Red Cell Disorders

Disorders of red cells can result in either anemia or polycythemia (an increase in the number of red cells).

Definition: Anemia is a reduction of the total circulating red cell mass below normal limits. Anemia reduces the oxygen-carrying capacity of the blood, leading to tissue hypoxia.

In practice, the measurement of red cell mass is not easy, and anemia is usually diagnosed based on a reduction in the:
- Hematocrit (the ratio of packed red cells to total blood volume) and the
- Hemoglobin concentration of the blood to levels that are below the normal range.

These values correlate with the red cell mass except when there are changes in plasma volume caused by fluid retention or dehydration.

Effects of anemia: The decrease in tissue oxygen tension that is associated with anemia triggers increased erythropoietin production (the exception is that of anemia related to chronic renal failure, in which erythropoietin-producing cells in the kidney are lost). Increased erythropoietin production leads to compensatory hyperplasia of erythroid precursors in the bone marrow and, in severe anemias, the appearance of extramedullary hematopoiesis within the secondary hematopoietic organs (the spleen, liver, and lymph nodes).

The hallmark of increased marrow output is reticulocytosis, the appearance of increased numbers of newly formed red cells (reticulocytes) in the peripheral blood. In contrast, disorders of decreased red cell production (a regenerative anemia) are characterized by reticulocytopenia.

Classification of anemia:

Morphologic classification: is based on the morphology of red cells; this is often correlates with the cause of their deficiency. Specific red cell features that provide etiologic clues include:
- Cell size (normocytic, microcytic, or macrocytic).
- Degree of hemoglobinization, which is reflected in the color of the cells (normochromic or hypochromic).
- Shape of the cells.

These features are judged subjectively by visual inspection of peripheral smears (blood film) and are also expressed quantitatively through the following indices:

Mean cell volume (MCV): the average volume per red cell, expressed in femtoliters (cubic microns)

Mean cell hemoglobin (MCH): the average content (mass) of hemoglobin per red cell, expressed in picograms
Mean cell hemoglobin concentration (MCHC): the average concentration of hemoglobin in a given volume of packed red cells, expressed in grams per deciliter.

**Iron Deficiency Anemia:**
It is the most common form of nutritional deficiency.
Iron deficiency anemia can result from a variety of causes:
1. Low intake and poor availability from predominantly vegetarian diets are an important cause of iron deficiency.
2. Malabsorption can occur with sprue and celiac disease or after gastrectomy.
3. Increased demands not met by normal dietary intake occur around the world during pregnancy and infancy.
4. Chronic blood loss is one of the most important causes of iron deficiency anemia. This loss may occur from the gastrointestinal tract (e.g., peptic ulcers, colonic cancer, hemorrhoids, hookworm disease) or the female genital tract (e.g., menorrhagia, metrorrhagia, cancers).

Regardless of the cause, iron deficiency develops insidiously. At first iron stores are depleted, leading to a decline in serum ferritin and the absence of stainable iron in the bone marrow. This is followed by a decrease in serum iron and a rise in the serum iron-binding capacity. Ultimately the capacity to synthesize hemoglobin is diminished, leading to anemia and even reduced immunocompetence.

**Lab findings:**
- Anemia (Reduced Hb or Hematocrit level).
- The red cells are microcytic and hypochromic, reflecting the reductions in MCV and MCHC.
- For unclear reasons, iron deficiency is often accompanied by an increase in the platelet count.
- Low serum ferritin and serum iron levels.
- Low transferrin saturation.
- Increased total iron-binding capacity.
- Normal Hb A2 level.
- Absent storage iron in the bone marrow.
- Although erythropoietin levels are increased, the marrow response is blunted by the iron deficiency, and thus the marrow cellularity is usually only slightly increased.

