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 (aregenerative anemias) 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.

Pathologic features (Lab findings):
● 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.
● Although erythropoietin levels are increased, the marrow response is blunted by the iron deficiency, and thus the marrow cellularity is usually only slightly increased.

Diagnostic criteria include:
● Anemia, hypochromic and microcytic red cell indices.
● Low serum ferritin and serum iron levels.
● Low transferrin saturation.
● Increased total iron-binding capacity.

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

The serum iron levels are usually low, and the red cells can be normocytic and normochromic, or, as in anemia of iron deficiency, hypochromic and microcytic. However, the anemia of chronic disease is associated with increased storage iron in the bone marrow, a high serum ferritin concentration, and a 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).

Pathologic features (Lab findings):
- The anemia is macrocytic (MCV >95 fL).
- The macrocytes are typically oval in shape.
The reticulocyte count is low.
• The total white cell and platelet counts may be moderately reduced, especially in severely anaemic 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.

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 B\textsubscript{12} to intrinsic factor.
    Binding antibodies that react with intrinsic factor-B\textsubscript{12} 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.
The diagnostic features of pernicious anemia include:
• Low serum vitamin B\textsubscript{12} levels.
Normal or elevated serum folate levels.
Serum antibodies to intrinsic factor.
Megaloblastic anemia.
Leukopenia with hypersegmented granulocytes.
A dramatic reticulocytic response (within 2-3 days) to parenteral administration of vitamin B₁₂.

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.
The diagnostic features of pernicious anemia include
Low serum vitamin B₁₂ levels.
Normal or elevated serum folate levels.
Serum antibodies to intrinsic factor.
Moderate to severe 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 pancytopenia.”

Etiology:
Aplastic anemia is divided etiologically in to:
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.

**Pathologic features (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.

**Pathologic features (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. β⁰, 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 β⁰ 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 diagnosis: (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.

**β-Thalassemia trait (minor):** This is a common, usually symptomless, abnormality characterized by:
- A hypochromic, microcytic blood picture (MCV and MCH very low) and mild anemia (hemoglobin 10-12 g/dL).
- 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.

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).

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 (TIP) 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. A.

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 as a result 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 immunohemolytic 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 B₁₂ 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.

The increases in erythropoietin that are seen in secondary polycythemias have a variety of causes:

- **Appropriate:** lung disease, high-altitude living, cyanotic heart disease
- **Inappropriate:** erythropoietin-secreting tumors (e.g., renal cell carcinoma, hepatoma, cerebellar hemangioblastoma).
HEMATOPATHOLOGY

WHITE CELL DISORDERS

Disorders of white cells include deficiencies (leukopenias) and proliferations (leukocytosis), which may be reactive or neoplastic.

NON-NEOPLASTIC DISORDERS OF WHITE CELLS

Leukopenia is a decrease in the number of white cells in the peripheral blood, most commonly the result of a decrease in neutrophils (the most prevalent circulating white cells).

- Neutropenia
  Neutropenia signifies a reduction in absolute neutrophil number below normal in peripheral blood; when severe (reduced less than 500 cells/µl), where affected persons are extremely susceptible to bacterial and fungal infections, which can be severe enough to cause death.

Etiology and Pathogenesis
The mechanisms that cause neutropenia can be broadly divided into two categories:

1. Inadequate or ineffective granulopoiesis, which is a manifestation of:
   A. Generalized marrow failure
      - Aplastic anemia
      - A variety of leukemias
   B. Isolated neutropenia;
      there is involvement of neutrophilic precursors only as is seen with
      - Congenital
      - Idiopathic benign (racial or familial)
      - Cyclical neutropenia syndrome (with 3-4 weeks periodicity)

2. Accelerated removal or destruction of neutrophils:
   - Acquired: drug-induced (immune-mediated or direct toxicity)
   - Overwhelming infections
   - Splenomegaly that leads to sequestration and accelerated removal of neutrophils

- Lymphopenias are associated with
  1. Congenital immunodeficiency diseases
  2. Acquired in association with:
     • advanced HIV infection
     • treatment with corticosteroids and other immunosuppressive therapy
     • Hodgkin disease
     • Widespread irradiation

Reactive Leukocytosis
An increase in the number of white cells is common in a variety of reactive inflammatory states caused by microbial and non-microbial stimuli.

