

SICKLE CELL ANEMIA

- Most common familial hemolytic anemia worldwide
- Common in Africa, Middle East, Saudi Arabia, African Americans
- Resistant to malaria falciparum infection
- Autosomal recessive
- Caused by single amino acid substitution (glutamic acid → valine) in β -chain
- In sickle cell disease (homozygous), Hg electrophoresis shows HgS and absent HgA
- In sickle cell carrier (heterozygous), Hg electrophoresis shows both HgA and HgS bands



PATHOGENESIS

- In deoxygenated case, HgS tends to polymerize in a longitudinal pattern, distorting cell shape and creating sickle shape
- The change is reversible by re-oxygenation, however, with repeated sicklings, cell membrane is damaged and hemolysis occurs
- The presence of normal HgA (carrier) and increased HgF (newborn) inhibits HgS polymerization
- Increased HgS concentration inside RBC promotes sickling (dehydration, acidosis), while the presence of additional α -thal decreases sickling



PATHOGENESIS

- Sickle-shaped RBCs take a longer time to pass through capillaries
- Removed by macrophages in spleen (extravascular hemolysis)
- Also adhere to endothelial cells, may create a thrombus



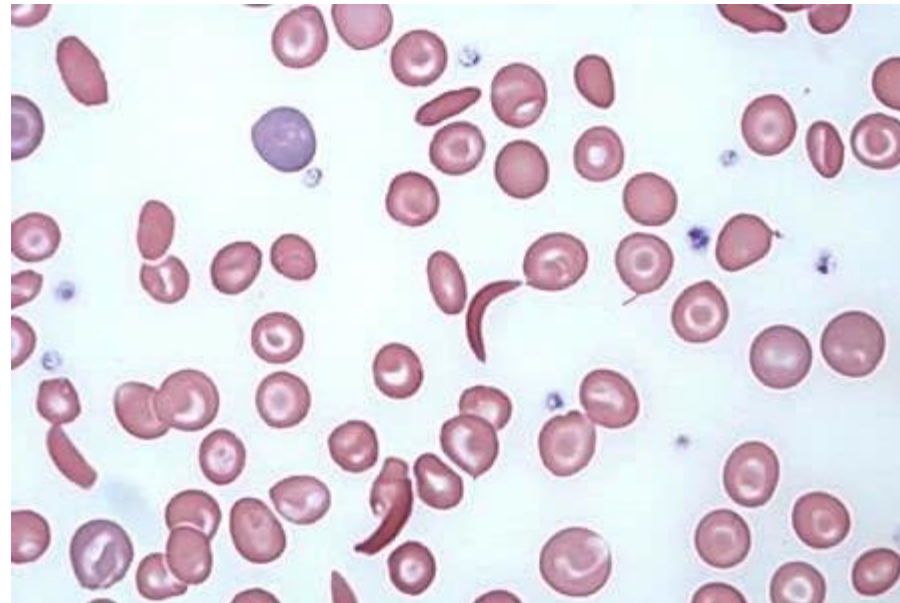
CLINICAL SYMPTOMS OF SSA

- Chronic moderate-severe hemolytic anemia, manifesting after the age of 6-months (dependent on fraction of sickled cells). The chronic course is interrupted by repeated sudden attacks of worsening anemia
- Vaso-occlusive crisis (independent on fraction of sickled cells), results in organ infarction. Commonly associated with systemic infection, inflammation, dehydration and acidosis.
- Hand-foot syndrome, acute chest syndrome, stroke, myocardial infarction, retinopathy, autosplenectomy
- Aplastic-crisis: infection by Parvovirus B19, causing worsening anemia, self-limited
- Susceptibility for encapsulated bacteria (pneumococcus, salmonella)
- Sickle cell carrier: asymptomatic



DIAGNOSIS

- Routine blood smear: presence of sickle cells, target cells
- Sickling test: adding hypoxic agent to RBCs promote sickling
- Hemoglobin electrophoresis
- DNA testing



