Subject: HLS Pathology

Topic: RBC Disorders 4

Hemolytic Anemias and Polycythemia

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Sickle Cell Anemia

General Info

- The most common familial hemolytic anemia worldwide.
- Common in Africa, Middle East (e.g. Saudi Arabia), and in African Americans.
- Patients resistant to Malaria falciparum infection.

Inheritance/Genetics

- Autosomal recessive
- Mutation: A single amino acid substitution in the β chain of codon 6: Glutamic acid (hydrophilic) is replaced by Valine (hydrophobic).
- Homozygous Patient → Sickle Cell Disease
- Heterozygous Patient → Sickle Cell Trait (carrier)

Pathogenesis

- Deoxygenated HgS tends to polymerize in a longitudinal pattern → Cell shape distorted (elongated, curved) → Cell becomes sickle-shaped → This change is reversible by reoxygenation. HOWEVER, with repeated episodes of sickling → Cells irreversibly sickled → Cell membrane becomes damaged → Hemolysis of cells (in the blood or spleen).
- Why does extravascular hemolysis occur (in the spleen)?
  - Sickled cells take a longer time to pass through capillaries, especially in the spleen. Therefore, they are taken out and destroyed by splenic macrophages (histiocytes).
- Sickled RBCs can adhere to endothelial cells and may create a spontaneous thrombus where the circulation is narrow.

Clinical Symptoms

- Chronic and life-long moderate-severe <u>hemolytic anemia</u> that manifests after the age of six months (when HbF drops). The chronic course is interrupted by repeated, sudden attacks of worsening anemia. **(severity dependent on fraction of sickled cells).**
- <u>Vasocclusive Crisis</u>: Life-threatening, painful condition. Thrombus can result in organ infarction. If a vital organ is affected it could be fatal. Commonly associated with systemic infection, inflammation, dehydration, and acidosis. **(severity independent of fraction of sickled cells).**
- <u>Hand-Foot Syndrome:</u> Severe pain in digits (ischemia), deformities (repeated infarction in bone and soft tissues of the digits), and abnormal growth of the fingers and toes.
- <u>Acute Chest Syndrome:</u> Affects the lungs+ribcage. Severe pain+shortness of breath (worsens hypoxia).
- <u>Stroke:</u> If cerebral circulation is affected.
- <u>Myocardial Infarction</u> (heart attack): Notorious complication, reason for shorter life expectancy of sickle cell patients, may occur early in life.
- <u>Retinopathy:</u> Thrombosis in blood vessels of retina. Hypoxia promotes growth of new blood vessels and this worsens symptoms as the entrance of light is blocked.
- <u>Auto-Splenectomy:</u> Repeated infarction causes the spleen to become fibrotic and disappear.
- <u>Aplastic Crisis:</u> Infarction to the bone marrow stops cell production and leads to aplastic anemia. Parvovirus B-19 infection causes pure red cell aplasia in chronic hemolytic anemia patients.
- <u>Susceptibility for Encapsulated Bacteria:</u> No spleen → Increased susceptibility to encapsulated bacteria (ex: pneumococcus, salmonella).

REMEMBER: Sickle cell **carriers** are **completely asymptomatic**.

Factors That Affect Sickling

FACTORS THAT INCREASE SICKLING:

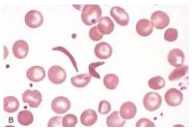
1. Any increase in HgS concentration: This occurs with dehydration and acidosis (appears in infection).

FACTORS THAT DECREASE SICKLING:

1. Carrier State: Patients have both HgS and HgA. HgA interacts weakly with deoxygenated HgS and inhibits polymerization.
2. Fetal Hemoglobin: HbF also inhibits polymerization.
3. Patients with additional α-thalassemia: They have less HbS.

Diagnosis

- Blood Smear: Sickled cells and target cells.



- Sickling test: Add a hypoxic agent to promote sickling (tests diseased and carrier patients).
- Hemoglobin Electrophoresis: Discriminates between diseased and carrier state.
  - Disease: HgS band present/HgA band absent.
  - Carrier: Both HgS and HgA bands present.
- DNA Testing

G6PD Deficiency

Glucose-6-Phosphate Dehydrogenase

- G6PD is an enzyme important for the reduction of $NADP^+$ to NADPH. NADPH is then needed for the production of the antioxidant glutathione, which protects the cell from reactive oxygen species (ROS).
- When G6PD is deficient, glutathione production is reduced. Oxidative stress is caused by the ROS and this puts the cell at risk of intravascular hemolysis.

Triggers of Hemolysis

- Infection: WBCs produce ROS to combat microbes.
- Drugs: Sulfonamides, Nitrofurantoin, large doses of Aspirin, vitamin K, Primaquine
- Fava beans: Vicine and convicine (molecules) are strong oxidants.
- Naphthalene – Deodorant, strong oxidant.
- Certain food coloring (aniline dye)

HOW DO THEY TRIGGER HEMOLYSIS?