- Neutrophilic Leukocytosis (Neutrophilia)
  a. Acute bacterial infections (especially pyogenic)
  b. Sterile inflammation caused by tissue necrosis (myocardial infarction, burns)
  c. Metabolic disorders (uremia, eclampsia, acidosis, gout)
  d. Neoplasms of all types
  e. Acute hemorrhage or hemolysis
f. Treatment with myeloid growth factors (G-CSF, GM-CSF)

- Eosinophilic Leukocytosis (Eosinophilia)
  a. Allergic disorders such as asthma, hay fever, allergic skin diseases (e.g., pemphigus, dermatitis herpetiformis)
  b. Parasitic infestations
  c. Drug sensitivity
  d. Collagen vascular disorders (polyarteritis nodosa, vasculitis)
  e. Certain malignancies (e.g., Hodgkin disease and some non-Hodgkin lymphomas)
  f. Hypereosinophilic syndrome
  g. Myeloproliferative neoplasms, Chronic eosinophilic leukemia
  h. Treatment with GM-CSF

- Basophilic Leukocytosis (Basophilia): this is rare, often indicative of myeloproliferative neoplasms (e.g., chronic myeloid leukemia). Reactive increase is seen in myxoedema, smallpox or chickenpox infections and in ulcerative colitis.

- Monocytosis
  a. Chronic bacterial infections (e.g., tuberculosis, brucellosis, endocarditis, typhoid)
  b. Collagen vascular diseases (systemic lupus erythematosus, rheumatoid arthritis)
  c. Hodgkin disease, AML and other malignancies
  d. Myelodysplastic syndrome (especially chronic myelomonocytic leukemia)
  e. Inflammatory bowel diseases (e.g., ulcerative colitis)

- Lymphocytosis
  a. Acute infections: infectious mononucleosis, rubella, pertussis, mumps, hepatitis A, cytomegalovirus, HIV, herpes, Epstein-Barr virus
  b. Chronic infections: tuberculosis, toxoplasmosis, brucellosis, syphilis
  c. Chronic lymphoid leukemias
  d. Acute lymphoblastic leukemia
  e. Non-Hodgkin lymphoma (some)
  f. Thyrotoxicosis

NEOPLASTIC PROLIFERATIONS OF WHITE CELLS

A. Lymphoid neoplasms, which include non-Hodgkin lymphomas, Hodgkin lymphomas, acute and chronic lymphoid leukemias, and plasma cell dyscrasias and related disorders.

B. Myeloid neoplasms arise from stem cells that normally give rise to the formed elements of the blood: granulocytes, red cells, and platelets. The myeloid neoplasms fall into three fairly distinct subcategories:

1. Acute myeloid leukemias, in which immature progenitor cells accumulate in the bone marrow (BM).
2. Chronic myeloproliferative neoplasms, in which inappropriately increased production of formed blood elements leads to elevated blood cell counts.
3. Myelodysplastic syndromes, which are characteristically associated with ineffective hematopoiesis and cytopenias.

ACUTE LEUKEMIATIONS (AL)

There are two major types of AL; acute lymphoblastic (ALL) and acute myeloblastic (AML).
**Acute leukemia** is usually an aggressive clonal malignant transformation involving the hematopoietic stem cells or early progenitors and characterized by uncontrolled proliferation of blasts in the BM with spillage into the peripheral blood and variable infiltration of other organs.

**Etiology of AL**

Several factors have been linked to the occurrence of AL including:

I. Environmental Agents

A. Ionizing Radiation

Exposure to atomic bomb explosions is associated with increased incidence of AL; younger age and those who are closer to the hypocenter are at particularly high risk. The predominant type is AML though ALL is reported in younger individuals. Infants whose mothers were exposed to X-rays during pregnancy are at higher risk. Exposure to diagnostic X-rays or radioisotopes at diagnostic levels (low dose) does not increase the risk.

B. Chemicals

Exposure to the following agents have been noted to be associated with a higher incidence

- **Benzene**
  - Benzene and other petroleum derivatives
  - Shoe makers and plastic glues
  - Handling buses and trucks
- **Alkylating agents**: (cytotoxic drugs used in the treatment of certain malignancies)

II. Host susceptibility to AL is determined by

A. Genetic factors

- Fraternal twins and siblings of affected children are at a 2-4 fold greater risk of leukemia during the first decade of life than are unrelated children.
- If one identical twin is affected, the other twin has a 20% chance of developing ALL.
- Those with Down's syndrome have 10-30 fold ↑ risk.

