Anemia II: Hemolytic Anemia

Introduction: Defined as anemia due to a shortened survival of circulating red blood cells (RBCs) because of their premature destruction.

Risk Factors: 1). Hereditary:
- RBC membrane defects (HS, HE, pyropoikilocytosis)
- RBC metabolic defects (EM pathway, HMP)
- Hemoglobin defects (thalassemia, abnormal variants)

2). Acquired
- Immune (autoimmune, isoimmune, drug)
- RBC fragmentation diseases
- PNH
- Secondary (renal and liver diseases)
- Misc (drug, infection, chemicals, toxins, physical agents)

Biology: The mature RBC generates energy through:
- Anaerobic glycolysis: Embden-Meyerhof (EM) pathway: 90% ATP
- Oxidative glycolysis: Hexose monophosphate (HMP) shunt: 10% ATP
- Nucleotide salvage pathway

Clinical: Hemolysis: see individual sections

Diagnosis: A) Diagnostic Evaluation:
1). H &P: anemia, jaundice, gallstones, splenomegaly
2). Labs:
   - ↑Retic, blood smear;
   - ↑ LDH, ↑ Indirect bilirubin, ↓ Haptoglobin
   - UA → hemoglobinuria if intravascular
3). Specific tests:
   - Direct Coombs test (DAT, Coombs)
   - Osmotic fragility test
   - Hb electrophoresis
   - G-6PD enzyme assays
   - RBC enzyme assays
   - Bone marrow aspirates/biopsy (reserved M:E ratio)

A). Classification:
1. Coombs-negative Hemolytic Anemia:
   - Hemoglobinopathies
   - Enzymopathies
   - RBC membrane defects
   - Drugs
   - Toxin/Wilson's disease
2. Autoimmune Hemolytic Disorders (Coombs-positive):
2.1 Warm-reacting antibodies (IgG, bind RBC at 37°C, RBC coated IgG or C3)
- Idiopathic
- Secondary
  * Autoimmune disorders
  * Immunodeficiencies
  * Lymphoproliferative disorders
  * Nonlymphoid malignancies
  * Viral infection
- Mixed warm and cold antibodies
- Drug induced.

2.2 Cold-reacting antibodies (bind RBC at <37°C, RBC coated with C3)
- Cold agglutinin disease (IgM)
  * Idiopathic
  * Secondary
  Infections
  Lymphoproliferative disorders
  Nonlymphoid malignancies

2.3 Paroxysmal cold hemoglobinuria (Donath-Landsteiner antibodies, IgG)
- Syphilis
- Viral infections

**Pathology:**
A. Peripheral blood smear:
- extremely valuable to rule out thrombotic microangiopathies (TMAs) such as thrombotic thrombocytopenic purpura (TTP) or drug-induced TMA (DITMA) or infections such as malaria or Babesia or Heinz bodies or bite cells to suggest possible glucose-6-phosphate dehydrogenase (G6PD) deficiency.
- Macrocytosis

**Prognosis:**
Various - can be life-threatening (TTP, etc)

**Tx Principle:**
A. RBC Enzyme Defects
1). G6PD Deficiency: X-linked, most common
- Classifications:
  * Class 1: Chronic hemolysis w/o precipitating cause
  * Class 2+3: Acute hemolysis, a/w exposure to oxidant drugs, stress or food (fava bean)
  * Class 4+5: harmless
- Sx: Jaundice more prominent than anemia; RBC oxidative sensitive, hemolysis can be fetal.
Two variants: Mediterranean variant: class 2
  G6PD A(-) variant (African American): 50% ↓ in enzyme half life
  Confirmation: \( \sqrt{G6PD} \) level (do not \( \sqrt{ } \) during acute hemolysis except for Mediterranean variant in which the G6PD level can be checked at any time). For non-Mediterranean G6PD deficiency, young RBC can have near normal level of G6PD).
2). Pyruvate Kinase (PK) Deficiency: 2nd most common (most common defect in glycolytic pathway), more in northern European
   - Sx: from severe hemolytoc anemia in neonates to fully compensated anemia
   - Dx: Initial test → RBC osmolarity normal. Blood smear → crenated cells (echinocytes)
     Confirmation: √ PK activity.
   - Tx: folic acid
     * Splenectomy, cholecystectomy for severe anemia. Alert: → postoperative thromboembolic events.

