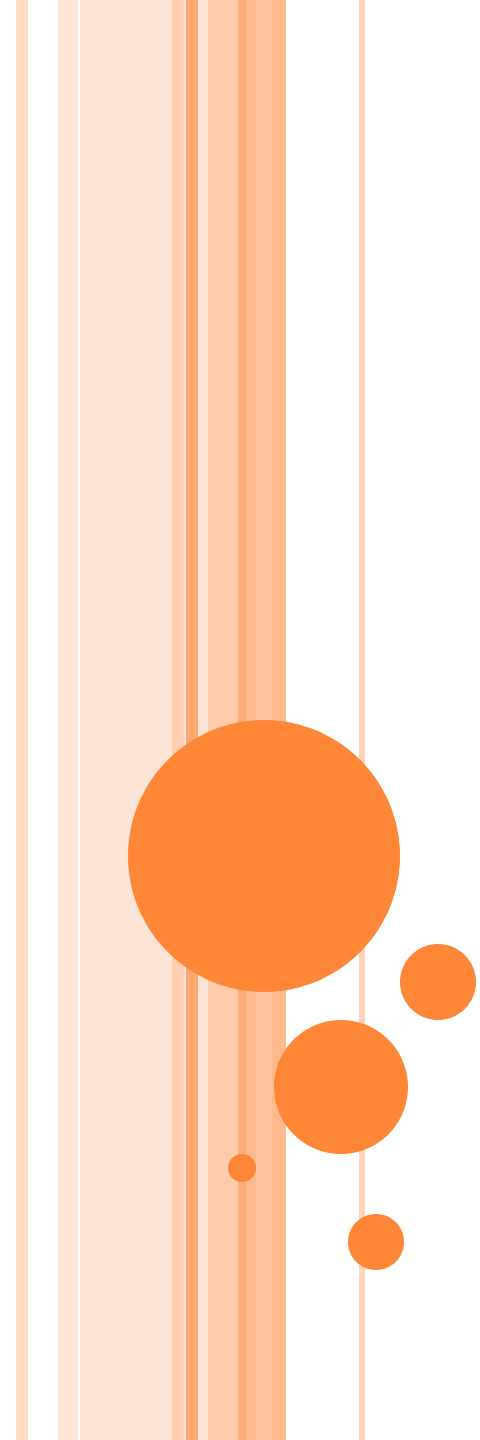




Pathology lab- Hemolytic Anemia

Types and etiologies of hemolytic anemia

Hemolytic anemias are characterized by an excessive breakdown of red blood cells (RBCs). They can be classified according to the cause of hemolysis (intrinsic or extrinsic) and by the location of hemolysis (intravascular or extravascular)

Some hemolytic anemias are both intra- and extravascular (e.g., paroxysmal nocturnal hemoglobinuria).



Type	Definition	Causes
By <u>RBC</u> pathology		
<u>Intrinsic hemolytic anemia</u>	Increased destruction of <u>RBCs</u> due to a defect within the <u>RBC</u>	<u>RBC</u> membrane defects: e.g., <u>hereditary spherocytosis</u>, <u>paroxysmal nocturnal hemoglobinuria</u> (PNH) - Enzyme defects: e.g., <u>glucose-6-phosphate dehydrogenase</u> deficiency (G6PD deficiency), <u>pyruvate kinase deficiency</u> (PKD) - Hemoglobinopathies: <u>sickle cell disease</u>, <u>thalassemia</u>, <u>hemoglobin C disease</u>
<u>Extrinsic hemolytic anemia</u>	Abnormal breakdown of normal <u>RBCs</u>	- Mechanical destruction in large vessels: e.g., prosthetic heart valves - Mechanical destruction in small vessels (i.e., <u>MAHA</u>): e.g., <u>HUS</u>, TTP, <u>DIC</u>, <u>HELLP syndrome</u> - Autoimmune reactions: <u>Autoimmune hemolytic anemia</u> (AIHA) - Alloimmune reactions: e.g., ABO/<u>Rh incompatibility</u> - Immune reactions due to infections (e.g., <u>Mycoplasma</u>) or tumors (e.g., <u>chronic lymphocytic leukemia</u>) - Infections causing increased destruction of <u>RBCs</u> (e.g., <u>Babesia</u>, <u>malaria</u>, <u>Bartonella bacilliformis</u>) - Increased degradation by the <u>spleen</u> (<u>hypersplenism</u>)

Intravascular hemolytic anemia

- Increased destruction of RBCs within the blood vessels

- Toxins (e.g., snake bites) and oxidizing agents (e.g., copper poisoning)^{[1][2]}
- G6PD deficiency
- Antibody-mediated hemolysis (transfusion ABO incompatibility, hemolytic anemia of the newborn, cold agglutinin disease)
- Complement-mediated hemolysis: PNH
- Macroangiopathic anemia (mechanical destruction by, e.g., prosthetic cardiac valves)
- Microangiopathic anemia (e.g., TTP, HUS, disseminated intravascular coagulation, HELLP syndrome, systemic lupus erythematosus)

Extravascular hemolytic anemia

- Increased destruction of RBCs by the reticuloendothelial system (primarily the spleen)

- RBC defects (e.g., sickle cell disease, spherocytosis, pyruvate kinase deficiency)
- Antibody-mediated hemolysis (warm and cold agglutinin disease)

Clinical features

Signs of anemia

Pallor

Fatigue

Exertional dyspnea

In severe cases: tachycardia, angina pectoris, leg ulcers

Signs of hemolysis

Jaundice

Pigmented gallstones (due to accumulation of indirect bilirubin in bile)

Splenomegaly (Extravascular hemolysis)

Back pain and dark urine in severe hemolysis with hemoglobinuria (Hgb is nephrotoxic)



Signs of increased hematopoiesis (mostly in severe chronic anemias, e.g., thalassemia)

Bone marrow expansion: widening of the diploic space of the skull, biconcave deformity of the vertebral bodies

Cortical thinning and softening of bone → ↑ risk of pathologic fractures

Extramedullary hematopoiesis: hepatosplenomegaly





Diagnostics

Perform initial laboratory studies to confirm anemia and hemolysis and classify anemia by morphology.

Anemia workup: CBC with MCV, reticulocyte count

Hemolysis workup: Add LDH, haptoglobin, bilirubin, urinalysis, and peripheral blood smear (PBS)

Obtain a direct Coombs test (i.e., DAT) to narrow the differential:

DAT positive: antibody-mediated hemolytic anemia

DAT negative: non antibody-mediated hemolytic anemia

Consider further investigations of the underlying etiology based on clinical suspicion and DAT results.



Routine laboratory studies

CBC

↓ Hb, Hct, and RBC count

MCV

Normocytosis: typical finding for most hemolytic anemias;
consider other anemia of chronic disease

↑ MCV: Can be due to severe reticulocytosis ; consider other
causes of macrocytosis (e.g., megaloblastic anemia)

Microcytosis: consider thalassemia and/or iron deficiency

WBC count: can be elevated due to inflammation or
malignancy

Platelets: decreased in MAHA and in Evan syndrome



Reticulocyte count: Use the corrected reticulocyte count for patients with anemia.

Reticulocytosis: most common ;

Can be normal in patients with:

- A delayed bone marrow response

- Concomitant bone marrow suppression or disease

Reticulocyte production index: usually $\geq 2\%$ in hemolytic anemia



Reticulocyte Count

- Used in anemia to determine if bone marrow response is adequate
- Reticulocyte count should rise if bone marrow responding normally
- Must be **corrected** for degree of anemia
- If $< 2\%$ $\rightarrow$ **inadequate bone marrow response**

Hct 45 (normal)	$\longrightarrow$	Hct 11
Retic 1% (normal)		Retic 8%

$$\text{Corrected RC} = 8\% * (11/45) = 2\%$$

Reticulocyte Production Index

- Normal reticulocytes circulate ~1 day
- In anemia → premature release of reticulocytes
- **Can circulate longer**
- RPI corrects for longer life of reticulocytes in anemia
- RPI > 3% is a normal marrow response to anemia
- RPI < 2% seen with bone marrow failure

$$\text{RPI} = \frac{\text{Corrected Retic \%}}{\text{Maturation Time}}$$

Hgb	MT
15	1
12	1.5
8	2
5	2.5



Hemolysis workup

While no single test can be used to confirm hemolysis, the finding of anemia in the presence of accelerated erythropoiesis (i.e., reticulocytosis) in addition to evidence of RBC destruction in serum and/or urine studies is highly suggestive of hemolytic anemia.

Typical biochemical findings in hemolysis include ↓ haptoglobin, ↑ LDH concentration, ↑ indirect bilirubin concentration, peripheral blood smear abnormalities (e.g., ↑ reticulocytes, schistocytes, spherocytes, polychromasia), and urinalysis abnormalities (e.g., hemoglobinuria, hemosiderinuria, and urobilinogen)



Serum studies

Haptoglobin

- ✓ Hb released from the breakdown of RBCs binds to haptoglobin → decrease in free circulating haptoglobin
- ✓ Not helpful to distinguish between intravascular hemolysis and extravascular hemolysis
- Lactate dehydrogenase (glycolysis enzyme)
- ✓ indicates increased cellular breakdown
- ✓ More prominent in intravascular hemolysis
- ✓ Can help distinguish between intravascular hemolysis and extravascular hemolysis
- ✓ Can be used as a marker of response to treatment



Indirect (unconjugated) bilirubin

- ✓ Hemolysis → Hb release → heme catabolized to indirect bilirubin
- ✓ More prominent in extravascular hemolysis
- ✓ Can be used as an early marker of response to treatment