*It is important to remember that in reasonably well-nourished persons, microcytic hypochromic anemia is not a disease but rather a symptom of some underlying disorder.*

**Anemia of Chronic Disease:**
This is the most common form of anemia in hospitalized patients. It superficially resembles the anemia of iron deficiency, but it stems from inflammation-induced
sequestration of iron within the cells of the mononuclear phagocyte (reticuloendothelial) system. It occurs in a variety of chronic inflammatory disorders, including the following:
● Chronic microbial infections, such as osteomyelitis, bacterial endocarditis, and lung abscess.
● Chronic immune disorders, such as rheumatoid arthritis and regional enteritis.
● Neoplasms, such as Hodgkin lymphoma and carcinomas of the lung and breast.

**Lab findings:**
● Anemia (Reduced Hb or Hematocrit level).
● The red cells can be normocytic and normochromic, or, as in anemia of iron deficiency, hypochromic and microcytic.
● The serum iron levels are usually low.
● Increased storage iron in the bone marrow.
● High serum ferritin concentration.
● Reduced total iron-binding capacity.
All of which readily rule out iron deficiency.

*This combination of findings is attributable to high concentrations of circulating hepcidin, which inhibits ferroportin and thereby block the transfer of iron from the mononuclear phagocyte storage pool to the erythroid precursors.*

**Megaloblastic Anemias:**
In megaloblastic anemia the red cells are abnormally large (MCV >95 FL).

There are two principal causes of megaloblastic anemia:
● Folate deficiency.
● Vitamin B₁₂ deficiency.

Both vitamins are required for DNA synthesis, and, hence, the effects of their deficiency on hematopoiesis are quite similar.

**Pathogenesis:** The morphologic hallmark of megaloblastic anemias is an enlargement of erythroid precursors (megaloblasts), which gives rise to abnormally large red cells (macrocytes). The other myeloid lineages are also affected. Most notably, granulocyte precursors are enlarged (giant metamyelocytes) and yield highly characteristic hypersegmented neutrophils. Underlying the cellular gigantism is an impairment of DNA synthesis, which results in a delay in nuclear maturation and cell division. Because the synthesis of RNA and cytoplasmic elements proceeds at a normal rate and thus outpaces that of the nucleus, the hematopoietic precursors show nuclear-cytoplasmic asynchrony. Erythrocyte, Granulocyte and platelet precursors are all affected. As a result, most patients with megaloblastic anemia develop pancytopenia (anemia, thrombocytopenia, and granulocytopenia).
Causes of vitamin B12 deficiency:
● Nutritional: Especially vegans.
● Malabsorption: Gastric causes = Pernicious anemia. Congenital lack or abnormality of intrinsic factor. Total or partial gastrectomy.
Intestinal causes: Intestinal stagnant loop syndrome-jejunal diverticulosis, blind-loop, stricture, etc. Chronic tropical sprue. Ileal resection and Crohn's disease.
(Pernicious anemia: This is caused by autoimmune attack on the gastric mucosa leading to atrophy of the stomach).

Pernicious anemia: This disease results from an autoimmune reaction against parietal cells and intrinsic factor itself, which produces gastric mucosal atrophy (autoimmune chronic gastritis).
Several associations favor an autoimmune basis:
● Autoantibodies are present in the serum and gastric juice of most patients with pernicious anemia.
Three types of antibodies have been found:
■ Parietal canalicular antibodies, which bind to the mucosal parietal cells.
■ Blocking antibodies, which block the binding of vitamin B₁₂ to intrinsic factor.
■ Binding antibodies that react with intrinsic factor-B₁₂ complex and prevent it from binding to the ileal receptor.
● An occurrence of pernicious anemia with other autoimmune diseases such as Hashimoto thyroiditis, Addison disease, and type I diabetes mellitus is well documented.
● The frequency of serum antibodies to intrinsic factor is increased in patients with other autoimmune diseases.
Patients with pernicious anemia have an increased risk of gastric carcinoma.

