**ANEMIA**

**Iron Deficiency Anemia**
- Anemia caused by iron depletion due to excessive iron loss
- Most common cause of anemia
- Typically presented as microcytic (MCV<80) and hypochromic (MCH<26) anemia
- Common causes are excessive menstruation, fibroids, GI bleeding due to malignancy or gastric/duodenal ulcer, reduced iron intake

**Blood morphology**: Microcytic hypochromic red cells, anisopoikilocytosis (increased RDW), elliptocytes, target cells, no polychromasia

**Bone marrow morphology**: Erythroid hyperplasia in chronic cases, reduced storage iron in bone marrow (Prussian blue stain)

**Blood work**:
- Iron: decreased (Serum iron concentration)
- Ferritin: decreased (Store iron, mostly within cells and small amount released to serum)
- TIBC: increased (Transferrin transport iron in serum. Total iron binding capacity measures amount of transferrin in serum)
- Transferrin: decreased (Transferrin saturation measures iron saturation in transferrin)
- Prussian blue stain on bone marrow: decreased (iron stored as hemosiderin in histiocytes)

**Anemia of Chronic Diseases**
- Anemia caused by ineffective utilization of iron. Presented with low serum iron and increased iron store
- Second most common cause of anemia
- Typically presented as normocytic normochromic anemia, may be microcytic or hypochromic.
- Common causes are infection, inflammatory diseases, trauma, malignancy etc
- Hepcidin (regulates iron homeostasis) plays a key role in ACD. Hepcidin negatively regulates ferroportin (delivers iron from storage to erythrocytes). Increased hepcidin production due to increased cytokines, that impairs mobilization of iron from reticuloendothelial storage to erythroid cells
- Cytokine-mediated suppression of erythropoietin production

**Blood morphology**: Typically normocytic and normochromic red cells, can be hypochromic and/or microcytic, minimal anisopoikilocytosis, no polychromasia

**Bone marrow morphology**: Normocellular, with normal to slightly reduced erythropoiesis, increased storage iron in bone marrow (Prussian blue stain)

**Lab test**:
- Iron: decreased
- Ferritin: increased (also increase as acute phase reactant in inflammation)
- TIBC: decreased
- Transferrin: normal or slightly decreased
- Prussian blue stain on bone marrow: increased
**Megaloblastic Anemia**

Macrocytic anemia caused by folate deficiency, vitamin B12 (cobalamin) deficiency, or other causes, resulting in impaired DNA synthesis, abnormal nuclear maturation, and ineffective hematopoiesis

- B12: absorbed in distal ilium, requires intrinsic factor which is secreted by gastric parietal cells
- Folate: absorbed in duodenum and jejunal
- Susceptible populations: elderly, breast feeding infants, low social economic, GI surgery, autoimmune gastritis

typically presented as gradual onset of macrocytic anemia, ineffective hematopoiesis, hemolysis (jaundice, elevated bilirubin, elevated LDH)

- B12 deficiency causes neuropathy (paresthesia, spastic ataxia, loss of position and vibration senses, cognitive impairment, psychosis)

**Blood morphology:** Macrocytosis, anisopoikilocytosis, no polychromasia, hypersegmented neutrophils (>5 lobes)

**Bone marrow morphology:** Hypercellularity, megaloblastic erythropoiesis (nuclear cytoplasmic dyssynchrony), megaloblastic granulocytes (giant bands and giant metamyelocytes), large than normal megakaryocytes

**Lab test:**
- MVC: >100
- Serum B12 or folate level: decreased
- Serum homocysteine: increased in B12 and folate deficiency
- Serum methylmalonic acid: increased in only in B12 deficiency

**Differential diagnosis:** Drugs and toxins, alcohol, myelodysplastic syndrome

**Autoimmune Hemolytic Anemia (AIHA)**

Anemia caused by abnormal production of antibodies against own RBC antigens that results in hemolysis

**Warm AIHA:** Caused by IgG antibodies that are most active at 37°C, may or may not be complement mediated, mostly extravascular hemolysis in spleen