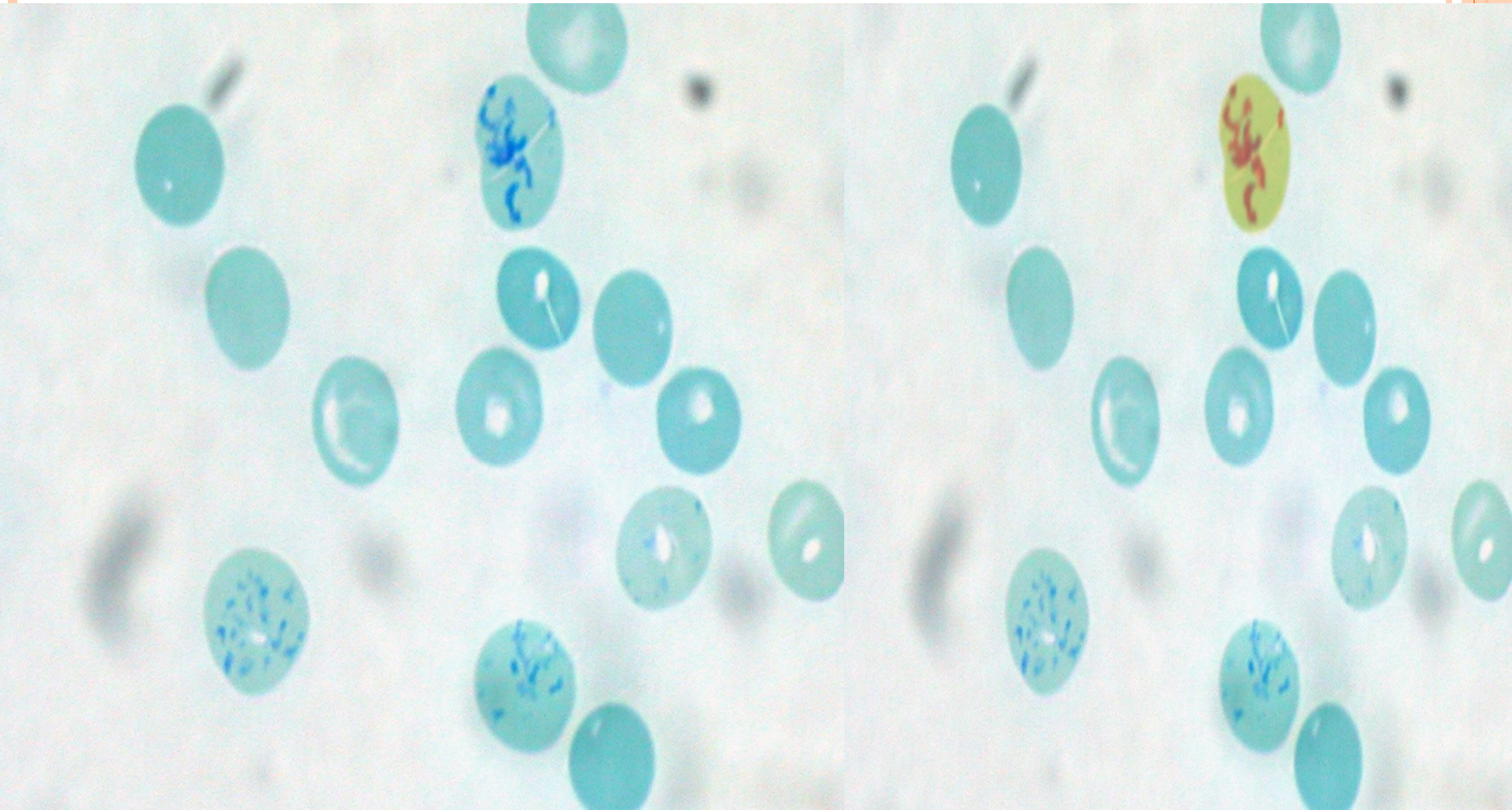


PBS

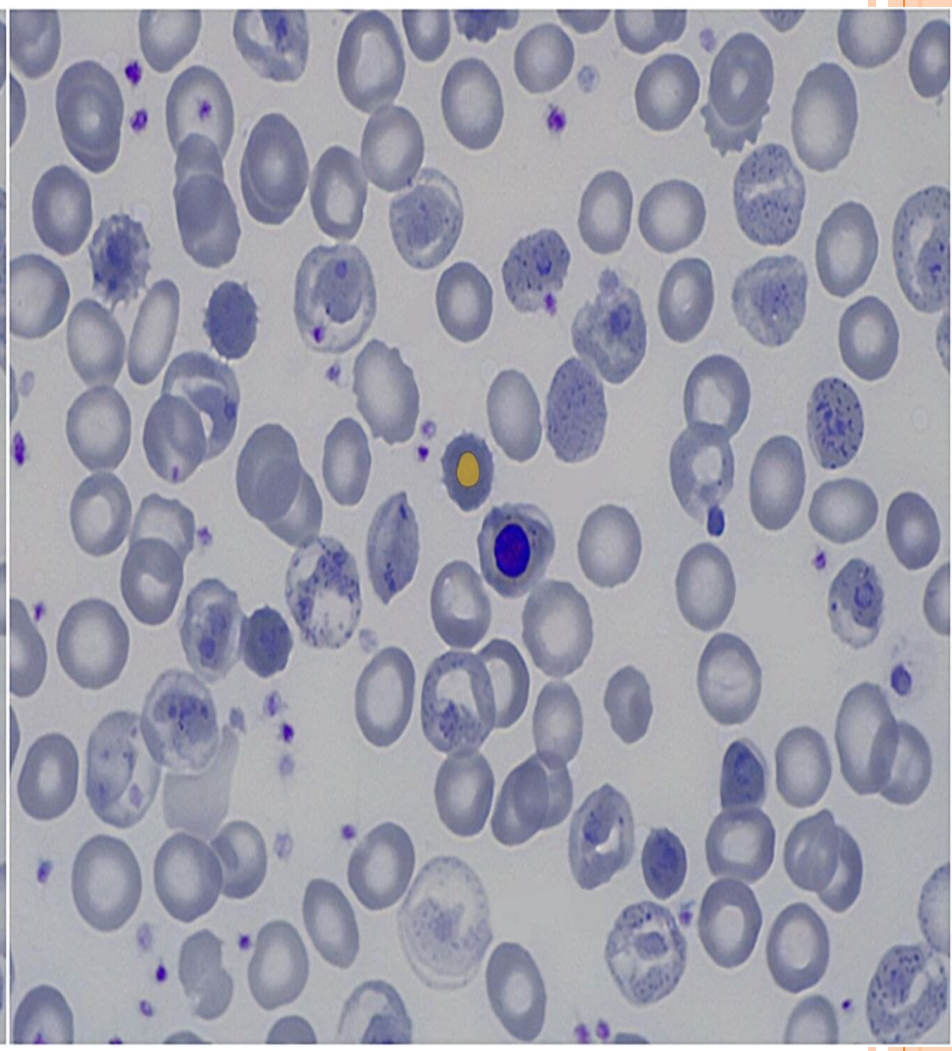
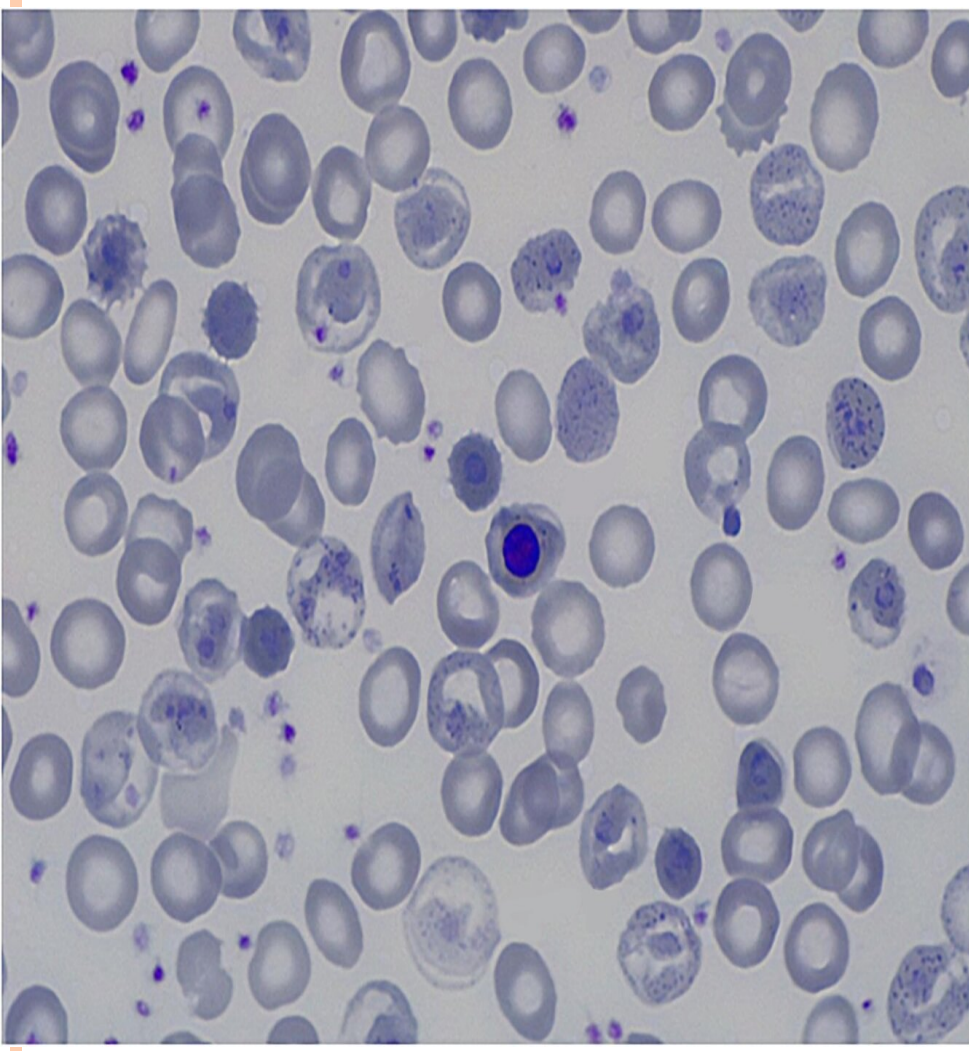
- ✓ Used to detect abnormal RBC forms, intracellular microorganisms, and inclusion bodies