Causes of folate deficiency:
● Nutritional: Especially old age, institutions, poverty, famine, special diets, goat's milk anemia, etc.
● Malabsorption: Tropical sprue, gluten-induced enteropathy (adult or child). Possible contributory factor to folate deficiency in some patients with partial gastrectomy, extensive jejunal resection or Crohn's disease.
● Excess utilization:
■ Physiological: Pregnancy and lactation, prematurity.
■ Pathological: Hematological diseases: hemolytic anemias, myelofibrosis.
■ Malignant disease: carcinoma, lymphoma, myeloma.
■ Inflammatory diseases: Crohn's disease, tuberculosis, rheumatoid arthritis, psoriasis.
● Excess urinary folate loss: Active liver disease, congestive heart failure.
● Drugs: Anticonvulsants, sulfasalazine.
● Mixed: Liver disease, alcoholism, intensive care.
**Lab findings:**
- The anemia is macrocytic (MCV >95 fL) and the macrocytic RBCs are typically oval.
- The reticulocyte count is low.
- The total white cell and platelet counts may be moderately reduced, especially in severely anemic patients.
- A proportion of the neutrophils show hypersegmented nuclei (with six or more lobes).
- The bone marrow is markedly hypercellular, as a result of increased numbers of megaloblasts.
- These cells are larger than normoblasts and have a delicate, finely reticulated nuclear chromatin (suggestive of nuclear immaturity) and an abundant, strikingly basophilic cytoplasm.
- The granulocytic precursors also demonstrate nuclear-cytoplasmic asynchrony, yielding giant metamyelocytes.
- Megakaryocytes, too, may be abnormally large.

**The diagnostic features (Lab findings) of pernicious anemia include:**
- **Serum antibodies to intrinsic factor.**
- Low serum vitamin B₁₂ levels.
- Normal or elevated serum folate levels.
- Megaloblastic anemia.
- Leukopenia with hypersegmented granulocytes.
- A dramatic reticulocytic response (within 2-3 days) to parenteral administration of vitamin B₁₂.

**Aplastic Anemia:**
Aplastic anemia is “a disorder in which multipotent bone marrow stem cells are suppressed, leading to:
- Marrow failure and Panctopenia.

**Pancytopenia:** Pancytopenia describes a reduction in the blood count of all the major cell lines: Red cells. White cells. Platelets.

**Etiology:**
Aplastic anemia is divided etiologically into:
- Primary (idiopathic) (50% of cases)
- Secondary to damaging agent to the BM:
  - Known toxic agent to the BM:
    * Predictable damage, which is dose related, and usually reversible. Included in this category are antineoplastic drugs, benzene, and chloramphenicol.
    * Unpredictable ("idiosyncratic" or hypersensitivity) damage to small doses of known myelotoxic drugs (e.g., chloramphenicol) or to drugs such as sulfonamides, which are not myelotoxic in other persons.
  - After certain viral infections, most often community-acquired viral hepatitis.
Marrow aplasia develops several months after recovery from the hepatitis and follows a relentless course.

**Pathogenesis:** Autoreactive T cells may play an important role in marrow failure. This is supported by the observation that in 70% to 80% of cases aplastic anemia responds to immunosuppressive therapy aimed at T cells. Perhaps viral antigens, drug-derived haptens, and/or genetic damage create neoantigens within stem cells that serve as targets for the T cells. A small fraction of patients with "acquired" aplastic anemia have inherited defects in DNA telomerase, which is needed for the maintenance and stability of chromosomes. In these settings, the outcome is direct damage to and senescence of hematopoietic stem cells.

**Lab findings:**
- The bone marrow is markedly hypocellular, with greater than 90% of the intertrabecular spaces occupied by fat.
- The limited cellularity often consists of only lymphocytes and plasma cells. These changes are better appreciated in bone marrow biopsy specimens than in marrow aspirates, which often yield a "dry tap."
- Thrombocytopenia and granulocytopenia may result in hemorrhages and bacterial infections, respectively.

It is important to distinguish aplastic anemia from anemias caused by:
- Marrow infiltration (myelophthisic anemia).
- Aleukemic leukemia.
- Granulomatous diseases affecting the BM.

Because pancytopenia is common to these conditions, their clinical manifestations may be indistinguishable, but they are easily distinguished by examination of the bone marrow.