B. Acquired factors; AL show increased incidence in association with the following:

- Myelodysplastic syndrome
- After chemotherapy ± radiotherapy
- Chronic myeloproliferative neoplasms
- Aplastic anemia

III. Oncogenic viruses: there is no good evidence except for HTLV-1, which may cause adult T-cell leukemia/lymphoma.

IV. Others: there is a significant correlation between infants with AL and alcohol intake, smoking, and exposure to benzene and petroleum derivatives of their mothers during pregnancy.

**Pathophysiology of Acute Leukemias**

- In acute leukemia there is a block in differentiation. This leads to the accumulation of immature leukemic blasts in the BM, which suppress the function of normal hematopoietic stem cells by physical displacement and other poorly understood mechanisms.
- Eventually BM failure results, which accounts for the major clinical manifestations of AL.
The acute leukemias have the following clinical characteristics:

- **Variable age of onset:** ALs can occur at any age, however, childhood AL (age <15 years) is usually ALL (80%) whereas adult AL (age ≥15 years) is usually AML (80%).

- **Abrupt stormy onset especially in children**

- **Symptoms and signs related to BM failure.** These include;
  a. Pallor, weakness, fatigue, lethargy, dyspnea on exertion, angina, and palpitation (due mainly to anemia)
  b. Fever (reflecting mainly infections resulting from neutropenia)
  c. Bleeding such as; petechiae, ecchymoses, epistaxis, and gum bleeding (secondary to thrombocytopenia).

- **Symptoms related to organ or tissue infiltration:**
  - Generalized lymphadenopathy, splenomegaly, and hepatomegaly, these are more pronounced in ALL than in AML.
  - Central nervous system manifestations these include headache, vomiting, and nerve palsies resulting from meningeal spread; these features are more common in children than in adults and are more common in ALL than AML.
  - Gum infiltration is more common in AML.
  - Testicular involvement is more common in ALL.
  - Arthralgia, bone pain and tenderness.

Laboratory diagnosis of Acute Leukemias

The diagnosis of AL is based on the presence of ≥20 % blasts in the BM and/or peripheral blood. However; it can be diagnosed with even <20 % blasts if specific leukemia-associated cytogenetic or molecular genetic abnormalities are present. Because of different responses to therapy, it is of great practical importance to distinguish ALL from AML. The nuclei of lymphoblasts have somewhat coarse and clumped chromatin and one or two nucleoli; myeloblasts tend to have finer chromatin with multiple nucleoli and more cytoplasm, which may contain granules or Auer rod(s).

- **Blood film (BF):**
  a. RBCs: anemia is usually normochromic normocytic and is almost always present.
  b. WBCs: the total WBC count is variable. There may be;
     - leukocytosis, where blasts are self-evident, or
     - leukopenia, blasts may be present or absent, or
     - it may show normal count.
  c. Neutropenia is also a common finding in the peripheral blood.
  d. Platelets: the count is reduced in most cases (i.e. <150,000/ L or <150 × 10⁹/L).

- **BM aspirate is necessary to confirm the diagnosis (especially when low counts).**

- **BM trephine biopsy is only essential when:**
  1. BM aspirate is inadequate; commonly due to BM fibrosis.
  2. To distinguish whether a poor aspirate is due to hypocellularity or persistent leukemia.

Investigations

- **Hematological:** BF and BM findings are already mentioned.
- **Biochemical tests** may reveal increased S. uric acid, S. LDH, and hypercalcemia.
- **Liver & Renal Function Tests** are performed as a baseline before treatment begins.
- **Radiological Examination** may reveal,
  a. Lytic bone lesions.
b. Mediastinal widening caused by enlargement of the thymus and/or mediastinal lymphadenopathy.  

*CSF examination* may show blast cells infiltration, indicating CNS involvement.  

*Cytochemistry* is useful if the leukemia is not obviously myeloid.  

*Immunophenotyping* is indicated in all patients in whom the leukemia is not obviously myeloid.  

*Cytogenetic analysis* is essential in all patients, best performed on BM aspirate.  