3). Glucosephosphate Isomerase (GPI) Deficiency: the third most common
   - Sx: Hemolytic anemia +/- myopathy, ataxia, mental retardation (all rare).
   - Dx: Blood smear → poikilocytosis, anisocytosis, marked polychromatophilia,
     reticulocytosis
     Confirmation: √ enzyme level

4). Hexokinase (HK) Deficiency:
   - Blood smear: normal RBC morphology, plus reticulocytosis, polychromatophilia,
     and a few spherocytes
   - Acquired HK deficiency: seen in Wilson's disease (↑ copper → inhibits HK → intermittent intravascular hemolysis)

5). Phosphoglycerate Kinase (PGK) Deficiency: only X-linked in EM pathway
   * Males develop normal until early childhood with neurologic manifestation

   - Phosphofructokinase (PFK) Deficiency:
     * First described as a muscle glycogen storage disease. Hemolysis is usually mild.

   - Triosephosphate Isomerase (TPI) Deficiency: TPI present in all tissues
     * Hemolytic anemia and progressive multi-system syndrome

   - Pyrimidine 5'-Nucleotide (P5N) Deficiency: defect of nucleotide metabolism
     * Deficiency → Basophilic stippling on wright-stained blood smear
     * Lead poisoning → inhibit this enzyme.

   - Hyperactivity of Adenosine Deaminase (AD): → ATP depletion → hemolysis
     * Autosomal dominant.

B. RBC Membrane Defects:
1). Basics:
   - Cytoskeleton: spectrin (75%), actin, ankyrin, protein 4.1 & adducin
     - "Spherocytes and Stomatocytes": ↓ surface to volume ratio; → osmotically sensitive.
     "Target cells and dehydrated RBC": ↑ surface to volume ratio; → osmotically resistant.

2). Hereditary Spherocytosis (HS):
   - Molecular mutations: AD, AR
     ** Ankyrin deficiency: most common in AD
* Spectrin deficiency: 30%, only in AR.
* Both ankyrin and spectrin deficiency: 30-45% cases.
* Band 3 mutations: 20%, in Japanese AR
- Pathogenesis: "Spectrin loss" is the key
* Primary spectrin deficiency:
* Secondary spectrin deficiency: ankyrin deficiency or other causes" → "Spectrin loss" attachment to the membrane → lipid bilayer destabilized → spherocyte.
- Sx: Anemia, Jaundice, splenomegaly
- Dx: Initial test: Coomb-negative hemolysis; Spherocytosis; Abnormal osmotic fragility test; Family history.
  Confirmation: √ specific membrane proteins
- Complications:
  * Hyperhemolysis Crisis: most common, a/w infecton → accelerated hemolysis & splenic enlargement
  * Aplastic Critis: rare, severe, pavovirus B19.
  * Cholelithiasis (Gallstone): 50% radioopaque. US required for diagnosis
  * Gout, indolent ankle ulcers, chronic erythematous dermatitis on the leg, etc
  * Distal renal tubular acidosis in HS pts with band 3 mutation (anion channel protein)
  * Spinocerebellar degeneration and familial myocardiopathy.
- Tx: Mild disease → observation.
  Severe anemia → Splenectomy; Relapse → look for accessory spleen.
  Gallstone → Splenectomy and cholecystectomy.

3). Hereditary Elliptocytosis (HE):
- Path: AD, Defect in the interaction of RBC membrane proteins with spectrin.
- Common HE: No anemia/splenomegaly or reticulocytosis.
  * Dx: Bioconcave elliptocytes; RBC fragments (in severe case); Normal osmotic fragility.
  - Spherocytic HE: all from Europe, mild to moderate hemolytic anemia
  * Dx: Round ellipocytes; No RBC fragments; Abnormal osmotic fragility.
  - Southeast Asia Ovalocytosis: Southeast Asia (Philippines, etc). Mild to no anemia. Band 3 mutation.
  * Dx: Round elliptocytes, with a transverse bar (spoon-shaped).

4). Hereditary Stomatocytosis
- Path: AD. ↑ RBC membrane permeability → erythrocyte Na ↑ and K ↓ → RBC swollen → MCV↑, MCHC↓
  * Other genetic causes: Rh-null disease, Tangier disease (low or absent HDL)
  * Acquired stomatocyes: liver dz or alcohol abuse.
  - Dx: Hemolytic anemia in young person, presence of stomocyte (up to 40-60%)
    Confirmation: √ erythrocyte Na and K (rarely done)
  - Tx: Splenectomy (Note: splenectomy a/w ↑ risk of thrombotic events).