**Hemoglobinopathies and Thalassemia:**
The hemoglobinopathies are “a group of hereditary disorders that are defined by the presence of structurally abnormal hemoglobins”. The prototypical (and most prevalent) hemoglobinopathy is caused by a mutation in the β-globin chain gene that creates sickle hemoglobin (HbS). The disease associated with HbS is sickle cell anemia. HbS, like 90% of other abnormal hemoglobins, results from a single amino acid substitution in the globin chain. On average, the normal adult red cell contains 96% HbA (α2β2), 3% HbA2 (α2δ2), and 1% fetal Hb (HbF, α2γ2). Substitution of valine for glutamic acid of the β-chain produces HbS. In homozygotes all HbA is replaced by HbS, whereas in heterozygotes only about half is replaced.

In parts of Africa where malaria is endemic the gene frequency approaches 30%, as a result of a small but significant protective effect of HbS against Plasmodium falciparum malaria. Worldwide, sickle cell anemia is the most common form of familial hemolytic anemia.
Sickle Cell Anemia:

Pathogenesis:
- Upon deoxygenation, HbS molecules undergo polymerization (gelation or crystallization). These polymers distort the red cell, which assumes an elongated crescentic, or sickle, shape.
- Sickling of red cells is initially reversible upon reoxygenation; however, membrane damage occurs with each episode of sickling, and eventually the cells accumulate calcium, lose potassium and water, and become irreversibly sickled.

Consequences of sickling:
Two major consequences of RBCs sickling.
- Repeated episodes of deoxygenation cause membrane damage and dehydration of red cells, which become rigid and irreversibly sickled. These dysfunctional red cells are recognized and removed by mononuclear phagocyte cells, producing a chronic extravascular hemolytic anemia.
- The sickling of red cells produces widespread microvascular obstructions, which result in ischemic tissue damage and pain crises.

Lab findings:
Homozygous disease:
- The hemoglobin is usually 6-9 g/dL.
- Sickle cells and target cells occur in the blood.
- Features of splenic atrophy (e.g. Howell Jolly bodies) may also be present.
- Screening tests for sickling are positive when the blood is deoxygenated.
- Hemoglobin electrophoresis: In Hb SS: No Hb A is detected. The amount of Hb F is variable and is usually 5-15%. Larger amounts are normally associated with a milder disorder.

Sickle cell trait:
- This is a benign condition with no anemia and normal appearance of red cells on a blood film.
- Hematuria is the most common symptom and is thought to be caused by minor infarcts of the renal papillae.
- Hb S varies from 25 to 45% of the total hemoglobin.

Diagnosis:
- In full-blown sickle cell disease, at least some irreversibly sickled red cells can be seen on an ordinary peripheral blood smear.
- In sickle cell trait, sickling can be induced in vitro by exposing cells to marked hypoxia.
- The ultimate diagnosis depends on the electrophoretic demonstration of HbS.
- Prenatal diagnosis of sickle cell anemia can be performed by analyzing the DNA in fetal cells obtained by amniocentesis or biopsy of chorionic villi.
Thalassemias:
The thalassemias are “a heterogeneous group of inherited disorders caused by mutations that decrease the rate of synthesis of α- or β-globin chains”. As a consequence there is a deficiency of hemoglobin, with additional secondary red cell abnormalities caused by the relative excess of the other unaffected globin chain.

Molecular Pathogenesis: A diverse collection of molecular defects underlies the thalassemias, which are inherited as autosomal codominant conditions. The adult hemoglobin, or HbA, is a tetramer composed of two α chains and two β chains. The mutations that cause thalassemia are particularly common among Mediterranean, African, and Asian populations.

β-Thalassemia: The β-globin mutations associated with β-thalassemia fall into two categories:
1. β^0, in which no β-globin chains are produced; and
2. β^+, in which there is reduced (but detectable) β-globin synthesis.

The majority of mutations consist of single-base changes.
Individuals inheriting one abnormal allele have thalassemia minor or thalassemia trait, which is asymptomatic or mildly symptomatic.
Most individuals inheriting any two β^0 and β^+ alleles have β-thalassemia major.