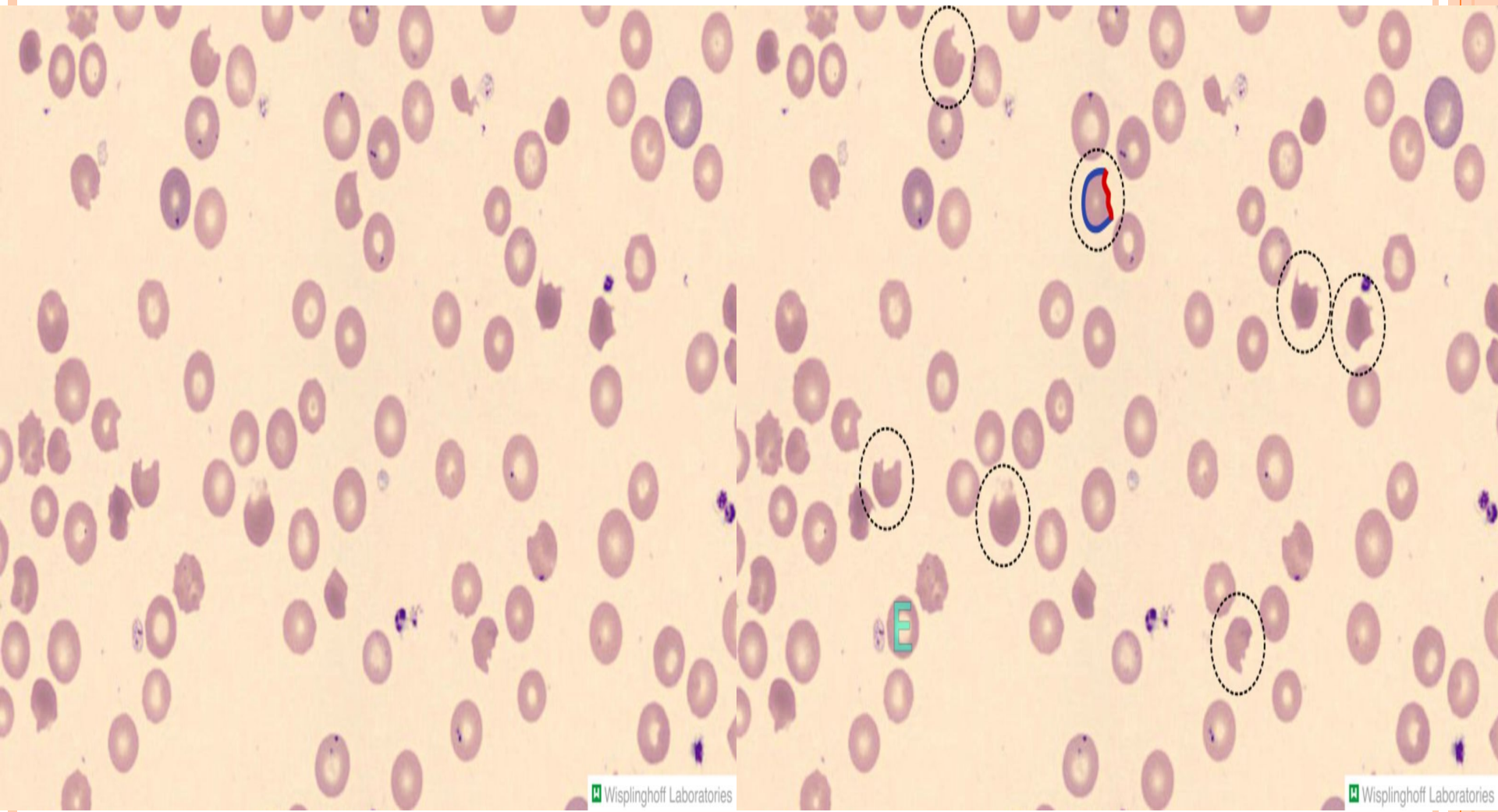
Reticulocytes in peripheral blood



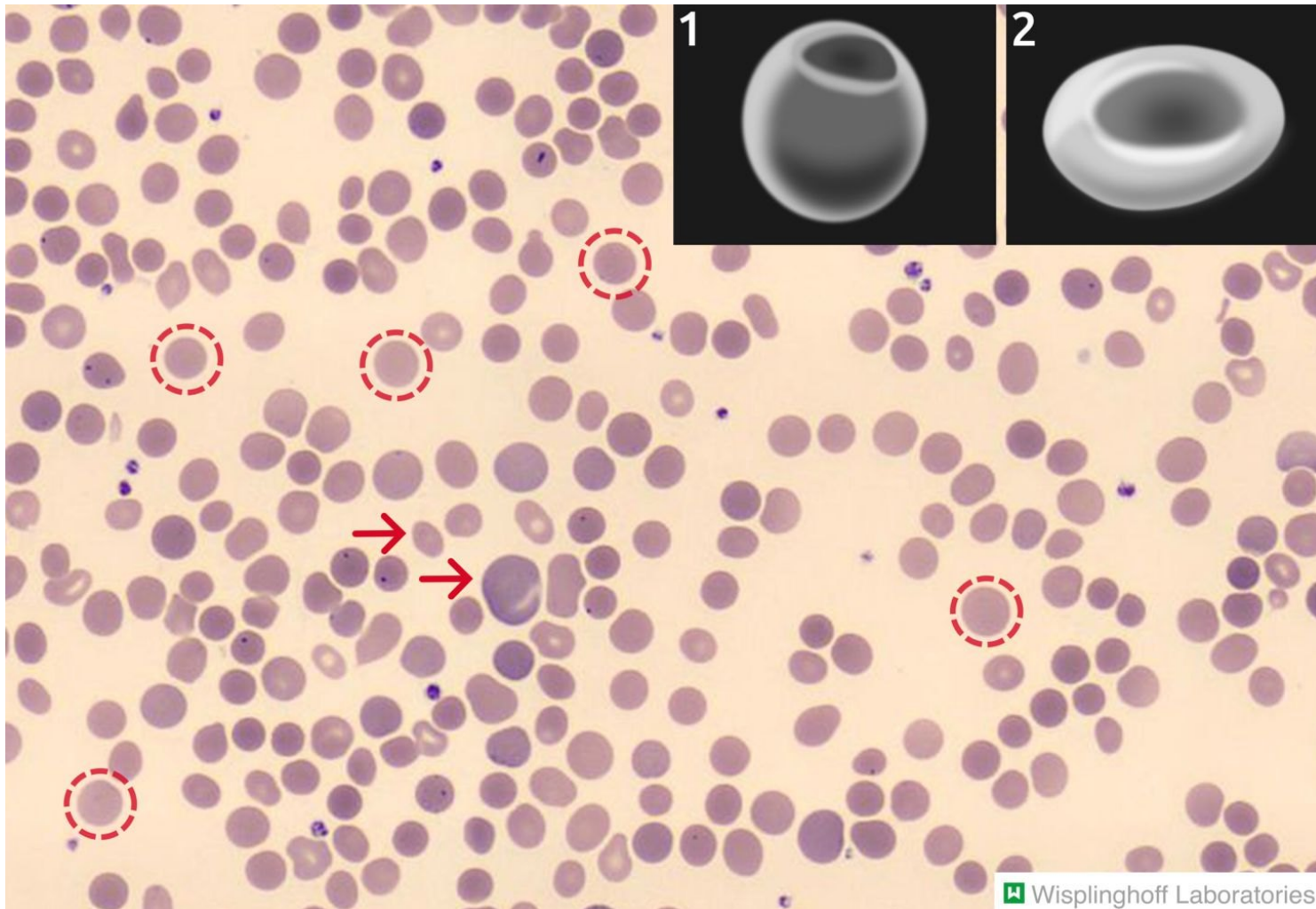
Heinz bodies



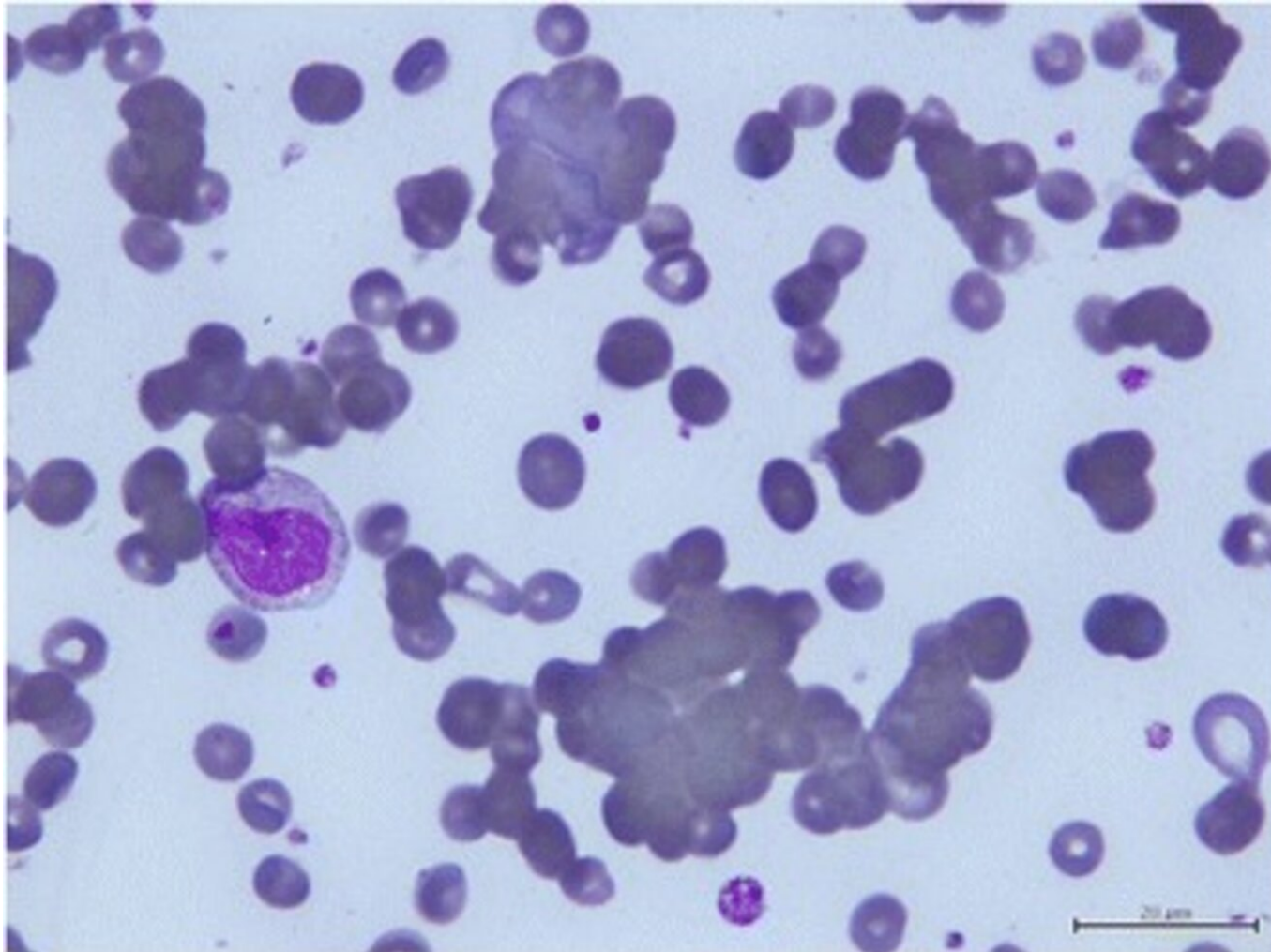
Schistocytes



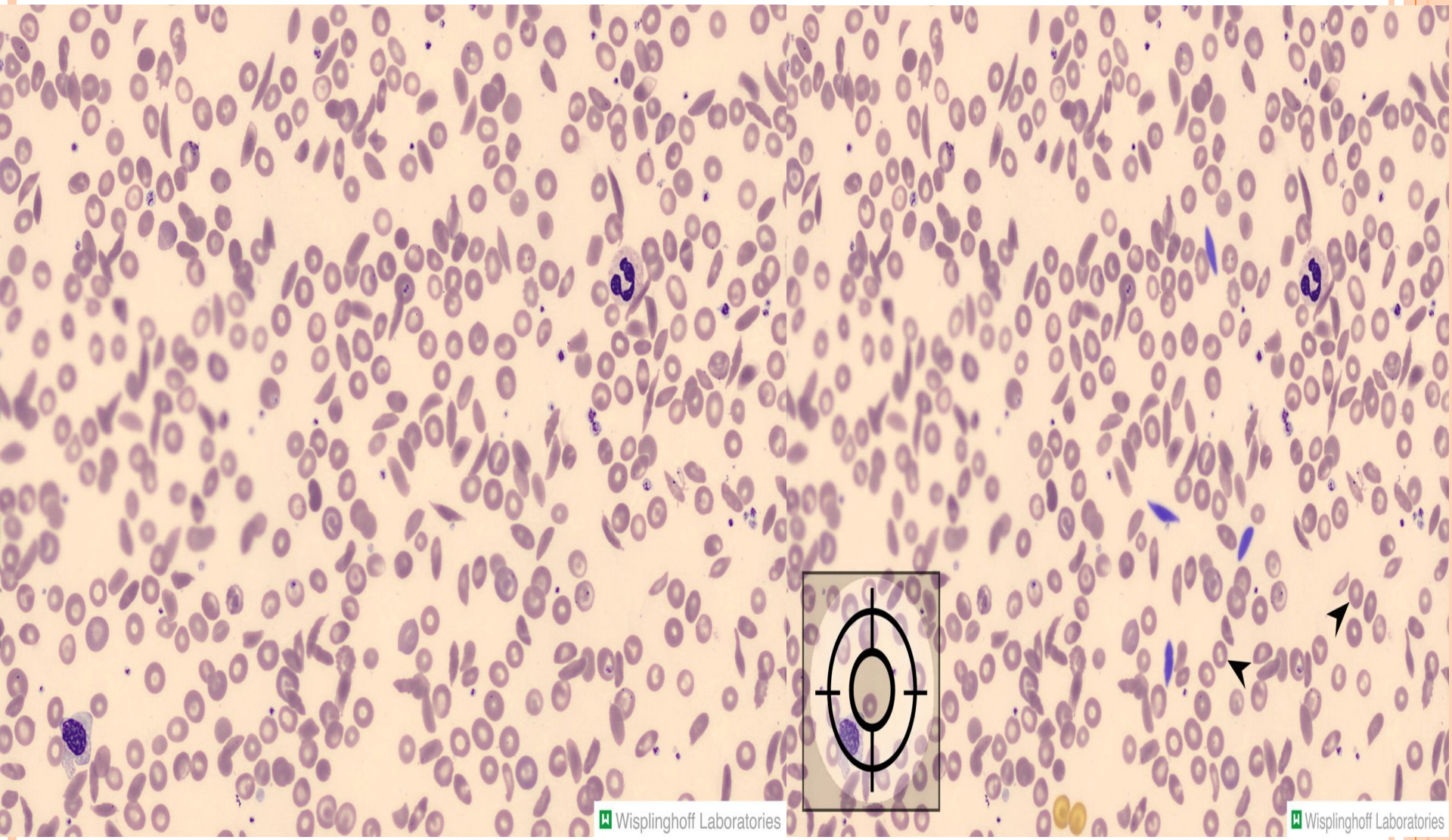
Spherocytosis



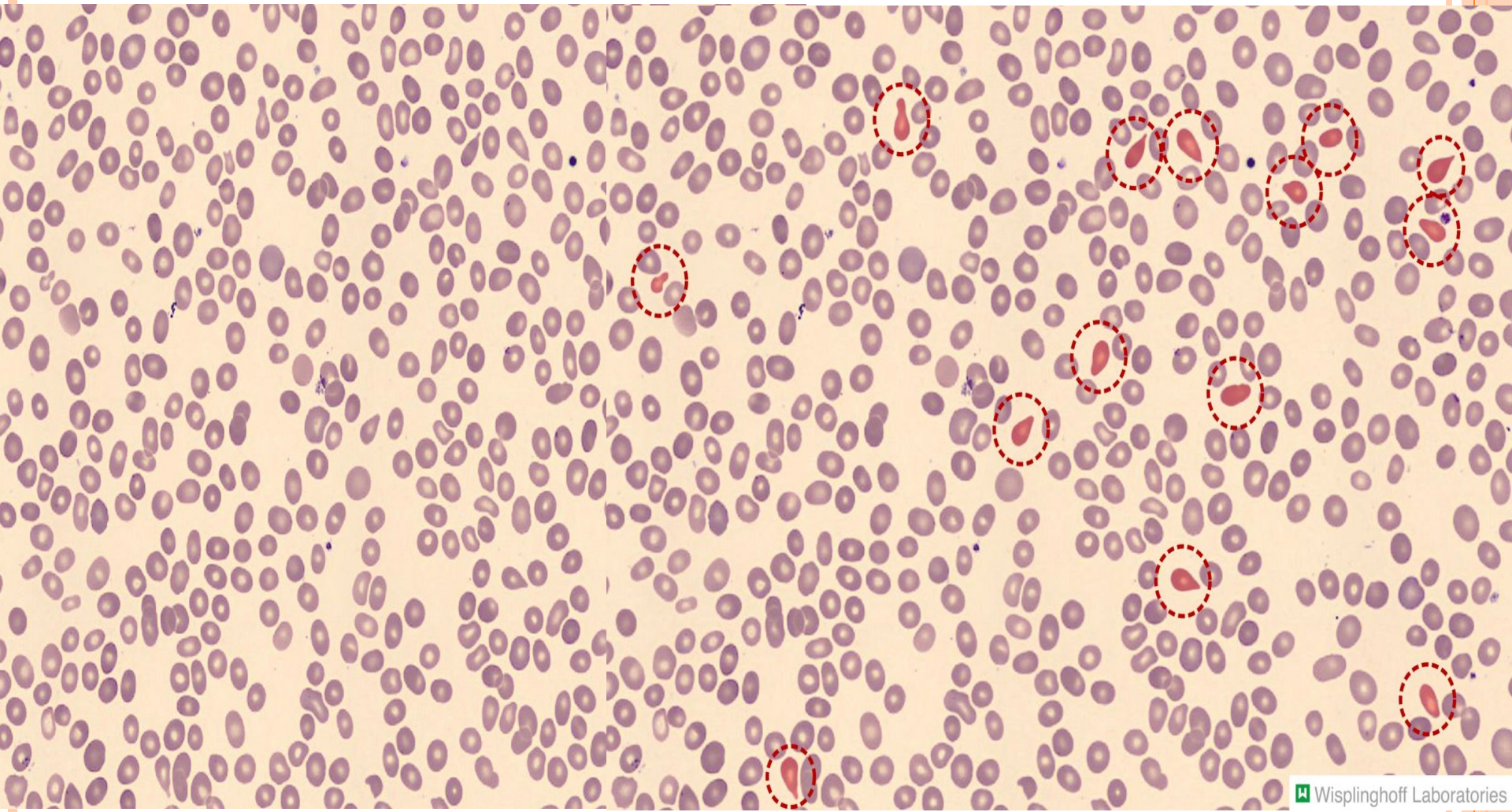
Agglutination of erythrocytes



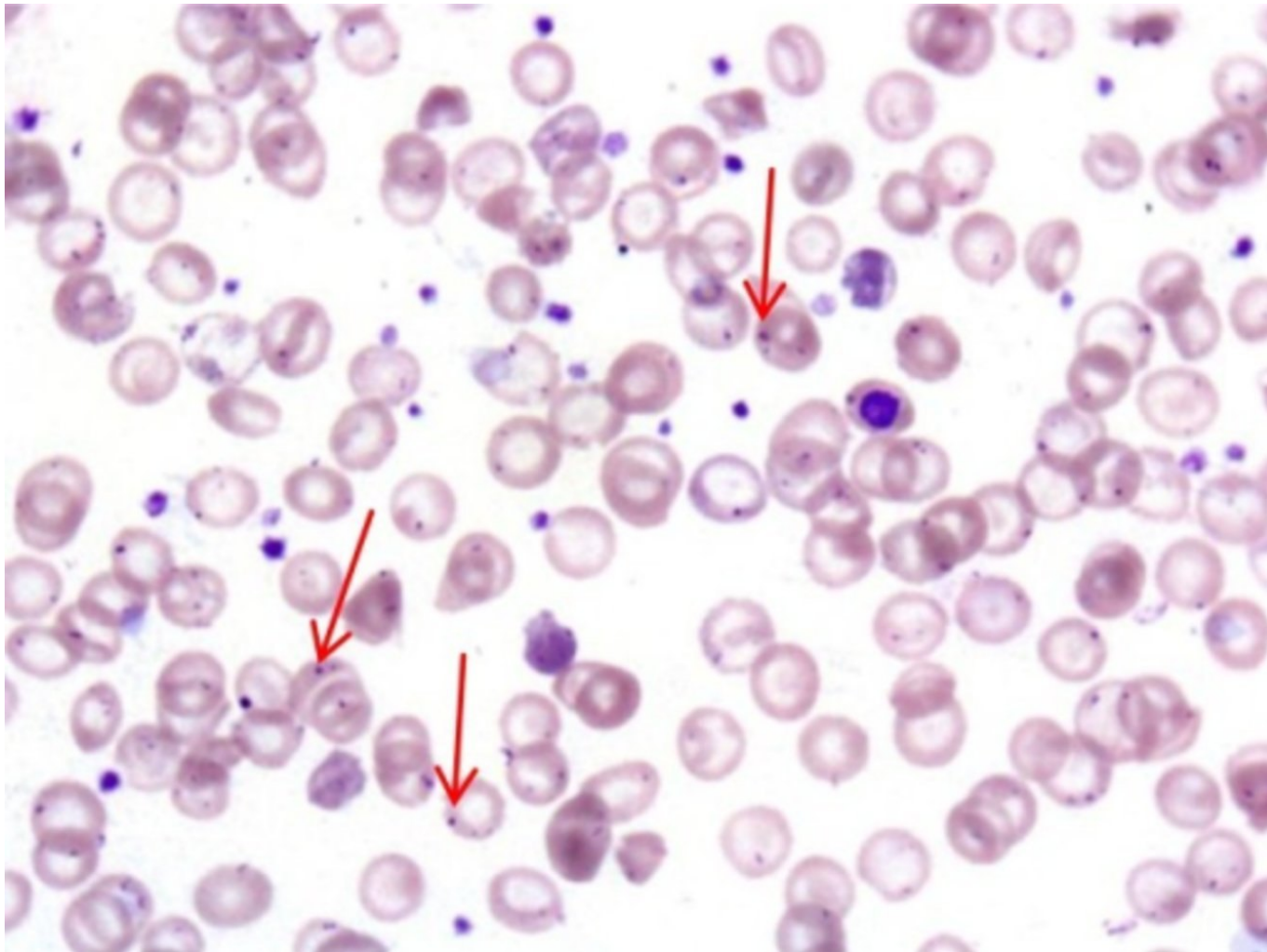
Erythrocyte morphologies in sickle-cell disease



Dacrocytes- TearDrop Cells



Howell-Jolly bodies in asplenia



- **Howell–Jolly bodies appear**
 - Some RBCs leave marrow with nuclear remnants (normal event)
 - Normally cleared by spleen
 - Presence in peripheral blood indicates splenic dysfunction or splenectomy



Urine studies

Hemoglobinuria

- ✓ Urine dipstick is positive for heme, but no RBCs are seen on microscopy.
- ✓ Early and transient marker of severe intravascular hemolysis
- ✓ Brown-colored urine
- ✓ Mostly occurs in intravascular hemolysis



Hemosiderinuria (intravascular)

Urobilinogen

- ✓ ↑ Indirect bilirubin → ↑ bilirubin breakdown in the gut → ↑ urobilinogen reabsorption into the enterohepatic circulation → excess urobilinogen enters the systemic circulation → urobilinogen is excreted in the urine
- ✓ Mostly occurs in extravascular hemolysis



Intrinsic hemolytic anemias

Hemolytic anemia that is caused by structural or functional RBC abnormalities can be further classified as one of the following based on pathogenesis:



RBC membrane defects

Paroxysmal nocturnal hemoglobinuria

Hereditary spherocytosis

Hereditary elliptocytosis

Enzyme defects

Pyruvate kinase deficiency

Glucose-6-phosphate dehydrogenase deficiency

Hexokinase deficiency

Adenylate kinase deficiency

Hemoglobinopathies

Hemoglobin C disease

Sickle cell disease

Thalassemia



Paroxysmal nocturnal hemoglobinuria

An acquired genetic defect of the hematopoietic stem cell characterized by a triad of hemolytic anemia, pancytopenia, and thrombosis



Pathophysiology

Physiologically, a membrane-bound glycosylphosphatidylinositol (GPI) anchor protects RBCs against complement-mediated hemolysis.

Acquired mutation on the PIGA gene located on the X chromosome → GPI anchor loses its protective effect → RBC destruction by complement and reticuloendothelial system → intravascular and extravascular hemolysis

The GPI anchor proteins involved in PNH are:

CD55/DAF (Decay-accelerating factor)

CD59/MIRL (Membrane inhibitor of reactive lysis)

PNH can also occur in patients with aplastic anemia and MDS.



Clinical features

Pallor, excessive fatigue , weakness

Intermittent jaundice

Episodes of hemoglobinuria causing pink/red/dark urine which usually occurs in the morning due to the concentration of urine overnight.