G6PD DEFICIENCY

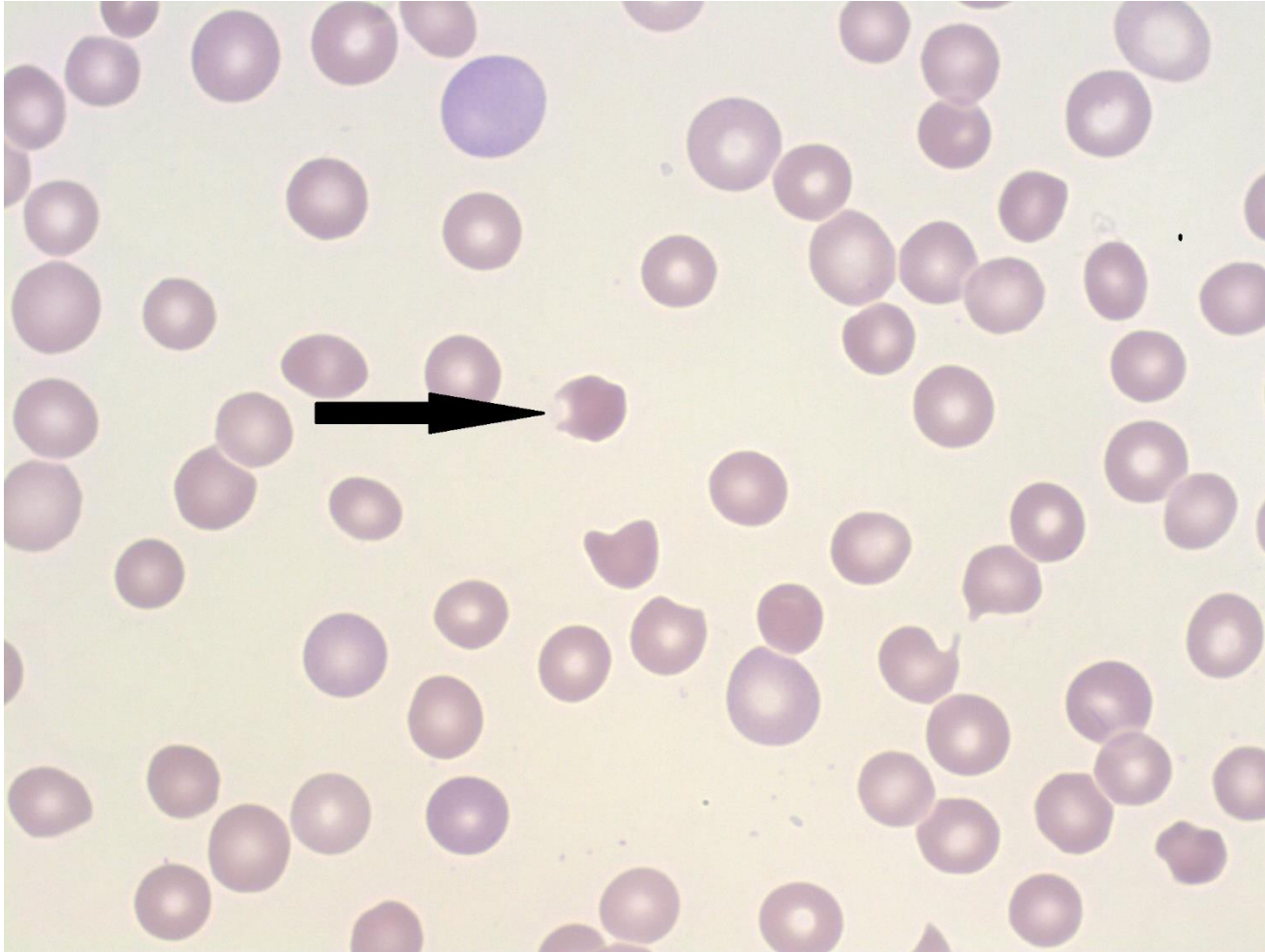
- Glucose 6-phosphate dehydrogenase deficiency
- X-linked inheritance
- Recurrent, transient episodes of intravascular hemolysis