Two conditions contribute to the pathogenesis of the anemia in β-thalassemia:
1. The reduced synthesis of β-globin leads to inadequate HbA formation, so that the MCHC is low, and the cells appear hypochromic and microcytic.
2. Red cell hemolysis is even more important is, which results from the unbalanced rates of β-globin and α-globin chain synthesis. Unpaired α chains form insoluble aggregates that precipitate within the red cells and cause membrane damage that is severe enough to provoke extravascular hemolysis. Erythroblasts in the bone marrow are also susceptible to damage through the same mechanism, which in severe β-thalassemia results in the destruction of the majority of erythroid progenitors before their maturation into red cells. This intramedullary destruction of erythroid precursors (ineffective erythropoiesis) is also associated with an inappropriate increase in the absorption of dietary iron, which often leads to iron overload.

Laboratory findings: (β-Thalassemia major):
● There is a severe hypochromic, microcytic anemia.
● Raised reticulocyte percentage.
● Normoblasts, target cells and basophilic stippling in the blood film.
● Hemoglobin electrophoresis reveals absence or almost complete absence of Hb A.
● Almost all the circulating hemoglobin being Hb F.

Laboratory findings: (β-Thalassemia minor):
This is a common, usually symptomless, abnormality characterized by:
● Mild anemia (hemoglobin 10-12g/dL).
● A hypochromic, microcytic blood picture (MCV and MCH very low). Some RBCs show Basophilic Stippling.
● The serum iron levels are usually normal.
● Normal storage iron in the bone marrow.
● Normal serum ferritin concentration.
● Normal total iron-binding capacity.
● *A raised Hb A2 (>3.5%) confirms the diagnosis.*

**α-Thalassemia syndromes:** These are usually caused by gene deletions.
As there are normally four copies of the α-globin gene:
The clinical severity can be classified according to the number of genes that are missing or inactive.
■ **Hydrops Fetalis:** Loss of all four genes completely suppresses α-chain synthesis. Because the α chain is essential in fetal as well as in adult hemoglobin: This is incompatible with life and leads to death in utero (hydrops fetalis).
■ **Hb H disease:** Three α gene deletions leads to a moderately severe (hemoglobin 7-11 g/dL) microcytic, hypochromic anemia with splenomegaly. This is known as Hb H disease because hemoglobin H (β4) can be detected in red cells of these patients by:
  ● Electrophoresis or
  ● In reticulocyte preparations.
In fetal life: Hb Barts (γ4) occurs.
■ **The α-thalassemia traits:** are caused by loss of one or two genes and are usually not associated with anemia, although the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) are low. Hemoglobin electrophoresis is normal.

**Hemolytic Anemias:**
Normal red cells have a life span of about 120 days. Anemias that are associated with accelerated destruction of red cells are termed hemolytic anemias. Destruction can be caused by:
1. Inherent (intracorpuscular) red cell defects, which are usually inherited, or
2. External (extracorpuscular) factors, which are usually acquired.

*There are certain general features of hemolytic anemias. All are characterized by:*
● An increased rate of red cell destruction.
● A compensatory increase in erythropoiesis that results in reticulocytosis. In severe hemolytic anemias, extramedullary hematopoiesis often develops in the spleen, liver, and lymph nodes.

**Extravascular and Intravascular hemolysis:**
There are two main mechanisms whereby red cells are destroyed in hemolytic anaemia.
● Extravascular hemolysis: There is excessive removal of red cells by cells of the reticuloendothelial system.
Intravascular hemolysis: The red cells are broken down directly in the circulation. Whichever mechanism dominates will depend on the pathology involved.

Non-Immune Hemolytic Anemia:

Glucose-6-Phosphate Dehydrogenase Deficiency (G6PDD):
The red cell is vulnerable to injury by endogenous and exogenous oxidants, which are normally inactivated by reduced glutathione (GSH). Abnormalities affecting the enzymes that are required for GSH production reduce the ability of red cells to protect themselves from oxidative injury and lead to hemolytic anemias. The prototype (and most prevalent) of these anemias is that associated with a deficiency of glucose-6-phosphate dehydrogenase (G6PD). The G6PD gene is on the X chromosome.