**Classification of acute leukemia** is based on:  
1. Morphology of blasts  
2. Cytochemistry through the use of special stains like; SBB, PAS, MPO, Estrases…etc  
3. Immunophenotyping (analysis by flow cytometry and immunohistochemistry).  
4. Genetic analysis includes;  
   - Cytogenetic analysis (applied by conventional karyotyping and FISH techniques)  
   - Molecular genetic analysis (applied by PCR and FISH techniques)  

**Morphological classification**  
I. French American British (FAB) classification (1976)  
A. **Acute Lymphoid Leukemia (ALL)** is classified into three subtypes:  
   - ALL- L1: Monomorphic blasts, majority are small, high nucleo-cytoplasmic (N/C) ratio, and scanty cytoplasm with small or inconspicuous nucleoli.  
   - ALL- L2: Heterogeneous blasts, variable sizes and N/C ratios, more prominent nucleoli with nuclear membrane irregularities.  
   - ALL- L3: Monomorphic large blasts with prominent nucleoli and strongly basophilic, vacuolated cytoplasm.  
B. **Acute Myeloid Leukemia (AML)** is classified into eight subtypes:  
   - M0: AML with minimal evidence of myeloid differentiation  
   - M1: AML without maturation  
   - M2: AML with maturation  
   - M3: Acute promyelocytic leukemia  
   - M4: Acute myelomonocytic leukemia  
   - M5: Acute monoblastic M5a/monocytic M5b leukemia  
   - M6: Acute erythroleukemia  
   - M7: Acute megakaryoblastic leukemia  

II. **WHO classification (2000-2002)**  
There is a consensus that FAB L1, L2 and L3 of ALL are no longer relevant, since L1 & L2 morphology do not predict immunophenotype, genetic abnormalities, or clinical behavior. ALL-L3 is generally equivalent to Burkitt lymphoma in leukemic phase and should be diagnosed as such.  

The WHO Classification of AML had reduced the blast threshold for diagnosis from 30% (in FAB classification) to 20% in the peripheral blood and/or BM. In addition, patients with certain clonal, recurrent cytogenetic abnormalities should be considered to have AML regardless of the blast percentage.  

**Cytochemistry of AL**
• ALL: is negative for Myeloperoxidase, Sudan Black B, and Non-specific estrases. Periodic Acid Schiff is positive in many cases.
• AML: is positive for Myeloperoxidase, Sudan Black B, and Non-specific estrases. PAS is positive in AML-M6.

Immunophenotyping of AL
This is very useful in typing and subtyping of AL. CD79a is a specific marker for B-cells and CD3 for T-cells. The most specific myeloid marker is anti-myeloperoxidase (MPO).

Karyotyping of AL
ALL: the most common karyotypic abnormalities in pre-B-cell ALL is hyperploidy (>50 chromosomes/cell), which is associated with t(12: 21) chromosomal translocation involving the TEL1 and AML1 genes. The presence of these aberrations correlates with a good prognosis. Poor outcomes are observed with pre-B-cell ALL that have translocations involving the MLL gene on chromosome 11q23 or the Philadelphia (Ph+) chromosome.
AML: good outcome correlates with t(8:21) & t(15:17). Conversely, poor outcome correlates with Ph+, t(6:9) and hyperploidy.

Course & Prognosis of AL
If untreated, patients will only survive for few months, and they will usually die either of severe infection or bleeding. ALL, in general, carries a better prognosis than AML. T-ALL patients have a better prognosis in adult than in children.

Treatment of childhood ALL (2-10 year age) represents one of the great success stories in oncology and has the best prognosis; most can be cured. Other groups of patients do less well.

CLASSIC CHRONIC MYELOPROLIFERATIVE NEOPLASMS (MPN)
This term covers a group of clonal disorders of the hematopoietic stem cells that lead to effective proliferation of one or more hematopoietic component in the BM, and in many cases, in the liver and spleen leading to elevated blood levels of one or more myeloid cell lineages (i.e. erythrocytosis, leukocytosis, and thrombocytosis). The classic MPNs include:
1. Chronic myeloid leukemia (CML - Ph⁺ve)
2. Polycythemia vera (PV)
3. Essential thrombocytemia (ET)
4. Primary myelofibrosis (MF)
These disorders are closely related to each other and transitional forms and evolution from one entity into another occurs during the course of the disease.

Karyotype and Molecular Features
• The vast majority of CML show the Philadelphia chromosome, in (90-95%) and M-BCR-ABL p210 in (99% of patients). Ph chromosome is a minute chromosome 22 from which the long arms are deleted (22q-). It is part of reciprocal translocation between chromosome 9 & 22 t(9; 22) in which part of 22 is clearly visible on 9 but the part of 9 on 22 is too small to be distinguished cytogenetically. This translocation is detected by PCR or FISH techniques.
• Almost all PV patients, and about 55% of ET and MF cases show an acquired mutation of cytoplasmic Janus-Associated Kinase 2 (JAK2) that occurs in the BM and in the peripheral blood granulocytes. JAK2 plays a major role in normal myeloid development.
**POLYCYTHEMIA**

True polycythemia refers to an *absolute increase in total body red cell volume* (mass), which usually manifests itself as a raised hemoglobin (Hb) concentration and/or packed cell volume (PCV) above the upper limit of normal for the patient's age and sex in specific population. A raised Hb (or PCV) can also be due to a reduction in plasma volume, *without an increase in total red cell volume*; this is known as apparent (or relative) polycythemia.