TRIGGERS OF HEMOLYSIS

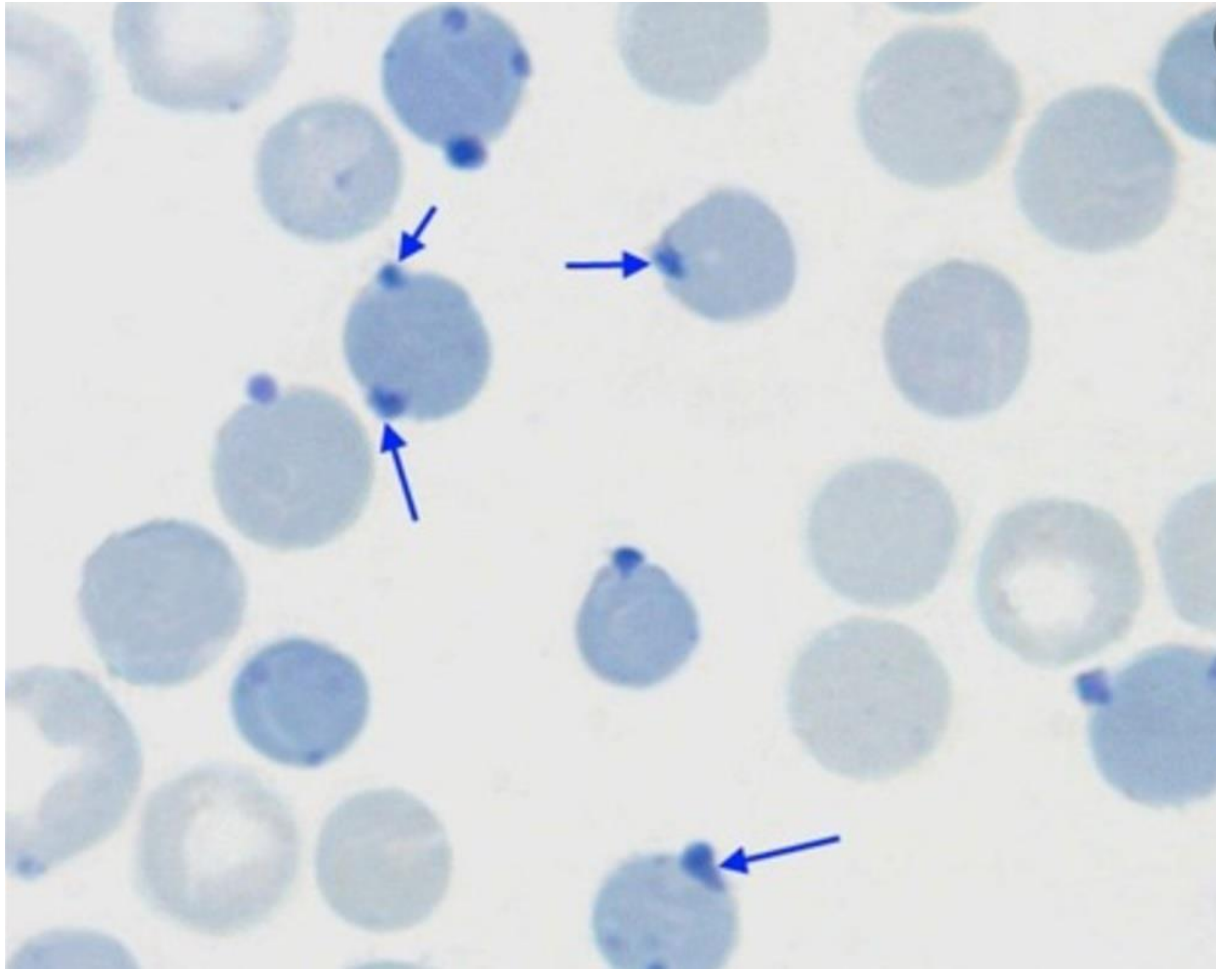
- Infection
- Certain drugs: sulfonamides, nitrofurantoin, large dose of aspirin, vitamin K, primaquine
- Fava beans
- In all, large amount of oxidants are generated, G6PD cannot neutralize them, causing hemoglobin denaturation and precipitate (Heinz bodies), damaging cell membrane and massive hemolysis of RBCs, 2-3 days after trigger
- Other cells lose deformability and partially phagocytosed inside spleen (bite cells)