Vasoconstriction

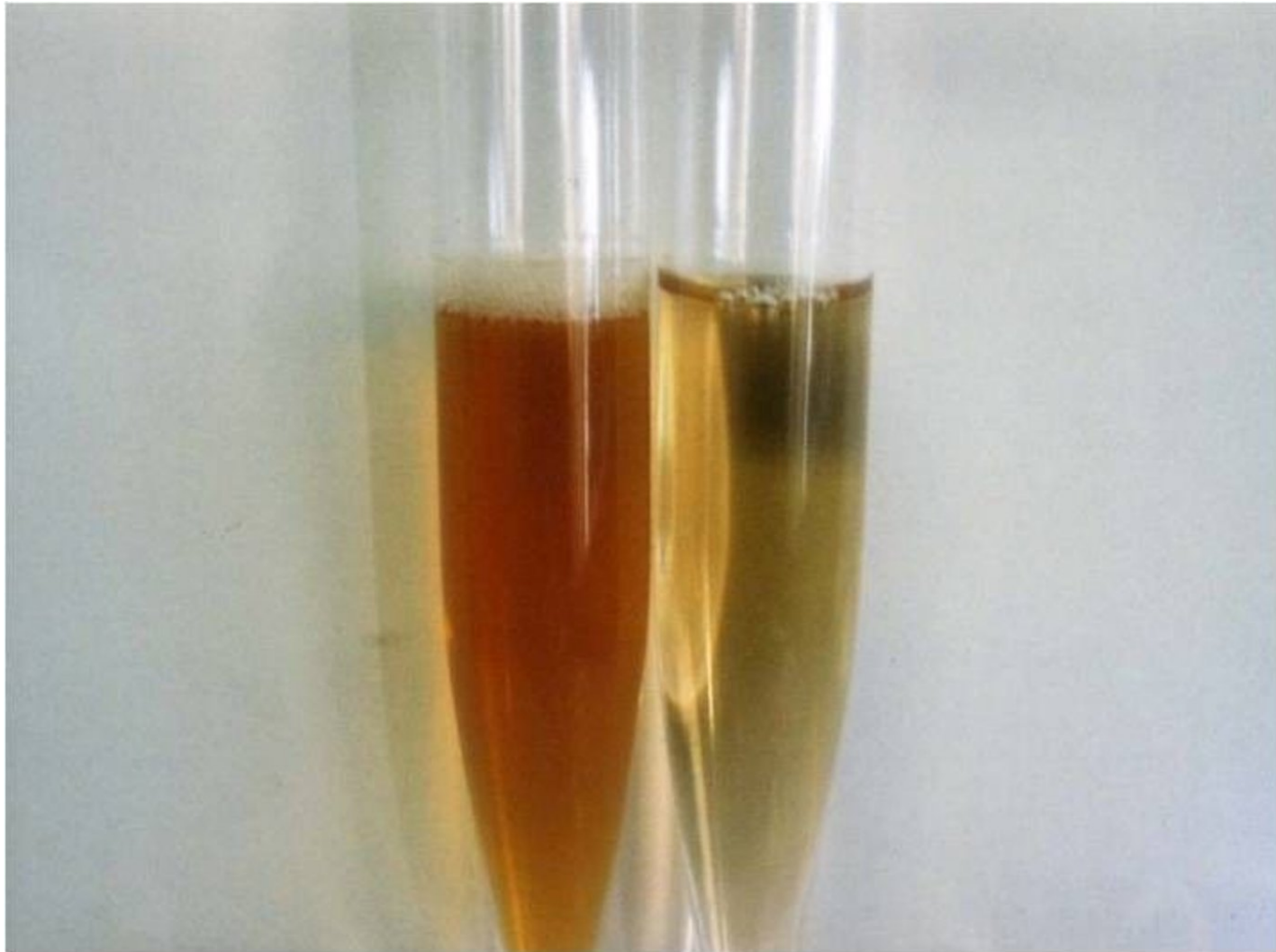
Headache, pulmonary hypertension

Abdominal pain, dysphagia, erectile dysfunction

Venous thrombosis in unusual locations (e.g., hepatic, cerebral, and/or abdominal veins)

Increased risk of infections (in case of pancytopenia)





Diagnostics

CBC: anemia, thrombocytopenia, and/or pancytopenia ; usually ↑ reticulocytes

Hemolysis workup: ↓ haptoglobin, hemosiderinuria, hemoglobinuria

Direct Coombs test: negative

Flow cytometry of peripheral blood (confirmatory test for PNH): can show deficiency of GPI-linked proteins on the surface of RBCs and WBCs (e.g., CD55, CD59)



Bone marrow biopsy: not required for the diagnosis of PNH, but indicated in patients with significant pancytopenia



Hereditary spherocytosis

Etiology

Congenital RBC membrane protein defect

Inheritance pattern

Autosomal dominant (~ 75% of cases)

Autosomal recessive (~ 25% of cases)

Family history often positive for relatives who required splenectomy and/or developed cholelithiasis at a young age



Pathophysiology

Genetic mutation → defects in RBC membrane proteins (especially spectrin and/or ankyrin) responsible for tying the inner membrane skeleton with the outer lipid bilayer → continuous loss of lipid bilayer components → decreased surface area of RBCs in relation to volume → sphere-shaped RBCs with decreased membrane stability → inability to change form while going through narrowed vessels →

- Entrapment within splenic vasculature → splenomegaly
- Destruction via splenic macrophages → extravascular hemolysis



Clinical features

Presentation is variable.
nemia



Diagnostics

Laboratory findings

Normocytic anemia: mean cell volume (MCV) within normal range (80-100 fL) or slightly decreased

Increased mean corpuscular hemoglobin concentration (MCHC)

↑ Red blood cell distribution width (↑ RDW)

↑ Reticulocytes (normal range: 0.5%–1.5% of total RBC count)

Findings of hemolytic anemia

↑ Unconjugated bilirubin

↓ Haptoglobin

↑ LDH



Negative Coombs test: to exclude autoimmune hemolytic anemia (positive Coombs test), since spherocytosis is seen in both

Positive osmotic fragility test

Measures the ability of RBCs to resist hemolysis when exposed to different degrees of salt dilution (e.g., RBCs swell and eventually lyse when incubated in hypotonic saline due to water influx)

Whether or not RBCs lyse depends on their surface area to volume ratio. This ratio is decreased in patients with HS, so their RBCs are more fragile and more vulnerable to osmotic stress.



Blood smear

Characteristic spherocytes (small round cells without central pallor)

Potentially anisocytosis

If splenectomy was done, Howell-Jolly bodies will appear in peripheral circulation



Treatment

Non-surgical treatments

Blood transfusions may be required in cases of aplastic or hemolytic crisis

Folic acid supplementation to maintain erythropoiesis

Splenectomy

Sole definitive treatment

Prior to the procedure, vaccinate against

Streptococcus pneumoniae, Haemophilus influenzae type B, and Neisseria meningitidis



Complications

Hemolytic crisis: esp. as a result of viral infection

Aplastic crisis: following infection with parvovirus B19 (erythema infectiosum); characterized by a low reticulocyte count (< 0.1% of total RBC count)

Megaloblastic anemia: folate and vitamin B12 deficiency may develop due to chronic hemolysis and high RBC turnover

Megaloblastic crisis: due to folate deficiency (although uncommon in developed countries, it might still be seen among pregnant women)

Other

Bilirubinate gallstone formation, possibly leading to cholecystitis, cholangitis, and pancreatitis

Growth retardation and skeletal abnormalities due to bone marrow expansion



Glucose-6-phosphate dehydrogenase deficiency (Favism)

Epidemiology

G6PD deficiency is the most common human enzyme deficiency.

Prevalence: ~ 400 million worldwide

Affects primarily males of African, Mediterranean, and Asian descent



Pathophysiology

X-linked recessive inheritance

G6PD is the rate-limiting enzyme of the pentose phosphate pathway (also known as the hexose monophosphate shunt). This pathway yields NADPH, which is essential for converting oxidized glutathione back to its reduced form. Reduced glutathione is capable of neutralizing reactive oxygen species (ROS) and free radicals and therefore protecting RBCs from oxidative damage.

In the absence of reduced glutathione (e.g., due to G6PD deficiency), RBCs become susceptible to oxidative stress that can damage erythrocyte membranes, resulting in intravascular and extravascular hemolysis.



Causes of increased oxidative stress are triggers of hemolytic crisis and include:

Fava beans

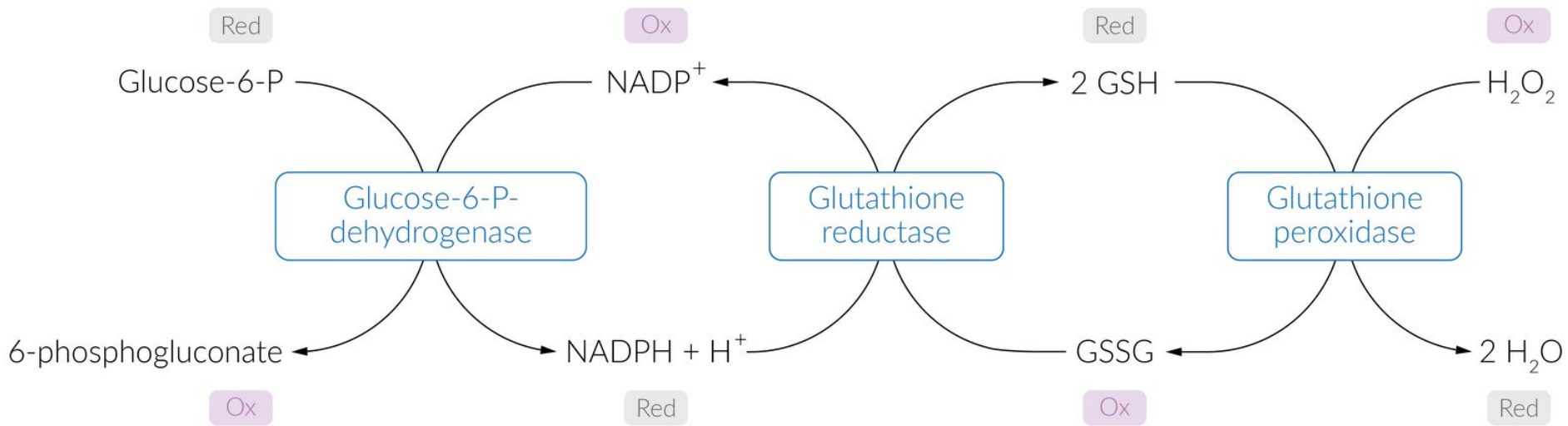
Drugs: antimalarial drugs (e.g., chloroquine, primaquine), sulfa drugs (e.g., trimethoprim-sulfamethoxazole), nitrofurantoin, isoniazid, dapsone, NSAIDs, ciprofloxacin, chloramphenicol

Bacterial and viral infections (most common cause):
Severe enzymatic deficiency can inhibit respiratory burst activity due to reduced NADPH production in phagocytes.

Inflammation: During an inflammatory reaction free radicals are produced and can diffuse into RBCs.

Metabolic acidosis





Clinical features

Most patients are asymptomatic.

Recurring hemolytic crises may occur, especially following triggers

- Arise within 2–3 days after increased oxidative stress

- Sudden onset of back or abdominal pain

- Jaundice

- Dark urine

- Transient splenomegaly



Diagnostics

Blood smear

Heinz bodies: Oxidative stress causes hemoglobin denaturation. Hemoglobin then precipitates as small inclusions within the erythrocytes.

Bite cells: due to macrophages selectively removing the denatured hemoglobin inclusions of RBCs

Signs of intravascular hemolysis

Normocytic anemia

↑ Reticulocyte count

↑ Unconjugated bilirubin

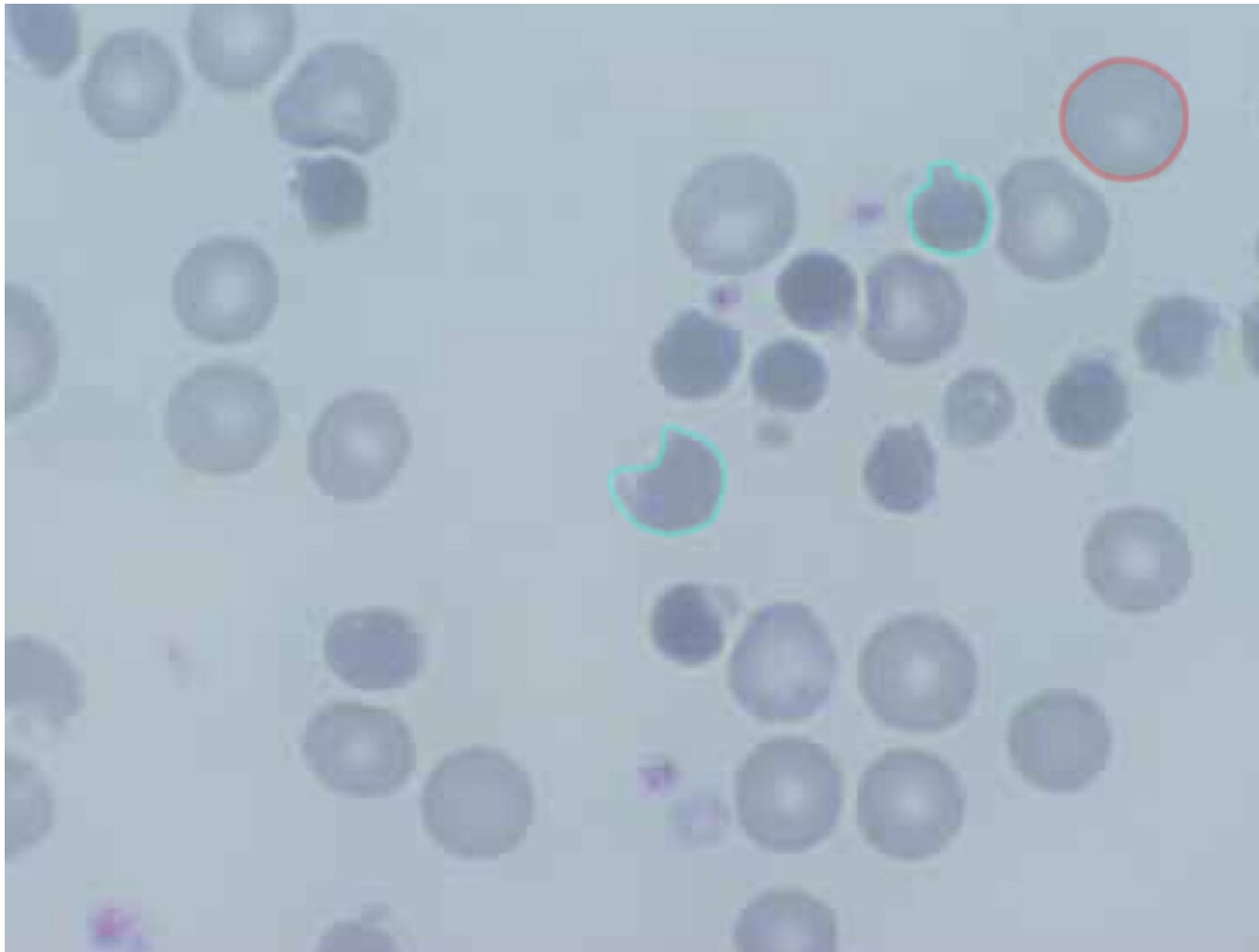
↑ LDH

↓ Haptoglobin

Hemoglobinuria



Bite cells in glucose-6-phosphate dehydrogenase (G6PD) deficiency



Confirmatory test: quantitative G6PD enzyme analysis

confirmatory tests should be performed during remission

During hemolysis, false-negative results may occur because older RBCs have been hemolyzed and replaced by reticulocytes and younger RBCs, which have increased G6PD activity.



Treatment

Avoid triggers

Blood transfusions should be performed only in rare, severe cases.