G6PD deficiency produces no symptoms until the patient is exposed to an environmental factor (most commonly infectious agents or drugs) that results in increased oxidant stress. The drugs incriminated include antimalarials (e.g., primaquine), sulfonamides, nitrofurantoin, phenacetin, aspirin (in large doses), and vitamin K derivatives. More commonly, episodes of hemolysis are triggered by infections, which induced phagocytes to produce free radicals as part of the normal host response. These offending agents produce oxidants such as hydrogen peroxide that are sopped up by GSH, which is converted to oxidized glutathione in the process. Because regeneration of GSH is impaired in G6PD-deficient cells, hydrogen peroxide is free to "attack" other red cell components, including globin chains, which have sulfhydryl groups that are susceptible to oxidation. Oxidized Hb denatures and precipitates, forming intracellular inclusions called Heinz bodies, which can damage the cell membrane sufficiently to cause intravascular hemolysis. Other cells that are less severely damaged nevertheless suffer from a loss of deformability, and their cell membranes are further damaged when splenic phagocytes attempt to "pluck out" the Heinz bodies, creating so-called bite cells. All of these changes predispose the red cells to becoming trapped in the splenic sinusoids and destroyed by the phagocytes (extravascular hemolysis). Drug-induced hemolysis is acute and of variable clinical severity. Typically, patients develop evidence of hemolysis after a lag period of 2 or 3 days. Because the G6PD gene is on the X chromosome, all the red cells of affected males are affected. Most carrier females are asymptomatic. In a variant known G6PD Mediterranean, found mainly in the Middle East, the enzyme deficiency and the hemolysis that occur upon exposure to oxidants are more severe.

Hereditary Spherocytosis (HS):
Is characterized by an inherited (intrinsic) defect in the red cell membrane that renders the cells spheroidal, less deformable, and vulnerable to splenic sequestration and destruction. It is transmitted most commonly as an autosomal dominant trait; approximately 25% of patients have a more severe autosomal recessive form of the disease.
**Pathogenesis:**
- HS is usually caused by defects in the proteins involved in the vertical interactions between the membrane skeleton and the lipid bilayer of the red cell. Various mutations involving spectrin and ankyrin that weaken the interactions between these proteins cause red cells to lose membrane fragments. The loss of membrane may be caused by the release of parts of the lipid bilayer that are not supported by the skeleton.
- The spleen plays a major role in the destruction of spherocytes. The marrow produces red cells of normal biconcave shape but these lose membrane and become increasingly spherical (loss of surface area relative to volume) as they circulate through the spleen and the rest of the RE system. Ultimately, the spherocytes are unable to pass through the splenic microcirculation where they die prematurely.

**Pathological features:**
- On smears, the red cells lack the central zone of pallor because of their spheroidal shape.
- Spherocytosis, though distinctive, is not diagnostic; it is seen in other conditions, such as immune hemolytic anemias, in which there is a loss of cell membrane relative to cell volume.
- Because of their spheroidal shape, HS red cells show increased osmotic fragility when placed in hypotonic salt solutions, a characteristic that is helpful for diagnosis.
- The excessive red cell destruction and resultant anemia lead to a compensatory hyperplasia of marrow red cell progenitors and an increase in red cell production, which is marked by peripheral blood reticulocytosis.
- The other general features of hemolytic anemias described earlier are also present, pigmented gall stone, which occurs in up to 50% of HS patients.

**Traumatic Hemolytic Anemia:**
These arise through physical damage to red cells either on:
- Abnormal surfaces: (e.g. artificial heart valves or arterial grafts), arteriovenous malformations or
- A microangiopathic hemolytic anemia.
This is caused by red cells passing through abnormal small vessels.
The latter may be caused by:
- Deposition of fibrin strands often associated with disseminated intravascular coagulation (DIC) or
- Platelet adherence as in thrombotic thrombocytopenic purpura (TTP) or
- Vasculitis (e.g. polyarteritis nodosa).
All of the above produce vascular lesions that predispose the circulating red cells to mechanical injury.
The morphologic alterations in the injured red cells (schistocytes) are striking and quite characteristic; "burr cells," "helmet cells," and "triangle cells" may be seen.