Bite cells: appears are indented defect in part of cell membrane of RBCs





Supravital special stain highlights Heinz bodies as membrane-bound, dark spots representing condensed and denatured Hg



CLINICAL SYMPTOMS

- Symptoms of intravascular hemolysis
- G 6PD-A type: decreased amount of G 6PD, bone marrow compensate by producing new RBCs
- G6PD-Mediterranean: qualitative defect of enzyme (low function), more severe symptoms
- Females: can have symptoms if random inactivation affects the normal X-chromosome



IMMUNE HEMOLYTIC ANEMIA (IHA)

- The presence of auto-antibody against RBC membrane protein
- These antibodies are detected by Coombs test
- Direct Coombs test: RBCs of patient are incubated with antibodies that target normal human antibodies (RBCs will agglutinate)
- Indirect Coombs test: patients serum is added to “test RBCs” that have certain surface proteins (identify the type of antigen)



WARM TYPE IHA

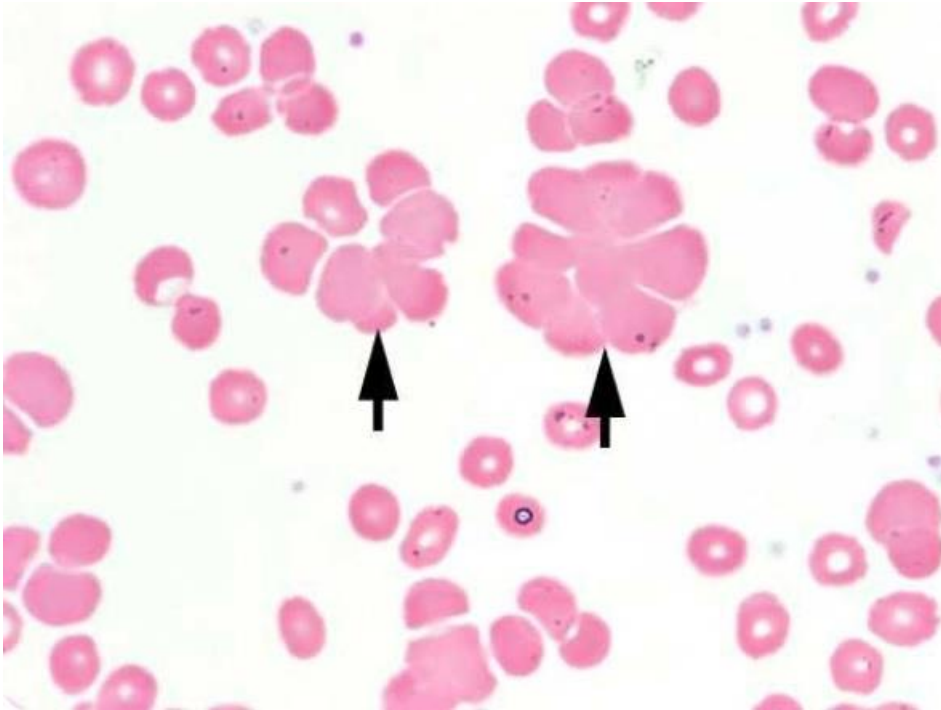
- High affinity auto-antibody (mostly IgG type)
- Binding occurs in core circulation (37°C)
- Removed by macrophages in spleen
- spherocytes develop, then destroyed by spleen (extravascular hemolysis)
- 60% are idiopathic, 25% associated with systemic lupus erythematosus, 15% by drugs (α -methyldopa, penicillin)
- Severity of anemia is variable, most patients have mild chronic anemia and splenomegaly



