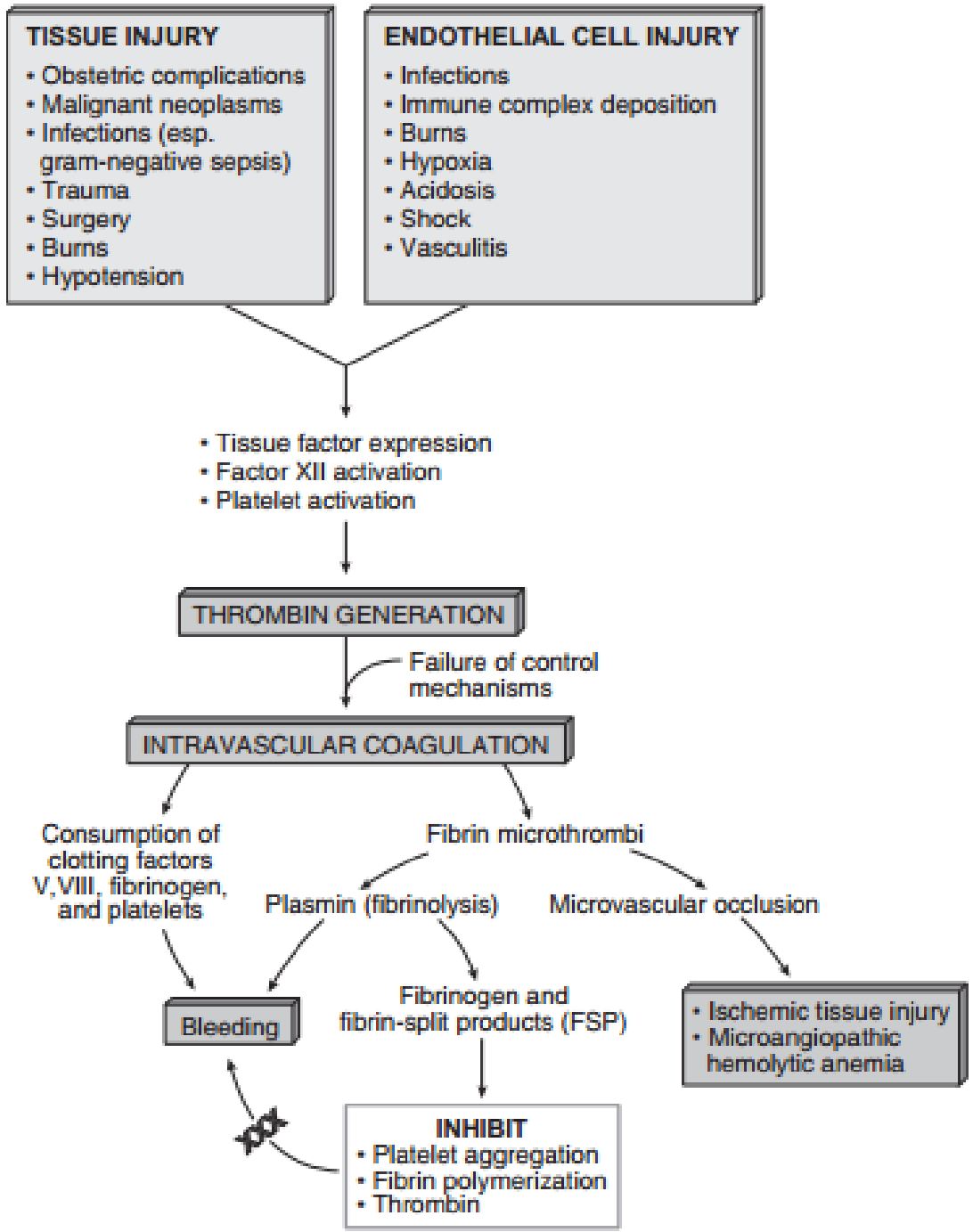
PTT is prolonged and the PT is normal

Diagnosed by assay of the factor levels

Disseminated Intravascular Coagulation (DIC)

DIC is a thrombohemorrhagic disorder characterized by the excessive activation of coagulation and the formation of thrombi in the microvasculature of the body, this happens through:

- Release of tissue factor and other procoaagulants, into the circulation, sources include the placenta in obstetric complications, burns, severe trauma
- Widespread injury to the endothelial cells e.g. In sepsis, TNF induces endothelial cells to express tissue factor on their cell surfaces and increase adhesion molecules, mediating endothelial damage by oxygen species from leukocytes



The pathophysiology of disseminated intravascular coagulation
(DIC)

DIC consequences

Widespread deposition of fibrin within the microcirculation leading to ischemia and microangiopathic hemolytic anemia

Consumption of platelets and clotting factors and the activation of plasminogen, leading to a hemorrhagic diathesis and accumulation of fibrin degradation products.

DIC Clinical Features

Onset can be acute or insidious based on underlying pathology

Common clinical presentations include microangiopathic hemolytic anemia, dyspnea, cyanosis, and respiratory failure, convulsions and coma, oliguria and acute renal failure; and sudden or progressive circulatory failure and shock

Diagnosis

- Clinical findings
- Low fibrinogen, high fibrin degradation products, low platelets and prolonged PT and PTT