**Immune Hemolytic Anemias:**

- **Autoimmune Hemolytic Anemias:** Autoimmune hemolytic anemias (AIHAs) are caused by antibody production by the body against its own red cells. They are characterized by a positive direct antiglobulin test (DAT) also known as the Coombs test. Divided into: ● Warm. ● Cold types.
  
  According to whether the antibody reacts more strongly with red cells at 37°C or 4°C.

**Classification of immune hemolytic anemias:**

**A. Warm type:**
- **Autoimmune:**
  - Idiopathic.
  - Secondary: SLE, other 'autoimmune' diseases. CLL, lymphomas. Drugs (e.g. methyldopa).
- **Alloimmune:**
  - Induced by red cell antigens: Hemolytic transfusion reactions. Hemolytic disease of the newborn.

**B. Cold type:**
- **Idiopathic.
  ● Lymphoma. ● Paroxysmal cold hemoglobinuria (rare, sometimes associated with infections, e.g. syphilis).

**Laboratory findings (Warm type):**

The hematological and biochemical findings are typical of an extravascular hemolytic anemia with spherocytosis prominent in the peripheral blood.

The DAT is positive because of Ig G, Ig G and complement or Ig A on the cells.

**Laboratory findings (Cold type):**

Are similar to those of warm AIHA EXCEPT that:
- Spherocytosis is less marked.
- Red cells agglutinate in the cold.
Laboratory Diagnosis of Anemias:
The diagnosis is established by:
● Decrease in the Hb and the hematocrit (PCV) to levels that are below normal.
● The red cell hemoglobin content and size of the RBCs are discriminatory in that the results can place the anemia into one of three major subgroups:
  * Normocytic Normochromic.
  * Microcytic Hypochromic.
  * Macrocytic.
● The presence of red cells with a particular morphology, such as spherocytes, sickled cells, and fragmented cells, provide additional etiologic clues.
● Specialized tests are particularly important in establishing the diagnosis of certain classes of anemia; these include:
  ■ Gel electrophoresis: used to detect abnormal hemoglobins, such as HbS.
  ■ Coombs test: used to diagnose immune hemolytic anemias.
  ■ Reticulocyte counts: used to distinguish between anemias caused by red cell destruction (hemolysis) and depressed production (marrow failure).
  ■ Iron indices (serum iron, serum iron-binding capacity, transferrin saturation, and serum ferritin concentrations): used to distinguish between hypochromic microcytic anemias caused by iron deficiency, anemia of chronic disease, and thalassemia minor.
  ■ Serum and red cell folate and vitamin B_{12} concentrations: used to identify the cause of megaloblastic anemia.
  ■ Plasma unconjugated bilirubin and haptoglobin concentrations: used to support the diagnosis of hemolytic anemia.

In isolated anemia, tests performed on the peripheral blood are usually sufficient to establish a cause. In contrast, when anemia occurs in combination with thrombocytopenia and/or granulocytopenia, it is much more likely to be associated with marrow aplasia or infiltration; in these instances, BM aspiration & biopsy are often important for diagnosis.
**Polycythemia (Erythrocytosis):**

Polycythemia (Erythrocytosis): This term signifies an increase in the blood concentration of red cells, which usually correlates with an increase in the hemoglobin concentration. Polycythemia are of two types:

- Relative polycythemia that is associated with hemoconcentration caused by dehydration, such as with water deprivation, prolonged vomiting, diarrhea, or the excessive use of diuretics.
- Absolute polycythemia, when there is an increase in the total red cell mass.

Absolute polycythemia is either:

- Primary when the increase in red cell mass results from an autonomous proliferation of the myeloid stem cells.
- Secondary when the red cell progenitors are proliferating in response to an increase in erythropoietin.

- Primary polycythemia (polycythemia vera [PCV]): is a clonal, neoplastic proliferation of myeloid progenitors.
- Secondary polycythemias: the increases in erythropoietin that are seen in secondary polycythemias have a variety of causes:
  - Appropriate: Lung disease, High-altitude living, Cyanotic heart disease.