- May be primary or associated with underlying conditions such as autoimmune diseases, immune deficiency, lymphoproliferative disorders or other malignancies, drugs.
- Presented with weakness, dizziness, dyspnea. Severity of symptom varies and correlates with severity of anemia
- Splenomegaly in nearly all patients, hepatomegaly in about half of the patients

**Cold AIHA (aka cold agglutinin disease):** Caused by IgM antibodies that are most active at cold temperature, complement mediated, either intravascular hemolysis or extravascular hemolysis in liver

- Less common than WAIHA. May be primary (typically in older female) or secondary (mostly associated with Waldenstrom macroglobulinemia and CLL), infection in younger patients (Mycoplasma pneumonia and infectious mononucleosis)

**Blood morphology:** Warm AIHA: Microspherocytes, prominent polychromasia
- Cold AIHA: Red cell agglutination

**Bone marrow morphology:** Normal

**Lab test:**
- Direct Coomb’s test: positive with polyspecific serum
- Warm AIHA: IgG and complement on RBC
- Cold AIHA: Complement on RBC, but not IgM
- Haptoglobin (binds free hemoglobin): Low
- LDH (released from lysed RBCs): High
Microangiopathic Hemolytic Anemia (MAHA)
Anemia caused by hemolysis resulting from mechanical disruption and fragmentation of RBC due to microvascular occlusion.

**Thrombotic thrombocytopenia purpura (TTP):** Caused by deficiency of enzyme ADAMTS13, either hereditary or acquired, which functions to cleave von Willebrand factor (vWF) from multimers to small molecules. Large vWF multimers are prone to thrombosis in small vessels. Some of the acquired forms are caused by inhibitors to ADAMTS13.

**Hemolytic uremic syndrome (HUS):** Children most common, affect kidney, commonly caused by infectious diarrhea due to O157:H7 E coli and other bacteria. Typical case involves binding of Shiga-toxin to ceramide trihexoside receptor on glomerular endothelium. This causes thrombogenic effect of the endothelial cells and inactivates ADAMTS13.

**Disseminated intravascular coagulation (DIC):** Hypercoagulable states throughout the body with wide spread clotting in small vessels. Common causes include sepsis, surgery, major trauma, cancer, complication of pregnancy. These conditions expose and/or release tissue factor (TF) that activates coagulation pathway and results in dysregulation of coagulation and fibrinolysis, which causes simultaneous clotting and bleeding.

**HELLP syndrome:** A complication of pregnancy, characterized by hemolysis, elevated liver enzymes, and low platelet count. Unknown cause.

**Blood morphology:** RBC fragmentation (schistocytes, helmet cells), spherocytes, polychromasia, thrombocytopenia

**Bone marrow morphology:** Normal

**Lab test:**
- TTP: ADAMTS13 level reduced, normal coagulation tests
- HUS: no specific tests, normal coagulation tests
- DIC: prolonged aPTT, PT/INR, reduced fibrinogen, increased D-dimers

Aplastic Anemia (AA), Acquired
Pancytopenia (anemia, leukopenia, thrombocytopenia) due to failure of hematopoiesis in bone marrow

Rare, affect mostly teenagers and young adults, with a second peak at 60s
Most cases are idiopathic (likely autoimmune). Others causes include toxins, drugs, chemicals, viral, radiation.

Symptoms include bruising and bleeding (low platelets), fatigue (anemia), infection (neutropenia)

**Bone marrow morphology:** Markedly hypocellular or acellular (<10%) with markedly reduced or absent all three lineages. No dysplasia

**Lab test:** Progressive pancytopenia, no polychromasia

**Cytogenetics:** Normal

**Differential diagnosis:** Hypocellular myelodysplastic syndrome: Bone marrow hypocellularity, morphologic dysplasia is present in 1 to 3 lineages. Cytogenetics may be abnormal

*Congenital aplastic anemia* (e.g. Fanconi anemia)
*Paroxysmal nocturnal hemoglobinuria* (PNH clones are frequent in AA)