Thalassemia

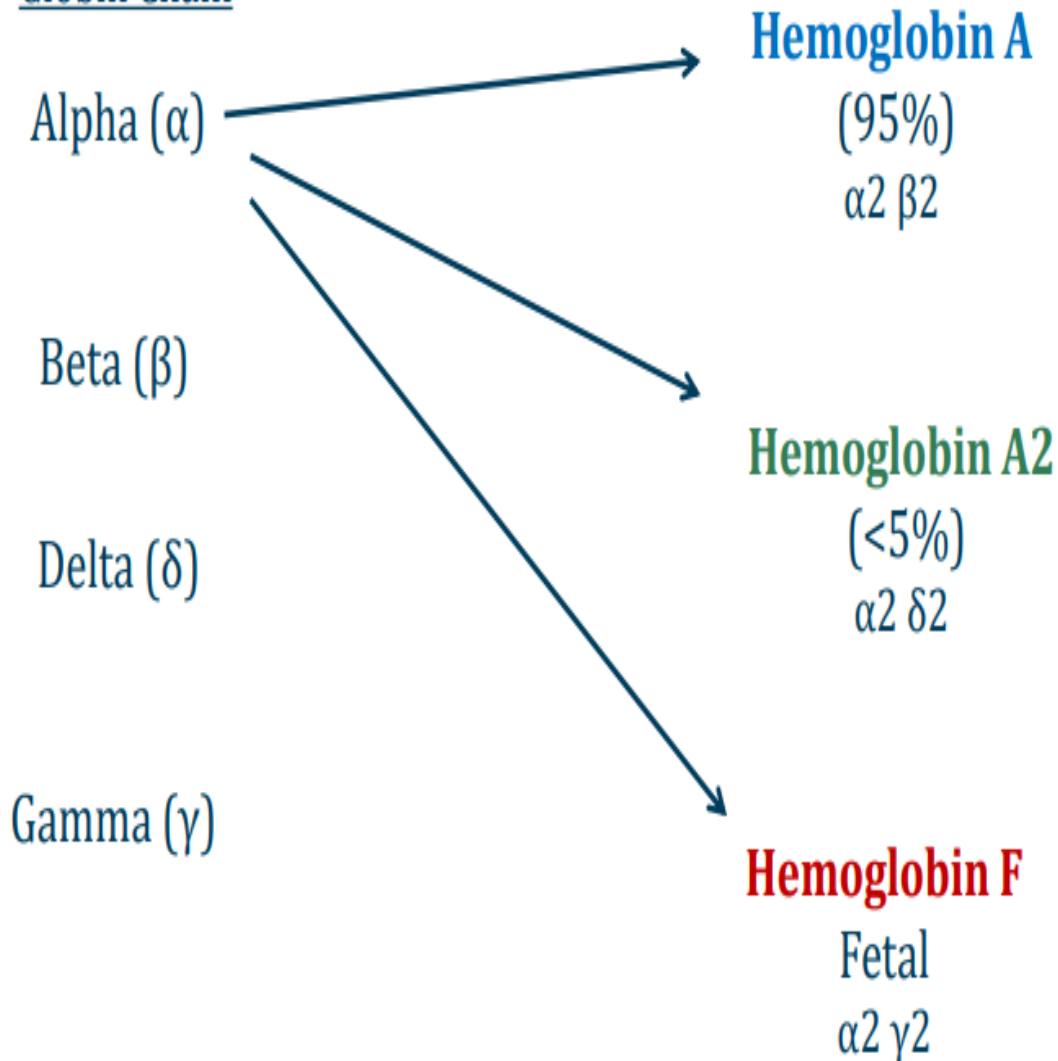
Hemoglobinopathies

- Abnormal hemoglobin
- Structural disorders: sickle cell disease
- Thalassemias: decreased globin chain production
- Alpha thalassemia: alpha globin
- Beta thalassemia: beta globin



Globin Chains and Hemoglobin

Globin Chain



All Hgb has two alpha globins
Other chain determines type

Globin Chains and Hemoglobin

Globin Chain

Alpha (α)

Beta (β)

Delta (δ)

Gamma (γ)

Hemoglobin A

(95%)

$\alpha_2 \beta_2$

Hemoglobin A2

(<5%)

$\alpha_2 \delta_2$

Hemoglobin F

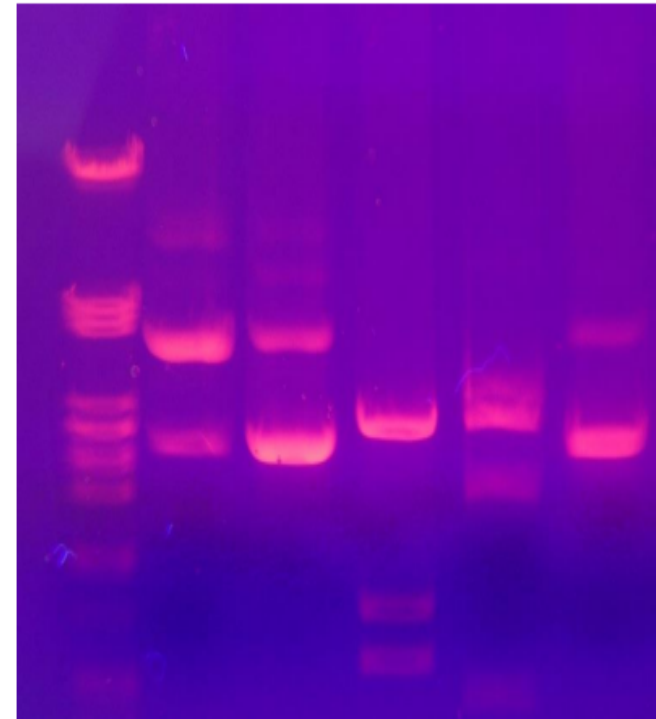
Fetal

$\alpha_2 \gamma_2$

All Hgb has two alpha globins
Other chain determines type

Hgb Electrophoresis

- Used to diagnose **hemoglobinopathies**
 - Thalassemia
 - Sickle cell disease
- Electrical charge applied to sample on gel
- Different hemoglobins → different distances moved
- Determines HgbA, HgbA2, HgbF
- Often done for microcytic anemia + no iron deficiency



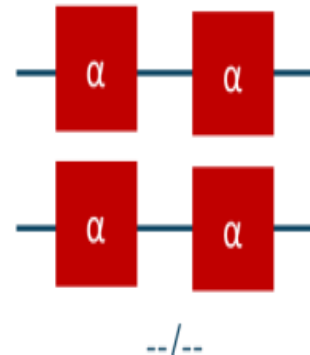
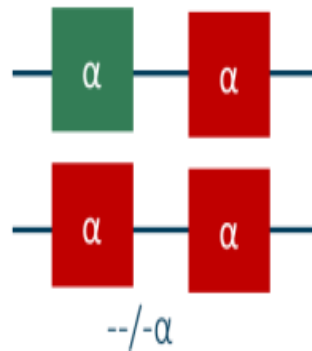
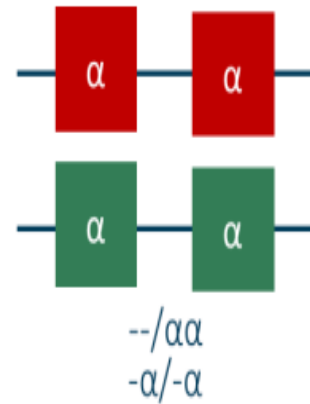
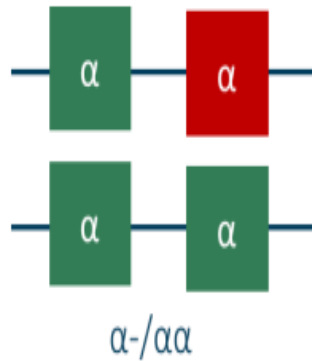
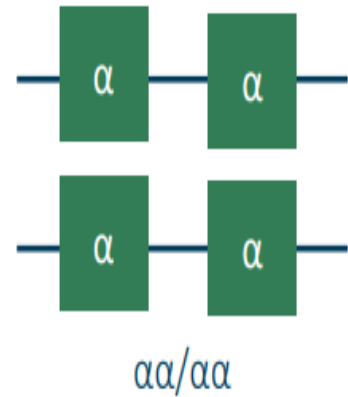
Thalassemias

- Spectrum of severity
- Thalassemia **minor**
 - Often asymptomatic
 - Identified on routine blood testing or blood smear
 - May have **microcytosis with ↑ red cell count**
 - Contrast with iron deficiency: microcytosis with ↓ RBC
- Thalassemia **major**
 - Severe loss of globin production
 - Lifelong transfusions or death



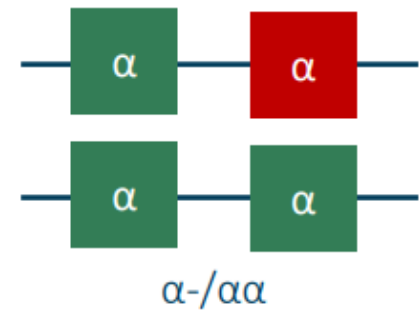
Alpha Thalassemia

- **Four genes** code for alpha chains
- Two on each copy of chromosome 16
- Often caused by **gene deletions** $\rightarrow$ $\downarrow$ α chains $\rightarrow$ alpha thalassemia



Alpha Thalassemia Minima

- Normal red cells
- No symptoms
- Carrier state



Alpha Thalassemia Minor

Alpha Thalassemia Trait

- No symptoms
- $\downarrow$ MCV/MCH/MCHC
- Electrophoresis: usually normal
- Diagnosis: genetic testing

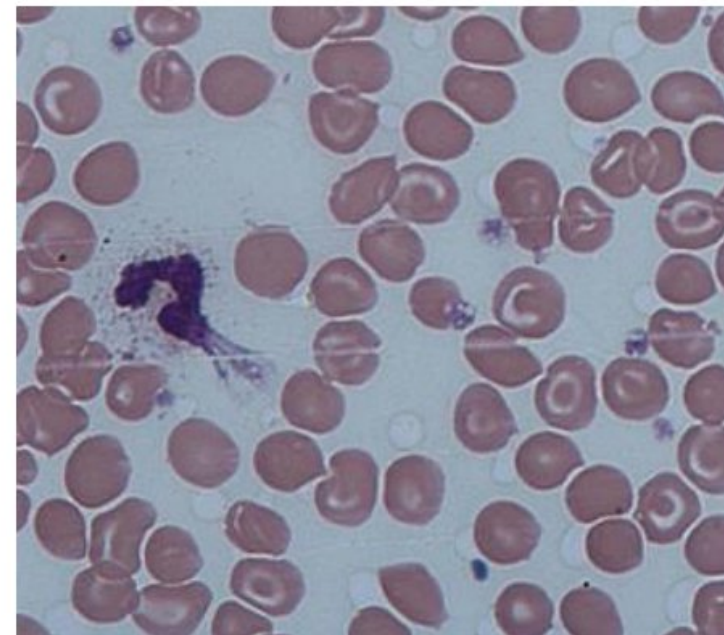


Thalassemia Minor

Differential diagnosis

- Alpha and beta minor can mimic **iron deficiency**
- Both may lead to low MCV and MCH
- Key distinguishing factors for thalassemia:
 - **Red cell count normal or increased**
 - RDW normal
 - Iron and ferritin normal or increased ($\uparrow$ turnover)
- **Target cells**
 - Classic finding in thalassemia
 - Caused by increased surface to volume ratio
 - Rarely seen in iron deficiency

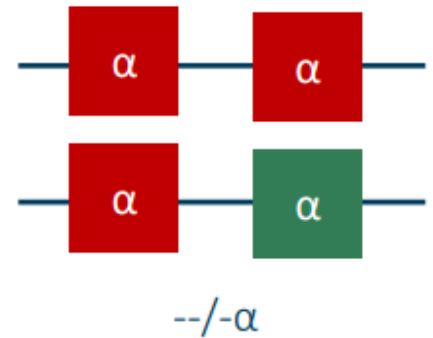
Target Cells



Hemoglobin H Disease

Alpha thalassemia intermedia

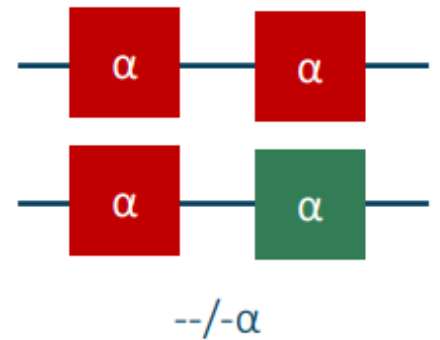
- Very little alpha globin production
- Excess beta globin
- **HbH forms: four beta chains**
 - Easily damaged
 - Affinity for oxygen 10x HbA
 - Poor oxygen delivery



Hemoglobin H Disease

Clinical features

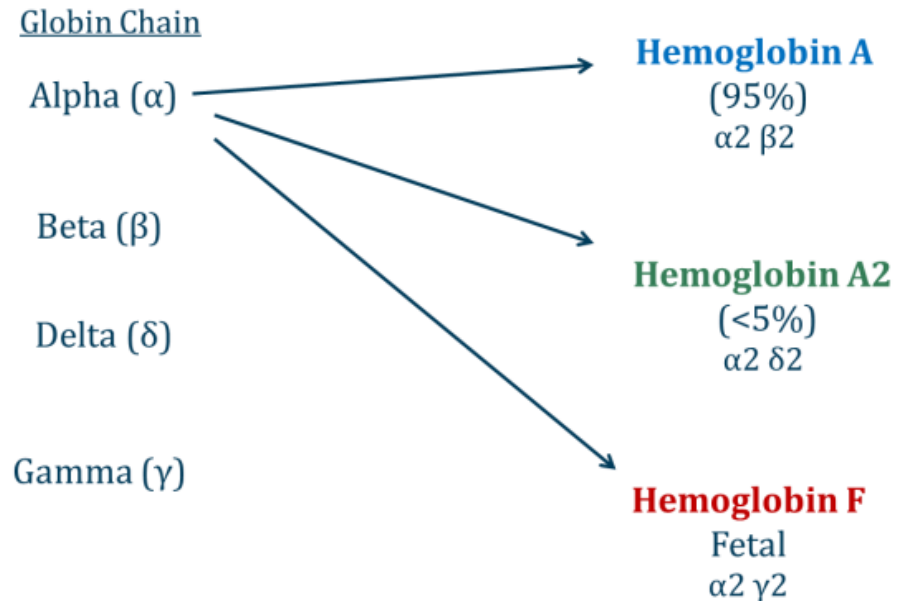
- Highly variable presentation
- Often symptomatic in infancy
- Hypochromic, microcytic anemia
- Low MCV, MCH, MCHC
- **Extravascular hemolysis**
 - Abnormal RBC deformability
 - Splenomegaly
 - Indirect hyperbilirubinemia
 - May cause neonatal jaundice
- Bizarre red cell morphologies



Hemoglobin H Disease

Diagnosis

- **Electrophoresis**
 - HBH
 - Decreased HbA, HbA₂, HbF
- **DNA testing**



Hemoglobin H Disease

Treatment

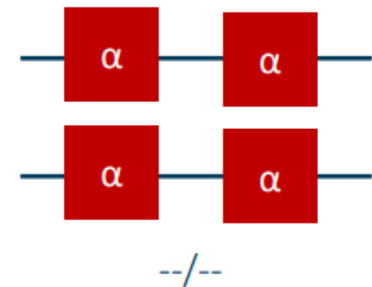
- **Blood transfusions**
 - Usually not transfusion dependent
 - May require **episodic transfusions** with stress
 - Especially during infection or pregnancy
 - Or with aplastic crisis from B19 infection
- Splenectomy
- Bone marrow transplantation
- Long term risk: **iron overload**
 - Ineffective erythropoiesis → ↑ intestinal iron uptake
 - Also from transfusions



Hemoglobin Barts

Alpha thalassemia major

- **No α globin**
- Cannot form HbF
- **Hgb Barts**
 - Forms in utero
 - Four gamma globin chains
 - Cannot release oxygen to tissues
- **Hydrops fetalis**
 - Massive total body edema
 - High output heart failure
- In utero death or death hours after birth



Beta Thalassemia

- $\downarrow$ β globin chain synthesis
- Two genes code for beta chains
- One on each copy of chromosome 11
- Often caused by mutations (not deletions)
- Wide spectrum of disease depending on mutation
 - β^0 = no function; β^+ = some function