COLD TYPE IHA

- Low-affinity autoantibody (IgM)
- Binding occur in peripheral areas of body ($<30^{\circ}\text{C}$)
- After IgM binding, few C3b molecules bind RBCs
- When RBCs return to core circulation, IgM dissociates, but C3b stays, identified by splenic macrophages and removed
- IgM binds 5 RBCs, thus creating in vivo agglutination, might block small capillaries in fingers and toes causing Raynaud phenomenon
- Transient forms of cold-IHA occur in recovery of infections by mycoplasma pneumonia and infectious mononucleosis (mild, self-limited)
- Chronic persistent form occur in B-cell lymphoma or idiopathic





Left: RBC agglutination: RBC clumps in different directions
Right: spherocytes appear as small, round hyperchromatic RBC



Hereditary Spherocytosis

- Autosomal Dominant, sometimes recessive
- Mutation is RBC cell membrane skeleton
- Most commonly affects ankyrin, band 3 or spectrin
- Cell membrane becomes unstable, keeps losing parts of it as the RBC age
- Little amount of cytoplasm is lost
- With decreasing surface area, the RBC loses its normal biconcave morphology and becomes a smaller sphere



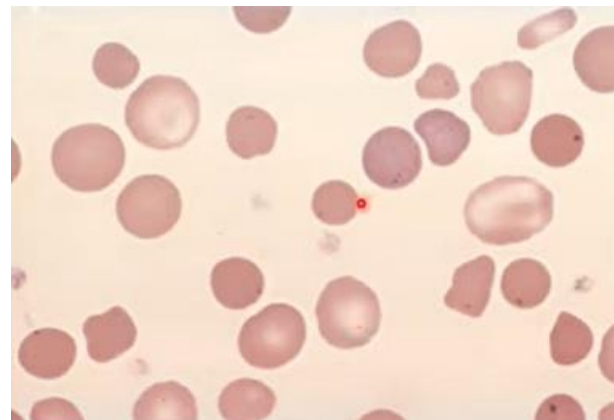
PATHOGENESIS

- Spherocytes are nondeformable
- Entrapped in small vessels in spleen, engulfed by histiocytes and destroyed (extravascular hemolysis)
- If spleen is removed, spherocytes persist in peripheral blood, thus, anemia is corrected
- The degree of anemia is variable (depends on the type of mutation)
- Some patients are asymptomatic, while others might have severe hemolysis



DIAGNOSIS

- Appearance of spherocytes in peripheral blood
- Spherocytes have a smaller size (low MCV)
- Little cytoplasm is lost, normal amount of Hg (normal MCH)
- MCHC is increased
- Spherocytes show increased fragility when put in hypotonic solution (increased osmotic fragility)



PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

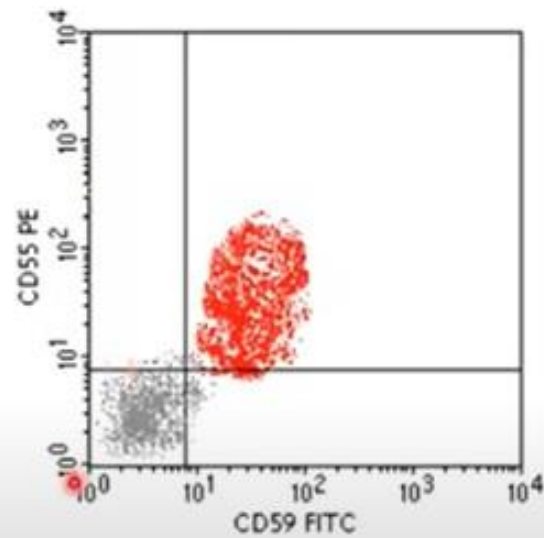
- Rare, acquired disease
- Mutation in PIGA gene, results in deficiency in phosphatidylinositol glycan (PIG), a structural protein on cell membrane that anchors many other proteins
- Mutation occurs in bone marrow stem cell (leukocytes, RBCs and platelets are all affected)