5). Hereditary Pyropoikilocytosis (HPP):
- Path: AR. Mutation in Spectrin → Spectrin self-association defects → ↑ RBC thermal instability (Heat-sensitive RBC → fragmentation at 45 C).
- **Sx:** Severe hemolysis
- **Dx:** Bizarre poikilocytosis, RBC fragmentation, very low MCV (50-60 fl).
- **Tx:** Splenectomy

6). Acanthocytosis or Spur Cells:
- Severe liver disease (alcoholic cirrhosis, Wilson's disease, etc)
- Abetalipoproteinemia: AR. Apo-B deficiency.
- McLeod phenotype: X-linked. Absence of Kx protein in RBC → ↓ Kell antigen (Kx protein is encoded by X chromosome) → mild hemolysis (male), asymptomatic (female).

7). Rh Deficiency Syndrome (Rh-null):
- AR. Rh (null) → Stomatocytosis → ↑ Osmotic fragility → mild or moderate hemolysis
- **Tx:** Splenectomy.

C. AutoImmune Hemolytic Anemia (AIHA)
1). Warm AIHA:
- **Dx:** Direct Coomb test positive for IgG +/- C3d.
  * Strength of the Ab does NOT correlate with clinical hemolysis
- **Etiology:**
  * Idiopathic
  * Viral Infection (CMV, HSV, Influ A, etc)
  * Lymphoproliferative neoplasia (NHL, CLL, etc)
  * Autoimmune diseases (SLE, etc)
  * Drug-induced (Methydopa, Quinidine, Penicillin, Purine Analog, etc)
  * Transfusion-associated AIHA
  * Allo-SCT-associated AIHA: Auto-Ab produced by donor lymphocytes (delayed onset)
  * Solid Organ Transplant Associated AIHA: Passenger lymphocyte syndrome
(Heart-lung > liver > kidney)
- **Clinical:** Extravascular hemolysis, plus ↑ risk for VTE (esp in splenectomized pts)
- **Treatment:**
  * Treat underlying disease, remove drugs, folic acid
  * Steroid: Prednisone 1mg/kg/d
  * Steroid-refractory AIHA:
    → Splenectomy (preferred): 1/2 - 2/3 respond, but relapse occur. low dose steroid post splenectomy for maintenance.
    → Rituximab (less preferred): for recurrent AIHA after splenectomy.
    → Cytotoxic agents (cyclophosphamide, etc): for recurrence after Rituximab or insufficient response.
  * Treatment options beyong second line:
    → Immunesuppressive agents: Cyclosporin A, Mycophenolate (MMF)
    → Danazol
  * Transfusion: difficult to identify fully compatible blood product b/c of panagglutinin.

2). Cold Agglutinin Disease: almost all are secondary!
Follow-Up: Monitor CBC, LDH, haptoglobin, retic.

- Dx: IgM antibody binds RBC at lower temp → removal of c3b-coated RBC by liver
  * Direct Coomb positive for C3b
  * High titer of cold agglutinin (>1:1000 at 4 C and 1:16 at 37 C).
- Clinical: Intravascular hemolysis, plus RBC agglutination, spurious very ↑ MCV
  * Mycoplasma (IgM against the I) → hemolysis 5-10 days after recovery from infection, self limited.
  - most common
  * EBV infection (IgM against the i) → hemolysis is rare
  * Chronic cold agglutinin syndrome (seen in pt >60yrs): "benign" monoclonal IgM (MGUS) → indolent for many yrs → develop malignant clone (5-10%) producing Anti-I against κ light chains (cold agglutinin).
  * Lymphoproliferative Disorders:
    → In pts with cold-reactive hemolysis, trisomy 3 has been a/w progression to a lymphoproliferative disorder.
    → Anti- I, a/w indolent lymphoma
    → Anti- i, a/w high grade lymphoma
- Tx: Avoid cold exposure, transfuse through a blood warmer, treat underlying dz
  * Cytotoxic/Immunosuppressive therapy (cyclophosphamide, chlorambucil, etc)
  +/− Rituximab
  * Plasmaphresis: useful (for emergency!)
  * Corticosteroids, splenectomy: NOT effective.

3). Paroxysmal Cold Hemoglobinuria (PCH):
- Donath-Landsteiner antibody: complement fixing cold-reactive IgG antibody:
  * Directed against the P antigen (receptor for parvovirus B19).
- Dx: all must be present
  * Intravascular hemolysis on rewarming after cold exposure (self-limited). In children, it occurs after a viral infection. In adult, seen in tertiary syphilis.
  * Bithermal hemolytic test: IgG antibody binds RBC at reduced temp but not at 37 C and causes hemolysis on rewarming.
    * Coombs test: positive for complement, but negative for IgG during hemolysis
  - Tx: Avoid cold. Self-limited in children.
    * Prednisone: 1mg/kg
    * Cyclophophamide, azathioprine: for steroid-refractory disease
    * Rituximab, Splenectomy, IVIg: NO clear role

4). Mixed AHA: IgM/IgG. Hemolysis is more severe.