The triggers cause the generation of large amounts of oxidants that glutathione can't neutralize → Hemoglobin denatures and precipitates (Heinz bodies form) → RBCs become less deformable → Cell membrane becomes damaged → Massive hemolysis 2-3 days after trigger.

Other RBCs lose deformability and are partially phagocytosed (histiocytes try to remove part of RBC with Heinz bodies) → Cells look like bitten cells.

General Info

- X-linked recessive disorder.
- Affects males more than females.
- Recurrent, transient episodes of **intravascular hemolysis**.
- RBCs are sensitive to this deficiency because they have no nucleus and have a relatively long life span.

Clinical Symptoms

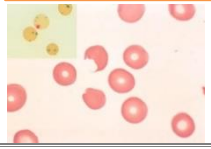
- On normal days patients do not have anemia.
- Recurrent attacks of intravascular hemolysis: sudden anemia, pain throughout body, and dark urine.

<u>MAJOR TYPES:</u>

- G6PD-A: Decreased amount of G6PD. Bone marrow produces new RBCs to compensate.
- G6PD-Mediterranean: Qualitative defect (low functioning enzyme). More severe symptoms (increased BM production can't fix problem).

Diagnosis

- Blood Film – 'Bitten' cells. With supravital stain you can see Heinz bodies.
- Enzyme Assay



Immune Hemolytic Anemia

General Info

- An autoimmune hemolytic anemia.
- Autoantibodies formed against RBC membrane proteins.
- Two types – Warm and Cold types – Based on site of agglutination.

Coomb's Test – Detecting Autoantibodies

Direct Coomb's Test: Patient's RBCs are incubated with synthetic antibodies (Igs) that target normal human antibodies. Positive test = RBCs will agglutinate.

Indirect Coomb's Test: Patient's serum is added to 'test RBCs' that have certain surface proteins known to be targeted by auto-Igs. Positive test = RBCs will agglutinate.

Warm Type IHA

- High affinity (mostly) IgG autoantibodies.
- Binding occurs in core circulation (37^{o}C)

PATHOGENESIS:

- When the Ig-coated RBCs reach the spleen, macrophages pinch off pieces of the RBCs to remove the autoantibodies.
- With incomplete consumption of the RBCs, the surface area decreases and the cells transform into spherocytes.
- Spherocytes are destroyed in the spleen → extravascular hemolysis.

CAUSES:

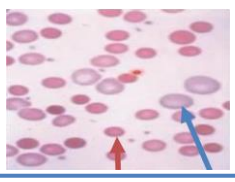
- 60% are idiopathic.
- 25% associated with another autoimmune disease (ex: SLE)
- 15% Secondary to drugs (α-methyldopa, penicillin)

CLINICAL PICTURE:

- Severity of anemia is variable, most patients have mild, chronic anemia.
- Reticulocytosis
- Splenomegaly

MORPHOLOGY:

- Spherocytes (very small, no pale center)
- Reticulocytes (larger, more bluish in color)
- Polychromasia (indicates reticulocytosis, because both colors present).



Cold Type IHA

- Low affinity IgM autoantibodies.
- Binding occurs in peripheral areas of the body (<30^{o}C) such as the digits, tip of the nose, ears.

PATHOGENESIS:

- After IgM binding, a few C3b molecules (complement protein) bind to the RBCs.
- RBCs return to the core circulation → IgM dissociates and C3b remains → C3b is identified and removed by splenic macrophages → spherocyte formation.
- IgM can bind to 5 RBCs and cause in vivo agglutination. This might block small capillaries in the fingers and toes and cause the **Raynaud phenomenon.** This agglutination may be seen in blood film.

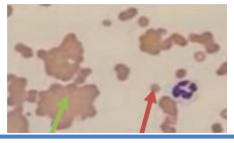
Raynaud Phenomenon: Digits become cold, painful, and blue in color secondary to decreased blood flow.

CLINICAL TYPES

- **Acute, transient IHA:** Occurs after infection by Mycoplasma pneumonia and Epstein-Barr virus (infectious mononucleosis). Mild and self-limited.
- **Chronic, persistent IHA:** Occurs in B-cell lymphoma or is idiopathic. Research suggests that malignant B cells stimulate the normal B cells to produce a huge amount of IgM.

MORPHOLOGY:

- Large clumps of RBCs (due to IgM)
- Small Spherocytes.



Hereditary Spherocytosis

General Info

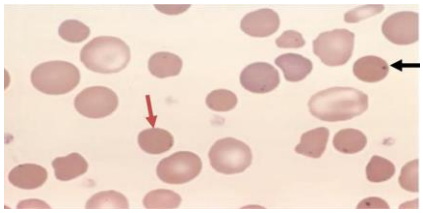
- Caused by inherited defect of RBC cytoskeleton-membrane proteins.
- Mutation most commonly affects ankyrin, band 3, or spectrin → Cell membrane unstable → Membrane fragments lost with aging → Surface area-to-volume ratio decreases → Cells become spherocytes.