PATHOGENESIS

- Complement system: circulating proteins that are part of immune system. They are activated (C5b-C9) and attack cell membrane to create pores, causing lysis
- Blood cells protect themselves by membrane proteins CD55 and CD59, that are normally attached to PIG
- In PNH: RBCs, and to a lesser degree WBCs and platelets, are spontaneously lysed inside blood
- During sleep, \uparrow CO₂, \downarrow blood PH, more active complement system, more hemolysis
- Thrombosis is common





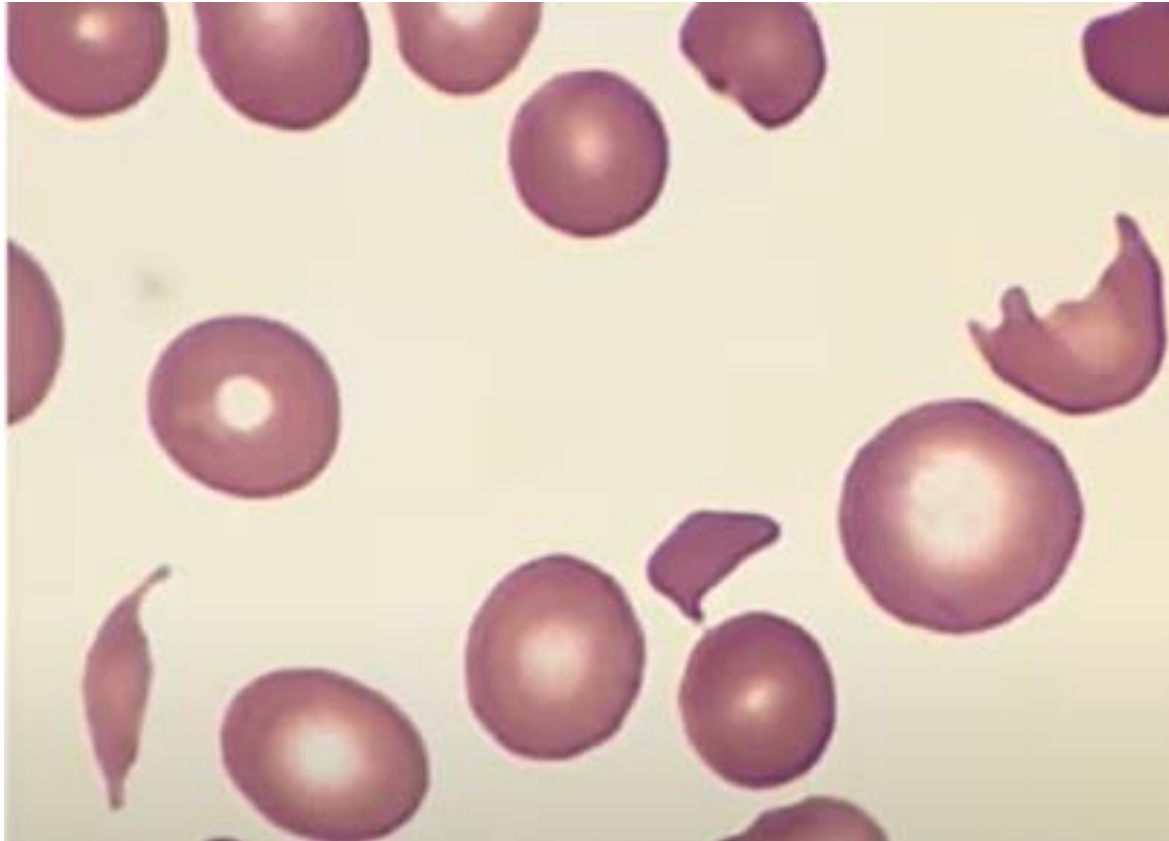
- Flow cytometry study: the red population shows expression of CD55 and CD59, while the gray one is negative for both (PNH clone)



TRAUMATIC HEMOLYSIS

- Direct physical force, or turbulence causing lysis of RBCs
- Prosthetic heart valves
- Repetitive physical pounding (marathon, boxing, marching)
- Disseminated thrombi (microangiopathic hemolytic anemia)
- Hallmark of traumatic hemolysis: schistocytes