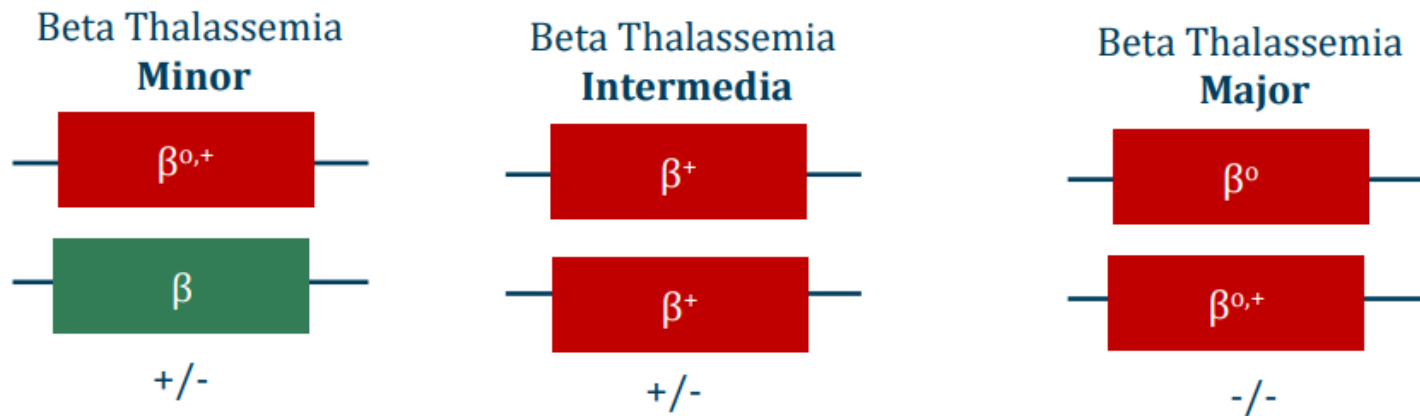


β



Beta Thalassemia

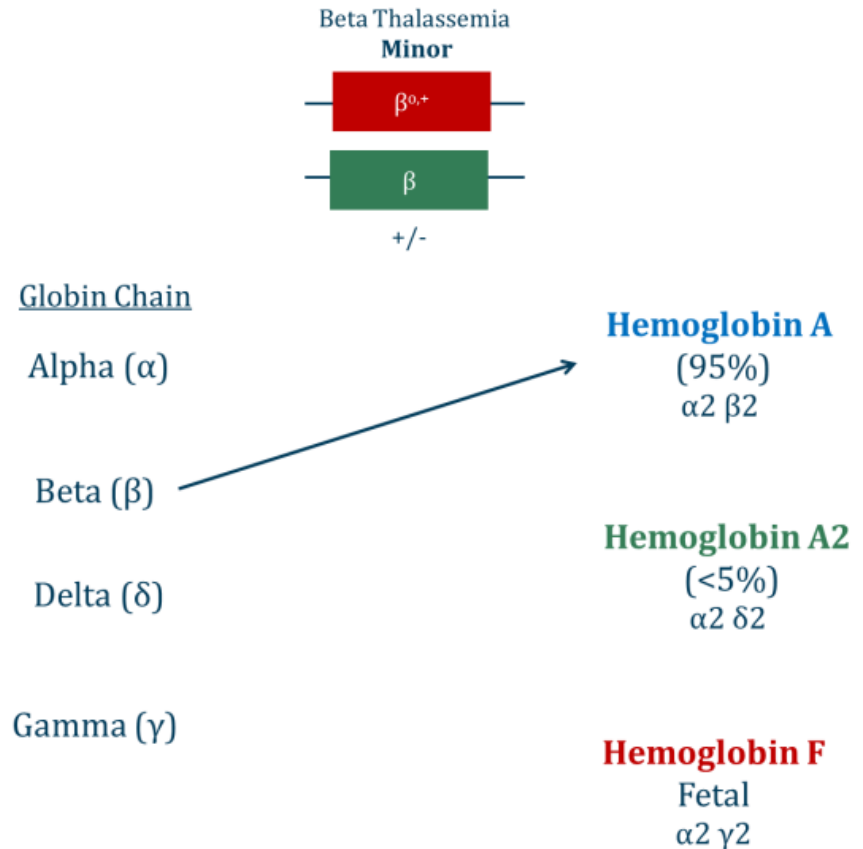
- Previously classified as minor, intermedia or major
- Now classified as **transfusion-dependent** or **transfusion-independent**



Beta Thalassemia Minor

Beta Thalassemia Trait

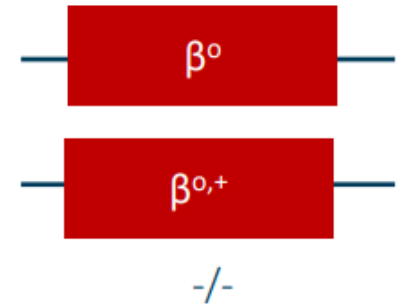
- Reduced β globin synthesis
- Asymptomatic
- Possible microcytosis with $\uparrow$ RBC
- Diagnosis by electrophoresis
- $\uparrow$ **HgbA2**
 - HgbA2: $\alpha_2\delta_2$ – no beta chains
 - Normal $\sim 3\%$



Transfusion-Dependent Beta Thalassemia

Beta Thalassemia Major

- No or severely limited β globin production
- Anemia beginning **1st year of life**
 - Usually begins 6 to 12 months
 - Occurs when HgbF ($\alpha_2\gamma_2$) production wanes
- Ineffective erythropoiesis
 - Alpha chains form tetramers
 - Precipitate $\rightarrow$ RBC damage
 - Failure to produce RBCs
- **Life-long transfusion-dependent anemia**

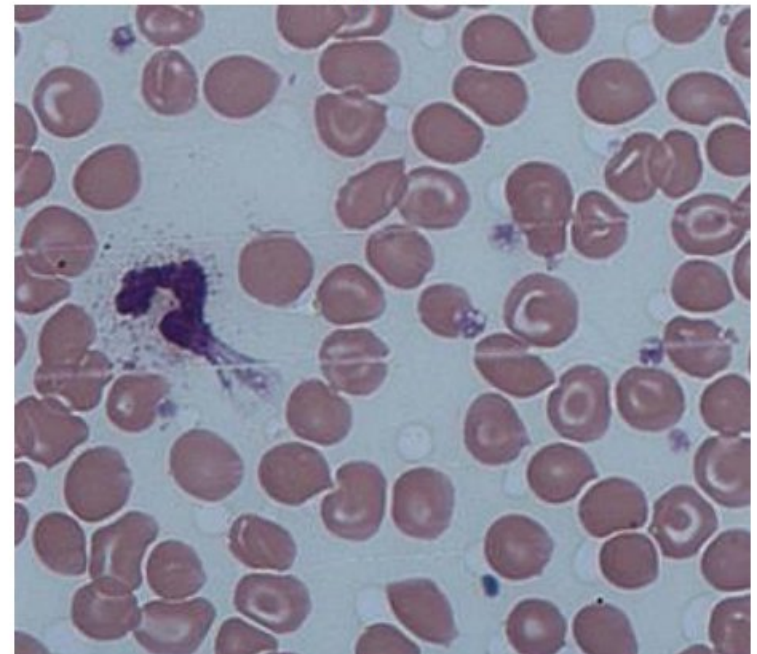


Transfusion-Dependent Beta Thalassemia

Clinical Features

- Hypochromic, microcytic anemia
- Bone changes
- Iron overload
 - Ineffective erythropoiesis → ↑ intestinal iron uptake
 - Also from transfusions
- Splenomegaly
 - Spleen clears abnormal RBCs
- Bizarre red cell morphology
 - Abnormal size and shapes
 - Many abnormalities possible

Target Cells

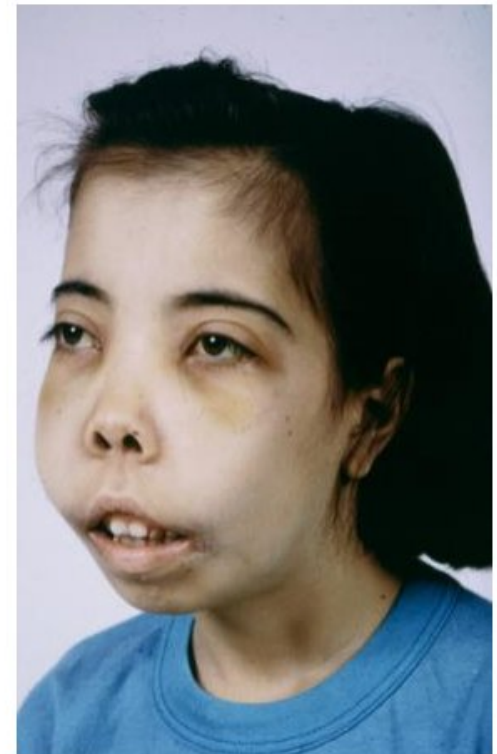


Transfusion-Dependent Beta Thalassemia

Bone Changes

- ↑↑ EPO without normal response
- Erythroid hyperplasia
- Massive expansion of bone marrow
- Abnormalities of **skull and facial bones**
- “Chipmunk facies”
- Delayed skeletal maturation
- Widening of marrow spaces → osteoporosis

Chipmunk Facies



Transfusion-Dependent Beta Thalassemia

Diagnosis

- **Electrophoresis**
 - ↓ or absent HbA ($\alpha_2\beta_2$)
 - ↑ HbA2 ($\alpha_2\delta_2$)
 - ↑ HbF ($\alpha_2\gamma_2$)
- **Genetic testing**

Globin Chain

Alpha (α)

Beta (β)

Delta (δ)

Gamma (γ)

Hemoglobin A

(95%)
 $\alpha_2\beta_2$

Hemoglobin A2

(<5%)
 $\alpha_2\delta_2$

Hemoglobin F

Fetal
 $\alpha_2\gamma_2$

Folate Supplementation

- All patients with **thalassemia major**



Needpix.com/Public Domain

Red Cell Distribution Width

RDW

- Spectrum of RBC size
- Normal RDW: 11.5 to 14.5%
- Often normal in thalassemia
- Wider in iron deficiency (often > 17%)
- Normal RDW makes iron deficiency unlikely
- Microcytic anemia + normal RDW: **almost always thalassemia**



Thalassemia

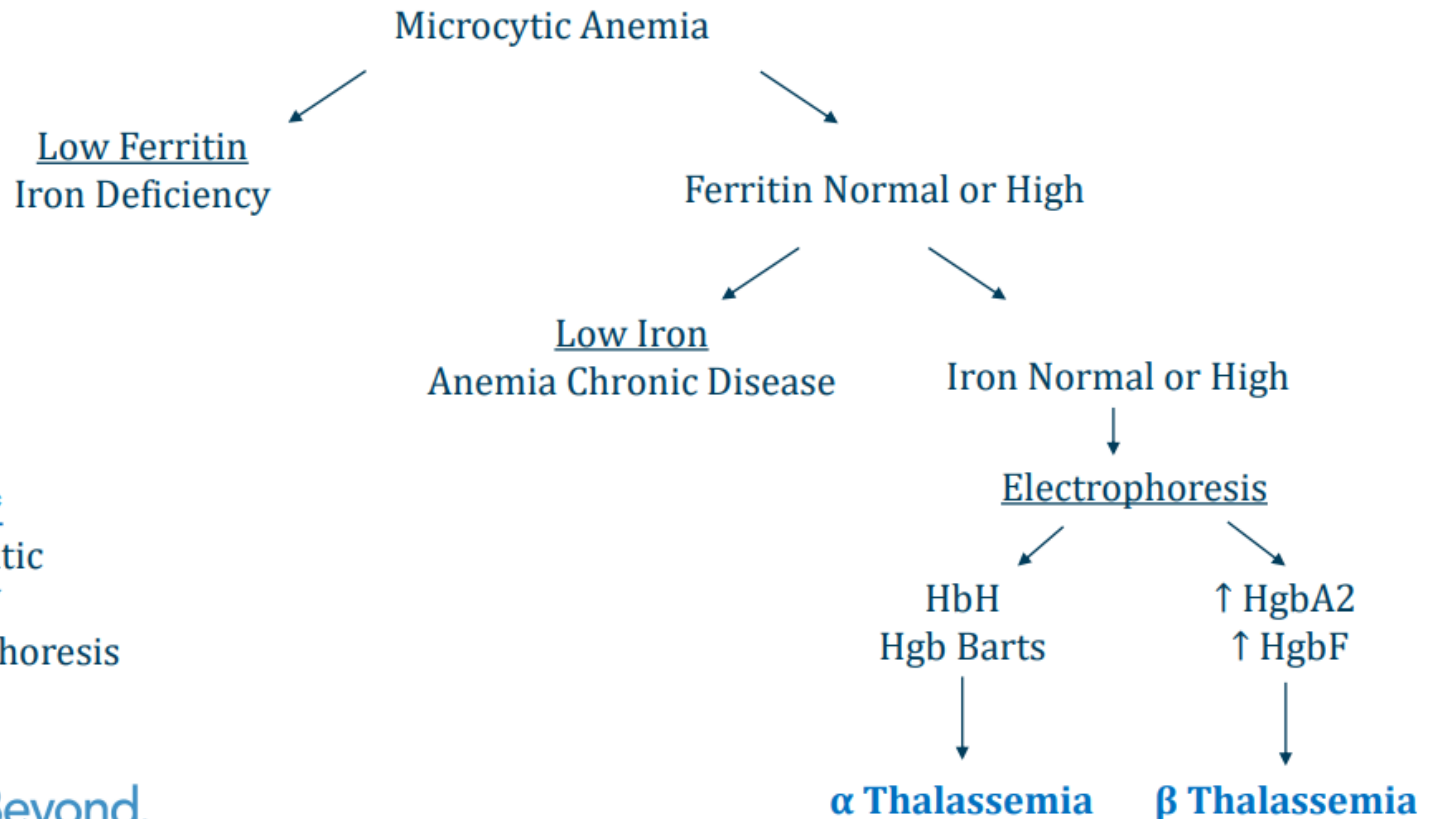
Iron Studies

- High red cell turnover
- **Serum iron level elevated** in severe forms
 - HbH, Beta major
 - Ineffective erythropoiesis → ↑ intestinal iron uptake
- Also may see **elevated ferritin**
- Low serum ferritin not seen in isolated thalassemia
 - Unless complicated by iron deficiency



Microcytic Anemias

Workup



α minor*

Asymptomatic

Low MCV

Normal electrophoresis

Sickle Cell disease

Sickle Cell Disease

- Autosomal recessive disorder
- **Abnormal β hemoglobin chains**
- Beta chains found in hemoglobin A ($\alpha_2 \beta_2$)
- Makes up 95% of adult Hgb

Globin Chain

Alpha (α)

Beta (β)

Delta (δ)

Gamma (γ)

Hemoglobin A

(95%)
 $\alpha_2 \beta_2$

Hemoglobin A2

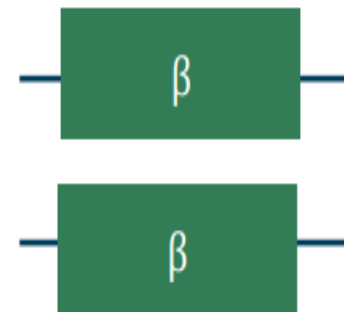
(<5%)
 $\alpha_2 \delta_2$

Hemoglobin F

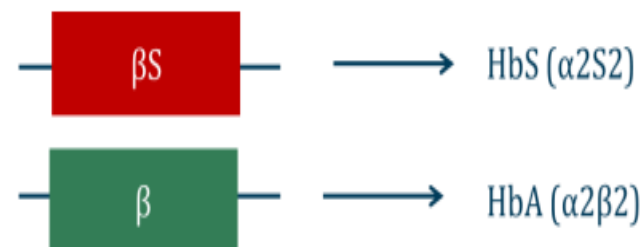
Fetal
 $\alpha_2 \gamma_2$

Sickle Cell Disease

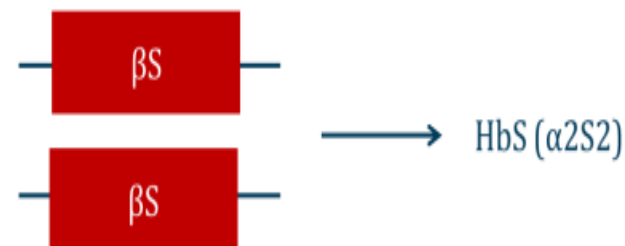
- Single base substitution in β globin gene
 - Adenine changed to thymine
- Produces **HbS** instead of HbA
 - HbA: glutamate
 - HbS: valine
- Altered shape of beta chains in HbS
- One abnormal gene: **sickle** cell trait (SA)
- Two abnormal genes: **sickle** cells disease (SS)