Polycythemia is classified according to its pathophysiology:

**A. Absolute**

1. **Primary**
   - Polycythemia (rubra) vera
   - Familial (congenital) Polycythemia.

2. **Secondary**
   - Caused by compensatory erythropoietin increase in:
     - High altitudes
     - Pulmonary disease and alveolar hypoventilation (sleep apnoea)
     - Cardiovascular disease, especially congenital with cyanosis.
     - Increased affinity hemoglobin (familial Polycythemia).
     - Heavy cigarette smoking
   - Caused by inappropriate erythropoietin increase in:
     - Renal diseases (e.g. hydronephrosis, vascular impairment, cysts, carcinoma)
     - Tumors (such as uterine leimyoma, renal cell carcinoma, and hepatocellular carcinoma).

**B. Relative (Stress or pseudopolycythemia):**

- Cigarette smoking
- Dehydration: water deprivation, vomiting.
- Plasma loss: burns, enteropathy.

**POLYCYTHEMIA RUBRA VERA (PV)**

PV is characterized by generalized hyperplasia of all marrow elements, but dominated by expansion of the red blood cell mass. Although the diagnostic finding is the increase in red cell volume (>125%), Hb >18.5 g/dl for men, >16.5 g/dl for women, in many patients there is also neutrophilia and thrombocytosis in 50% of cases.

The clinical features are headache, dyspnea, blurred vision, night sweats, pruritus (characteristically after hot bath) and plethoric appearance. Splenomegaly occurs in 75% of patients. Thrombosis and hemorrhage are the major clinical problems. Typically, the prognosis is good with a median survival of 10-16 years. Transition from PV to MF and AL may occur.

**ESSENTIAL THROMBOCYTHEMIA (ET)**

ET is characterized by a sustained increase in platelet count, because of megakaryocytic proliferation and overproduction of platelets.

A persisting platelet count > 400 × 10^9/L (400,000 /µL) is the central diagnostic feature but other causes of raised platelet count need to be fully excluded before the diagnosis can be made. Many cases are symptomless and diagnosed on routine blood counts. Thrombosis is a risk in about 25% of the patients. Hemorrhage as a result of abnormal platelet function. Erythromelalgia is a characteristic symptom (it is a burning sensation felt in hands or feet and relieved by aspirin). Up to 40% of patients will have palpable splenomegaly, whereas in others there may be splenic atrophy because of infarction. Abnormal large platelets and
megakaryocyte fragments may be seen on blood film. BM typically shows an excess proliferation of abnormal large and mature megakaryocyte, and no or little granulocyte or erythroid proliferation. Often the disease is stationary for 10-20 years or more and has a lesser risk to transform to MF, AL and PV.

PRIMARY MYELOFIBROSIS (MF)

MF is characterized by proliferation of multiple cell lineages and accompanied by progressive BM fibrosis, with development of hematopoiesis in the spleen and liver. The onset is insidious with symptoms of anemia. About ≥ of the patients have previous history of PV. Massive splenomegaly is the main physical sign.

Laboratory findings:
1. Anemia is usual.
2. The WBC and platelet counts are frequently high at presentation but with advanced disease, leucopenia and thrombocytopenia are common.
3. A leukoerythroblastic blood film is found and the red cells show characteristic 'tear-drop' poikilocytes.
4. BM is usually unobtainable by aspiration. Trephine biopsy shows hypercellular marrow; granulocytic proliferation and increased numbers of atypical megakaryocytes are frequently seen with often decreased erythropoiesis in pre-fibrotic phase with extensive marrow fibrosis in fibrotic phase.

Course & prognosis
MF has the poorest prognosis of the MPNs; the median survival is 3-5 years (range 1-15 years). Causes of death include: heart failure, infection and in 10-20% of cases transformation to AML.

CHRONIC MYELOID LEUKEMIA (CML)

CML is characterized by proliferation of a population of differentiated cells that leads to a greatly expanded total myeloid mass. CML represents about 15 % of leukemias.

CML has 3 phases during its course:
A. Chronic Phase (CP), B. Accelerated Phase (AP), C. Blastic Phase (BP).