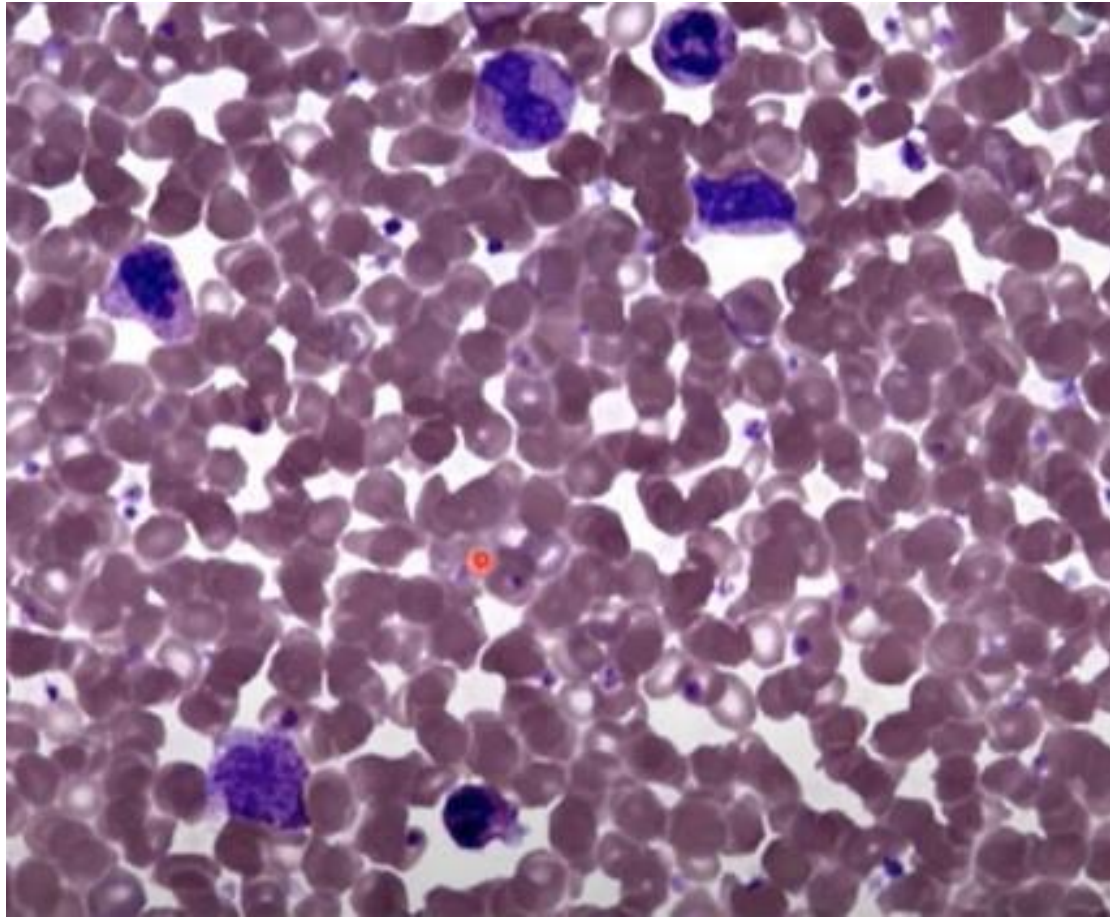
Schistocytes: torn and distorted RBCs



POLYCYTHEMIA

- Increase in total RBC mass
- Erythrocytosis: increased RBC s number
- Relative polycythemia: secondary to decreased plasma volume (water deprivation, severe diarrhea, diuretics)
- Absolute polycythemia: true increase in RBC mass, secondary to increased BM production (primary or secondary)
- Primary: polycythemia vera (low erythropoietin, splenomegaly)
- Secondary: adaptive (high altitude, cyanotic heart disease), paraneoplastic (renal cancer), surreptitious (endurance athletes). Erythropoietin in high, no splenomegaly





Polycythemia: packed RBCs in peripheral blood