Sickle Cell
Trait



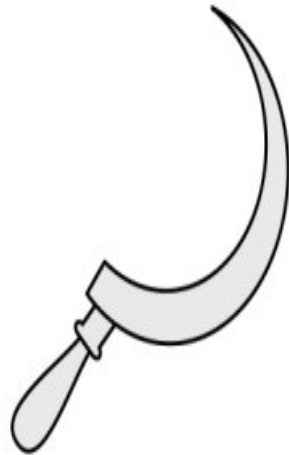
Sickle Cell
Disease



Sickle Cell Disease

Pathophysiology

- Deoxygenated HbS is poorly soluble
- **Polymerization** when oxygen low
 - Also in volume depletion, acidosis, infection
- Red blood cells form crescents
- Appearance of a **sickle**



Sickle Cell Anemia

- Two major problems result from sickle cells
- #1: **Hemolytic anemia**
- #2: **Vaso-occlusion of small blood vessels**



Sickle Cell Disease

Hemolysis

- Sickling is reversible
- Cells continuously **sickle**/de-**sickle** in circulation
- Leads to RBC membrane damage
- **Chronic hemolytic anemia**
 - Anemia, elevated unconjugated bilirubin, pigment gallstones
 - Extravascular and intravascular hemolysis
- Usually no/limited anemia symptoms
 - Increased EPO and adaptation to lower Hgb
- All patients: **folate supplementation**



Sickle Cell Disease

Aplastic crisis

- High reticulocyte count required to maintain Hgb
- Infection may suppress bone marrow function
- Classic cause: parvovirus B19
- Acute hemoglobin decrease
- Fatigue, pallor and dyspnea
- Treatment: **blood transfusion**
- Usually improves after infection



Sickle Cell Disease

Vaso-occlusion

- **Sickle** cells may **occlude microvasculature**
- May affect any organ
- Classic clinical manifestations:
 - Acute pain crises
 - Splenic sequestration
 - Acute chest syndrome
 - Swollen hands (“dactylitis”)
 - Spleen failure → infections
 - Renal dysfunction



Sickle Cell Disease

Pain Crises

- Sudden onset of pain
- Most common type of vaso-occlusive event
- May affect any part of body
- Abdomen, bones, joints, soft tissue, fingers, toes
- Swollen hands and/or feet especially in children
- Can be preceded by a trigger
 - Cold, volume depletion, stress, alcohol, menses
 - Patients can develop strategies to avoid triggers



Sickle Cell Disease

Dactylitis

- Vaso-occlusion of small bones in hands and feet
- Pain and swelling in hands or feet
- Fingers may look like “sausage” digits
- Common in infants and young children

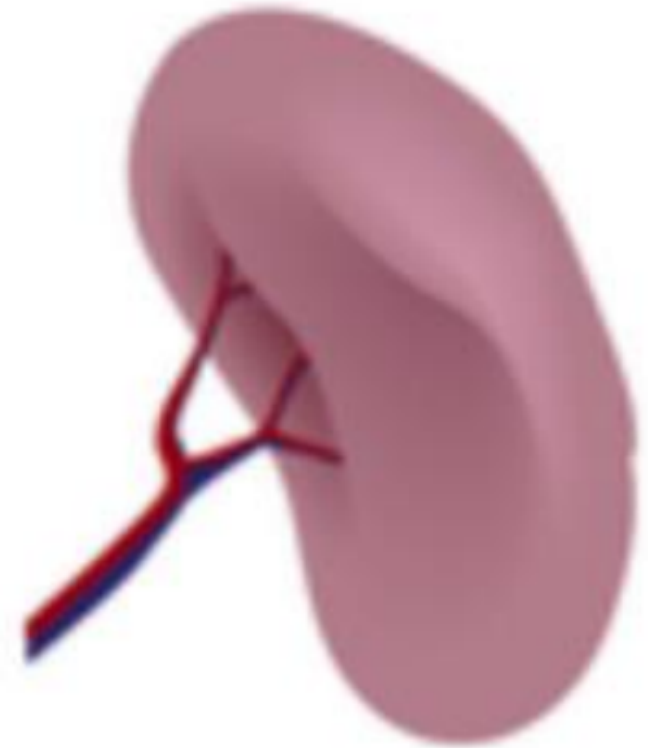


Sickle Cell Disease

Splenic sequestration crisis

- Vaso-occlusion in spleen
- Pooling of red cells
- Marked fall in **hemoglobin level**
- **Rapidly enlarging spleen**
- Left lower quadrant pain
- Risk of **hypovolemic shock**

Spleen



Sickle Cell Disease

Splenic sequestration crisis

- **Common in children**
 - Occurs in spleens yet to develop fibrosis
 - May occur before sickle cell disease is diagnosed
- **High reticulocyte count**
 - Contrast with aplastic crisis
- Acute treatment: **fluids and blood transfusion**
 - Treatment for shock and severe anemia
- Definitive treatment: **splenectomy**
 - May be done after first episode
 - Prevents recurrent episodes



Sickle Cell Disease

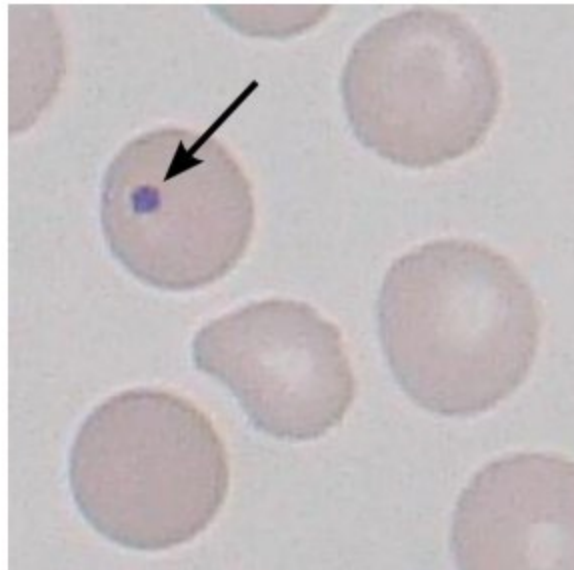
Splenic failure

- Splenic vaso-occlusion → splenic fibrosis
- Functional hyposplenism in early childhood
- Often before one year of age
- Patients functionally asplenic by four years old
- Increased risk of **infection with encapsulated organisms**
- Especially *S. pneumoniae* and *H. influenzae*



Splenic Failure

- Repeated splenic infarctions → functional asplenia
 - Early in disease: splenomegaly (macrophage hyperplasia)
 - Late in disease: Fibrosis and atrophy
- **Howell-Jolly** bodies will appear in peripheral blood



Paulo Henrique Orlandi Mourao /Mikael Häggström



Sickle Cell Disease

Chest syndrome

- Leading cause of death in adults with SCD
- Vaso-occlusion of pulmonary microvasculature
- **Clinical syndrome**
 - Fever and chest pain
 - Wheezing and cough
 - Hypoxemia and respiratory distress
 - New pulmonary infiltrate
- Often triggered by infection (pneumonia)
 - Increased sickling in lungs
 - Once begun → inflammation/acidosis → more sickling



Sickle Cell Disease

Renal dysfunction

- Vaso-occlusion of **vasa recta in renal medulla**
 - Medulla has low oxygen and high osmolality
 - Promotes sickling
- May impair concentrating ability
 - “Hyposthenuria:” dilute urine (lots of water)
 - Cannot raise urine osmolality
 - Causes nocturia and polyuria
- Hematuria
- Increased risk of chronic kidney disease

Renal Dysfunction

- Papillary necrosis
 - Sloughing of renal papilla due to renal vaso-occlusion
 - Usually painless
 - Gross hematuria and proteinuria



Sickle Cell Disease

Musculoskeletal complications

- **Avascular necrosis**
 - Bone collapse
 - Most commonly femoral or humeral head
- **Osteomyelitis**
 - Infection of infarcted bone
 - Most common Salmonella



Diagnosis in older children and adults

	Normal (AA)	Sickle Cell Disease (SS)	Sickle Cell Trait (AS)
HbA	97%	--	>50%
HbA2	3%	3%	3%
HbF	<2%	5-15%	<2%
HbS	--	>85%	30-40%

Treatment

- Immunizations
- Hydroxyurea
 - Raises amount of HbF
 - Mechanism unclear
- Transfusions
 - Iron overload may develop
- Bone marrow transplant is curative
- Median survival 42-48 years



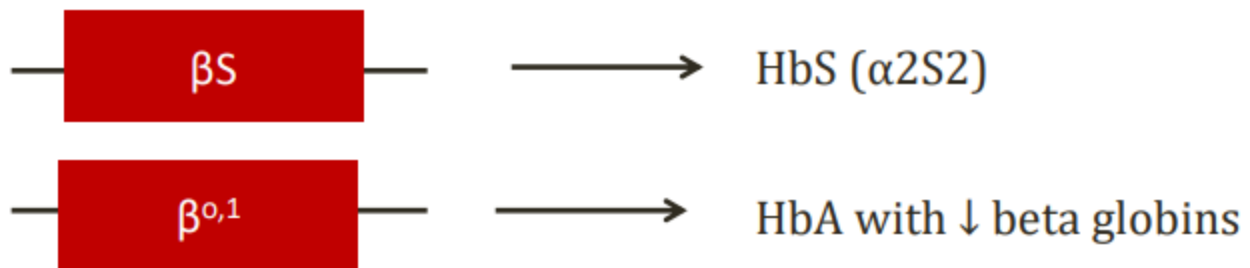
Sickle Cell Trait

- One mutated beta globin gene
- Usually no sickling
 - Normal beta gene more effective $\rightarrow$ $>50\%$ beta globins
 - Need $>50\%$ HbS for sickling
- One exception: **Renal medulla**
 - May see loss of concentrating ability
 - $\uparrow$ risk of **renal medullary carcinoma** ($>$ than sickle disease)



Sickle Cell/Beta Thalassemia

- One beta gene: sickle cell
- One beta gene: beta thalassemia
- Clinical manifestations similar to sickle cell
 - Vary depending on beta **thalassemia** gene function
 - β^0 : Similar to sickle cell disease
 - β^1 : Less severe



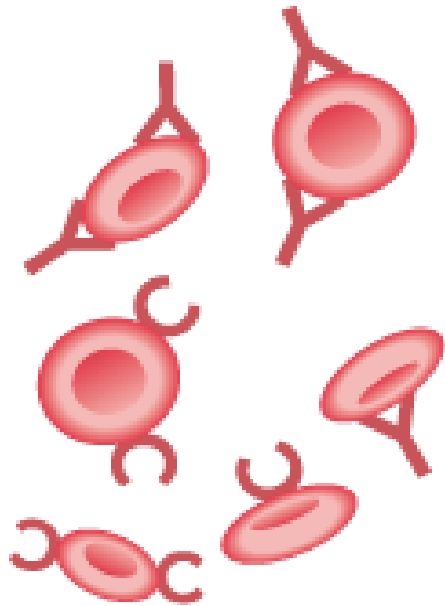
Hemoglobin C

- Rare mutation of beta gene (different from SCA)
- Glutamic acid replaced by lysine (not valine)
- Heterozygotes: Mild anemia (extravascular hemolysis)

Hemoglobin SC

- One HbS gene plus one HbC gene
- More common than homozygous HbC disease
- At risk for same complications as sickle cell disease
- Lower frequency of complications

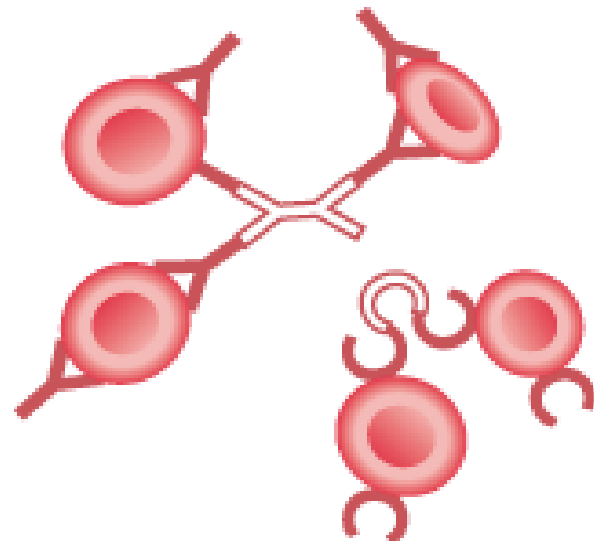
Extrinsic Hemolysis



RBCs with IgG (Y)
or C3 (C) bound to
membrane



Incubation with
antibodies to
human Ig (X)
and C3 (C)



Agglutination
(positive direct
Coombs' test)



AIHA

Red cell destruction from autoantibodies

- Results in extravascular hemolysis
- Red cell membrane removed in pieces by spleen
- Peripheral smear: spherocytes
- Smaller than normal RBCs
- Spherical
- Partial removal RBC membrane
- Can be “warm” or “cold”



Warm AIHA

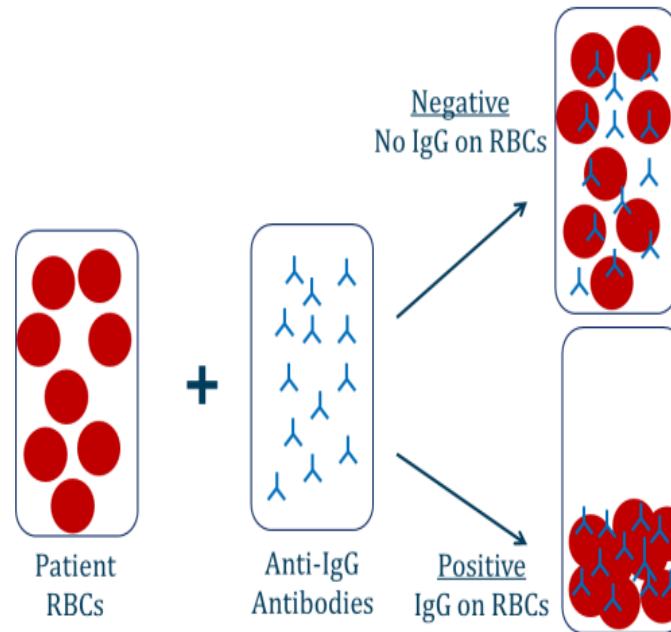
- Most common type of AIHA
- Antibodies bind at body temp 37°C (“warm”)
- IgG antibodies against RBC surface antigens
- Clinical features of hemolytic anemia
- Extravascular hemolysis
- Jaundice
- Splenomegaly
- Spherocytes



Direct Antiglobulin Test

DAT or Coombs Test

- Identifies red blood cells coated with antibodies or complement
- Warm AIHA: patient RBCs plus **anti IgG antiserum**
- Positive if agglutination occurs
- Indicates patient's **RBCs covered with IgG**