**Pure red cell aplasia:** A variant of aplastic anemia with markedly reduced to absent erythropoiesis. Myelopoiesis and megakaryocytes are not affected. Some cases are associated with parvovirus B19 infection. Bone marrow shows markedly reduced to absent erythropoiesis.
**Paroxysmal Nocturnal Hemoglobinuria (PNH)**

A clinical syndrome with hemolytic anemia, thrombosis, and/or bone marrow failure secondary to loss of GPI-anchor proteins on cell membrane

Somatic mutations in *PIG-A* gene, which is required for synthesis of GPI-anchors. GPI-anchors are responsible for membrane expression of CD55, CD59. Lack of these membrane proteins results in complement mediated red cell lysis and platelet activation

Rare, most commonly affects middle aged adults

Presented with fatigue and lethargy (anemia due to hemolysis), early morning dark urine (hemoglobinuria), thrombosis (platelet activation), petechial and bleeding (low platelets)

**Blood morphology:** May present with hemolytic picture (polychromasia, microspherocytes) or aplastic picture (macrocytosis, no polychromasia).

**Bone marrow morphology:** Variable, normal in most cases, but could be hypercellular or hypocellular

**Lab test:**

*Ham’s test:* Measures red cell lysis, sensitive to complements in acidic condition.

Replaced by flow cytometry

*Flow cytometry* (method of choice): Lack of GPI-anchor proteins CD55 and CD59. Other GPI-anchor proteins include CD16, CD24. Flaler binds to these proteins and is a sensitive marker used as surrogate for GPI-anchor proteins

Usually tests red blood cells, neutrophils, and monocytes

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**Hemoglobinopathy**

Anemia caused by structural or quantitative abnormalities of alpha or beta globin proteins resulting in anemia and other associated organ damages

**Sickle cell anemia:** Caused by mutation of 6th position of beta-globin (substitute valine with glutamic acid). The abnormal beta-globin forms abnormal polymers, resulting in rigid, deformed RBCs

Clinical features include hemolytic anemia, vaso-occlusion resulting in pain crisis, ulceration, infarction (spleen), acute chest syndrome, stroke, acute sequestration syndrome

Early mortality common, median life span 40-50 years

**Blood morphology:** Sickle cells and variants. Target cells, Howell-Jolly bodies.

Polychromasia, nucleated RBCs

**Bone marrow morphology:** Non-specific, may show erythroid hyperplasia

**Lab test:**

Hemoglobin electrophoresis: Hb S predominant, Hb F variable (1-20%), no Hb A

**Differential diagnosis:** Hemoglobin SC and C diseases: milder clinical symptoms, fewer sickle cells, more target cells, C crystals

**Thalassemia:** Anemia caused by quantitative reduction of either alpha-globin (alpha-thalassemia) or beta-globin (beta-thalassemia)

Severity of disease depends on amount of globin reduction

Beta-thalassemia major: nearly absent beta-globin

Beta-thalassemia intermediate: moderate reduction of beta-globin

Beta-thalassemia minor: mild reduction of beta-globin

Hydrops fetalis: deletion of all 4 alpha-globins. Hemoglobin Barts (4 gamma globins)

Hemoglobin H: deletion of 3 alpha-globins

Alpha-thalassemia: deletion of 2 alpha-globins

Silent alpha-thalassemia: deletion of 1 alpha-globin
Clinical features include still birth or death after birth (hydrops fetalis), death in childhood without transfusion (beta-thalassemia major), mild anemia to asymptomatic (remaining)

**Blood morphology:** Microcytic RBCs, target cells, no polychromasia

**Bone marrow morphology:** None specific, may have erythroid hyperplasia

**Lab test:**
- Low hemoglobin with increased RBC, low MCV, normal to slightly high RDW
- Hemoglobin electrophoresis: Increased Hb A2 (beta-thal), normal (alpha-thal)
- Hemoglobin H: H inclusions on blood smear when stained with brilliant cresyl blue stain

**Differential diagnosis:** Iron deficiency anemia

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