Pathogenesis

- Non-deformable spherocytes get entrapped in the small vessels in the spleen → RBCs engulfed and destroyed by histiocytes **(extravascular hemolysis).**
- If the spleen is removed, spherocytes persist and symptoms will be corrected.
- Degree of anemia is variable depending on the mutation. Some patients asymptomatic, others have severe hemolysis.

Diagnosis

- Blood Film: Spherocytes in peripheral blood.
- Increased MCHC: MCV decreases while MCH remains the same.
  - *Note: Increased MCHC indicates spherocytes and sickle cell disease.*
- Osmotic Fragility Test: Spherocytes show increased fragility when placed in a hypotonic solution (burst easily, unlike normal RBCs). Test not specific because other abnormal shapes can cause the same result.
- Family history is important.
- Small black dots inside the RBCs (Howel-Jolley bodies, remnants of DNA) indicate the patient has undergone splenectomy.



Paroxysmal Nocturnal Hemoglobinuria

General Info

- An acquired, rare disease.
- Caused by a mutation in the PIGA gene (X-chromosome). This leads to a deficiency in the anchor protein phosphatidylinositol glycan **(PIG).**
- The mutation occurs in a bone marrow stem cell → RBCs, WBCs, and platelets are affected.

Diagnosis

- Flow Cytometry Study: Fresh blood is taken and tested for antigens on the cell membrane. Here, we test for CD55 and CD59.
- More commonly only a portion of the cells are affected (i.e. only some stem cells, and resulting progeny, are mutated). However, sometimes all cells may be effected.

Pathogenesis

- Blood cells protect themselves from the complement system with the membrane proteins **CD55 and CD59**, which are normally attached to PIG → When PIG is absent, so are they.
- The cells are now sensitive to lysis by the C5b-C9 membrane attack complex (creates pores in cell membrane and destroys the cell).
- RBCs are most affected and are suddenly lysed, WBCs and platelets are lysed to a lesser degree.
- WBC lysis → Leukopenia
- Platelet lysis → Thrombocytopenia + lysed platelets release their contents and this causes **thrombosis (a common and serious symptom).** This can be fatal.

WHY IS THE DISEASE CALLED NOCTURNAL?

During sleep there's more CO_2 → Blood becomes more acidic → Higher activation of the complement system.

**For more on the results of flow cytometry, please check the sheet.*

Traumatic Hemolysis

- Lysis of RBCs is caused by direct physical force or turbulence. This may occur with a defective cardiac valve prosthetic or repeated physical pounding (marathon, boxing, marching).
- Disseminated thrombi (microangiopathic hemolytic anemia).
- Hallmark of traumatic hemolysis: Schistocytes (mechanical fragments of RBCs).

Polycythemia

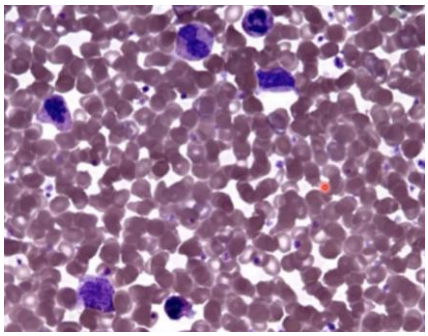
Definition

An increase in total RBC mass.

Note: This increase in mass does not necessarily mean an increase in cell number (erythrocytosis).

Types

1. **Absolute Polycythemia:** True increase in RBC mass caused by increased BM production.
2. **Relative Polycythemia:** Secondary to decreased plasma volume, RBCs become more concentrated. Causes: water deprivation, severe diarrhea, and diuretics.



Notice how the blood film is very crowded with RBCs.

Absolute Polycythemia

1. Primary – Polycythemia Vera
   - A neoplasm in the bone marrow produces large amounts of RBCs.
   - Erythropoietin is low (negative feedback).
   - Splenomegaly
2. Secondary
   - Erythropoietin is high due to hypoxia.
   - No splenomegaly.
   - Causes:
     I. Adaptive Process – Such as in people who live in high areas or have cyanotic heart disease (have hypoxia early in life).
     II. Alcoholism – Hypoxia and acidosis in blood. They also sleep and urinate a lot, leading to a state of hypoxia.
     III. Paraneoplastic Syndrome – Occurs in patients with renal cancer (ex: renal cell carcinoma or the pediatric Wilm's tumor), which leads to more erythropoietin production. Also seen in liver carcinoma.
     IV. Surreptitious (hidden) Cases – Endurance athletes who take erythropoietin to enhance their performance.