Associated Conditions

- Most cases idiopathic
- Associated with many disorders
- Lupus
- Non-Hodgkin lymphoma
- Chronic lymphocytic leukemia (CLL)

Penicillin & methyldopa



Treatment

- Glucocorticoids
- Immunosuppressants
- Splenectomy



Cold AIHA

Less common type of AIHA

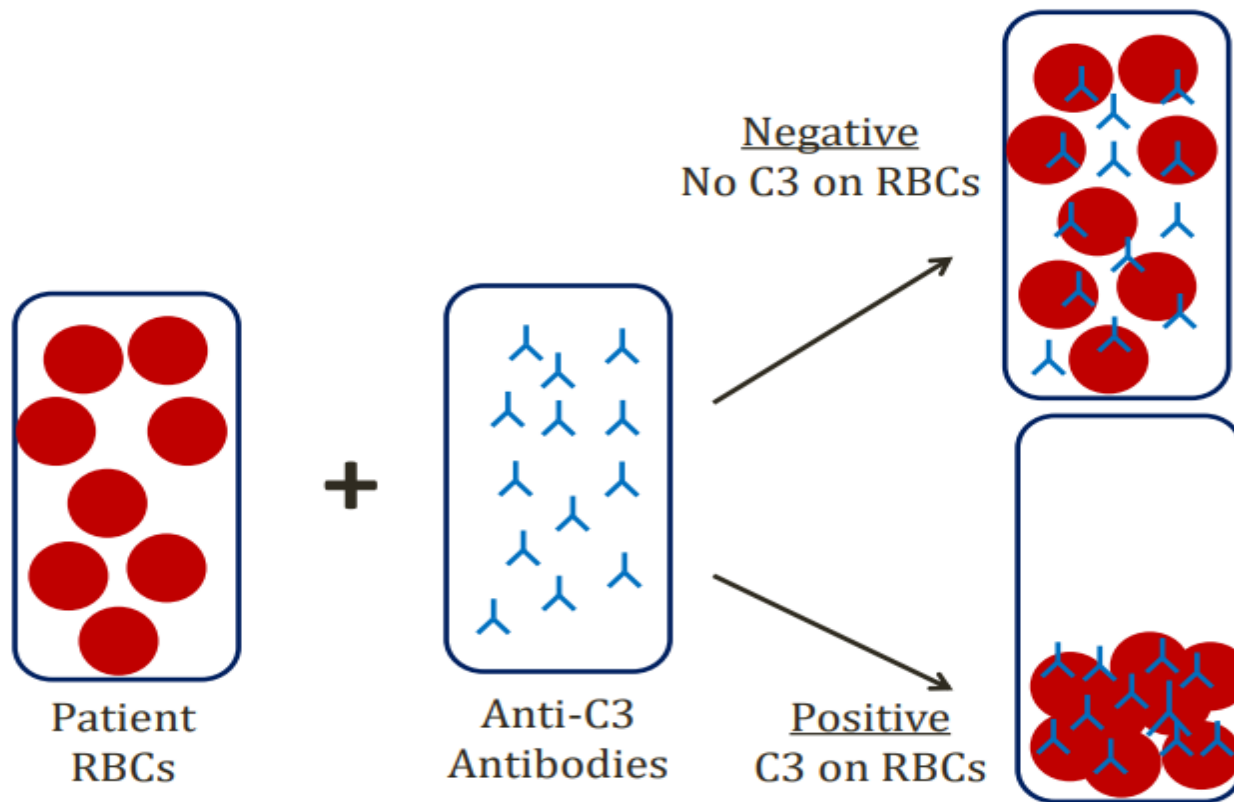
- Antibodies bind at $<30^{\circ}\text{C}$ (“cold”)
- Occurs in limbs
- Also fingertips, toes, nose, ears
- May present with painful fingers/toes
- Purple discoloration
- Symptoms associated with cold exposure
- Extravascular +/- intravascular hemolysis
- IgM activates complement
- Complement-coated red cells cleared by spleen
- Complement-mediated red cell intravascular lysis
- Spherocytes less common



Caused by IgM antibodies that agglutinate RBCs

- RBCs warmed in central organs → IgM lost
- Leaves bound C3 on RBCs
- DAT positive only for C3

DAT or Coombs Test



Associated conditions

- Can be seen in chronic lymphocytic leukemia (CLL)
- Often occurs secondary to infection
- Mycoplasma pneumonia
- Epstein–Barr virus (Infectious mononucleosis)

Treatment

- Avoid cold (stay warm!)
- Immunosuppressants



Drug-Induced Hemolytic Anemia

- Subtype of immune hemolytic anemia
- Occurs when drug binds to RBC surface
- Induces immune response (hapten)
- Commonly-associated drugs
 - Diclofenac (anti-inflammatory)
 - Piperacillin
 - Ceftriaxone
- Labs indicate hemolysis
- DAT positive
- Treatment: stop offending drug

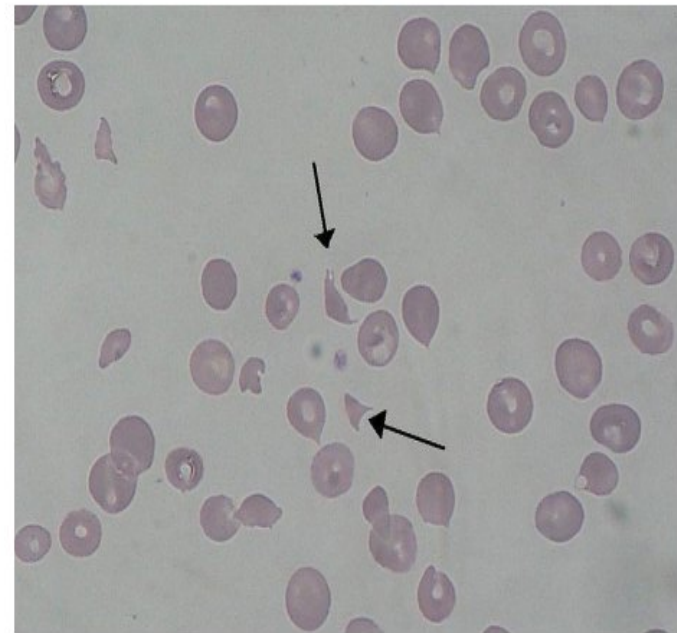


MAHA

Microangiopathic hemolytic anemia

- Non-immune hemolysis
- Shearing of RBCs in small blood vessels
- Thrombi in microvasculature → narrowing
- Blood smear: **schistocytes**
- Thrombotic thrombocytopenic purpura (TTP)
- Hemolytic uremic syndrome (HUS)
- Disseminated intravascular coagulation (DIC)
- Severe hypertension
- Preeclampsia/HELLP

Schistocytes

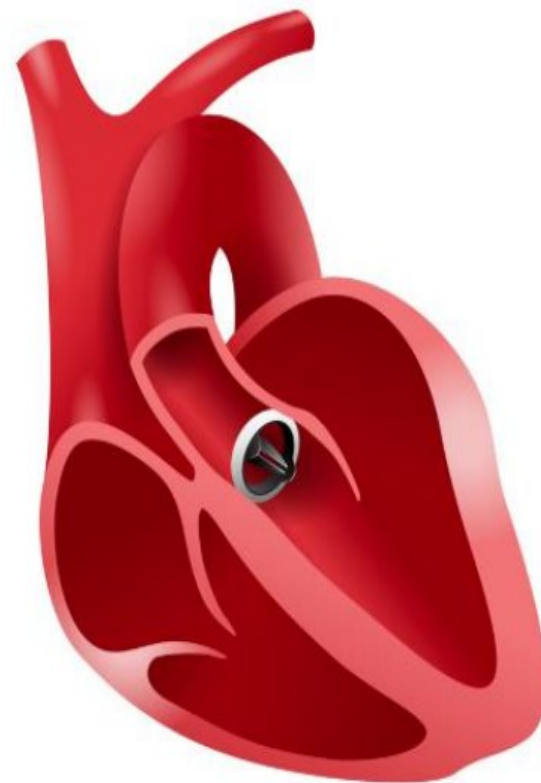


Paulo Henrique Orlandi Mourao

Mechanical Hemolysis

- Shear forces destroy RBCs in large blood vessels
- Aortic stenosis
- Mechanical heart valves
- Left ventricular assist devices
- Hemolytic anemia may occur
- **Schistocytes** on blood smear
- Similar features to MAHA

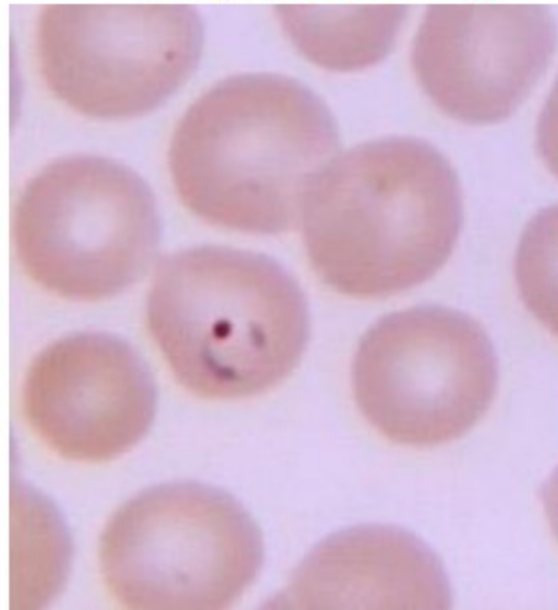
Mechanical Aortic Valve



Red Blood Cell Infections

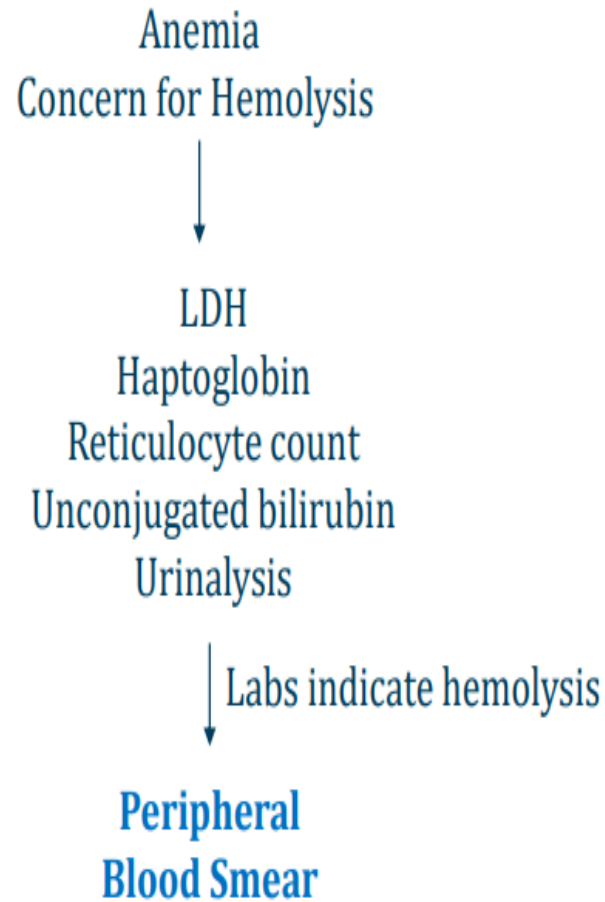
- May cause hemolytic anemia
- Classic infectious agents: malaria and babesia

Malaria
Trophozoite Ring



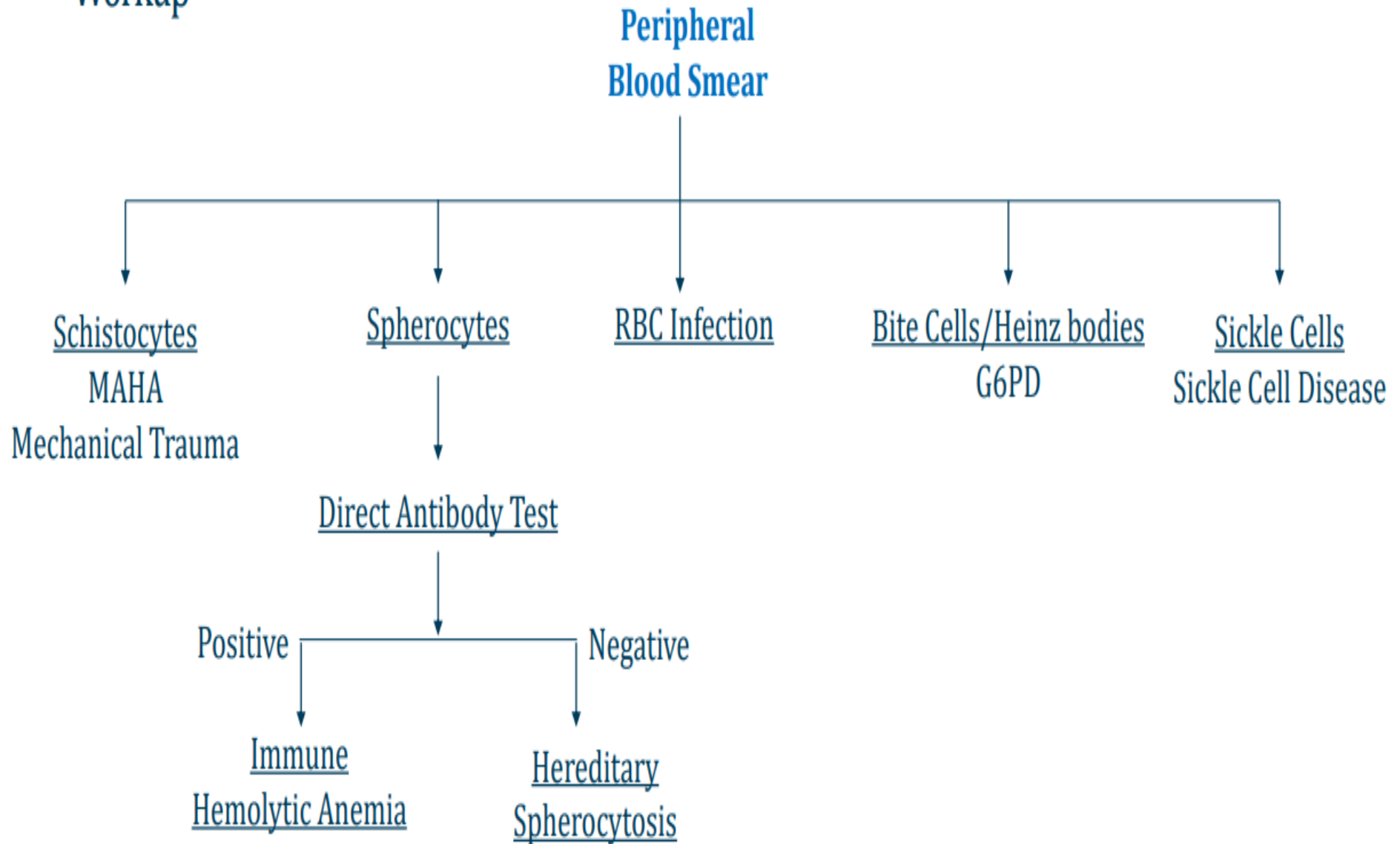
Hemolysis

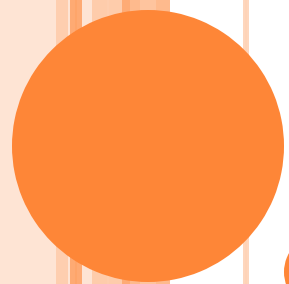
Workup



Hemolysis

Workup





Thank You