The Chronic phase (CP) usually lasts 2-7 years and in 50% of cases it is transformed to BP directly. In up to 50% of cases the diagnosis is made incidentally from a routine blood count (asymptomatic). There may be features of anemia (pallor, dyspnoea, and tachycardia) and of abnormal platelet function (bruising, epistaxis, and menorrhagia). Splenomegaly is nearly always present and is frequently massive.

Laboratory findings
- Anemia; usually normochromic normocytic.
- Leukocytosis; usually in the range of 20-200 ×10⁹/L.
- Blood film shows a full spectrum of granulocytic cells, ranging from blasts (usually 2-10%) to mature neutrophils, with intermediate myelocytes and neutrophils predominating.
- Eosinophils and basophils are usually increased.
- Platelet count is usually increased.

BM Aspirate:
- Markedly hypercellular marrow
- Blast cells < 10% of all nucleated cells (ANC).
- Megakaryocytes are small, hypolobed and increased in numbers.
**BM Biopsy** shows complete loss of fat spaces due to dense hypercellularity.

**In the Advanced disease (AP & BP) the clinical features are quite variable:**
- Asymptomatic; the diagnosis is based entirely on blood and marrow findings.
- Patients may develop fever, excessive sweating, anorexia and weight loss or bone pain.
- Occasionally, patients present with generalized lymphadenopathy; where LN biopsy shows nodal infiltration with blast cells that may be myeloid or lymphoid.
- Localized skin infiltrates may be seen. Discrete masses of blast cells may develop at almost any site; these are sometimes referred to as "Myeloid Sarcomas".

**Laboratory findings**
In AP: Blasts range (10-19%) in peripheral blood and/or BM, basophils ≥20%. Platelet count is < 100 × 10⁹/L or persistently >1000 × 10⁹/L, increasing spleen size and WBCs unresponsive to therapy. There may be megakaryocytic proliferation in sizable sheets and clusters, associated with marked fibrosis.
In BP: Blasts ≥20% in peripheral blood and/or BM, or extramedullary blast proliferation (LN, skin, elsewhere), or detection of large foci or clusters of blasts in BM biopsy.

**Course & prognosis**
- CML patients in chronic phase usually show an excellent response with the use of imatinib, the 5-year survival is around 90%.
- The 5-year survival after SCT is approximately 50-70%, providing that SCT done within the first year following diagnosis.
- The average survival of patients in AP is 1–2 years.
- Transformation to acute leukemia that ends with death within 2-6 months.
- Death occurs from terminal blastic transformation or intercurrent hemorrhage or infection.

**MYELODYSPLASTIC SYNDROME (MDS)**
MDS is characterized by increasing BM failure with quantitative and qualitative abnormalities of megakaryocytes, erythroid and myeloid cells. MDS is either primary or is secondary to chemotherapy ± radiotherapy.

**Pathogenesis**
There is increased stem cell proliferation with ineffective differentiation and maturation, resulting in a hypercellular BM with peripheral blood pancytopenia; this is the hallmark of the disease.

**Clinically:** patients may present with anemia (transfusion-dependent), recurrent infections and easy bruising or bleeding (neutrophils and monocytes, and platelets are often functionally impaired).

**Laboratory findings**
A. Peripheral Blood:
- Pancytopenia is frequent
- Anemia; is usually macrocytic.
- Granulocytes are often decreased in number and frequently lack granulation.
- Pelger abnormality (neutrophil with single or bilobed nucleus) is often present.
- Platelets may be improperly large or small and are usually decreased in number.
- Blasts in variable numbers are present in poor prognosis cases.
**B. Bone Marrow:**
- Usually hypercellular.
- Multinucleate normoblasts and other dysplastic (dyserythropoietic) features are seen.
- Ring sideroblasts may be seen (>4 perinuclear iron granules/normoblast or covering ≥ of the nuclear circumference).
- Granulocytes and megakaryocytes are dysplastic with abnormal morphology.

At least 10% of the cells in a lineage should be dysplastic to consider the diagnosis of MDS.

**THE CHRONIC LYMPHOID LEUKEMA**

A number of lymphoproliferative disorders (LPD) are included in this group characterized by accumulation in the blood of mature lymphocytes of either B- or T- cell type. In general the diseases are incurable but tend to run a chronic and fluctuating course.

**Diagnosis**
This group is characterized by a chronic persistent lymphocytosis. Subtypes are distinguished by:
1. Morphology.
2. Immunophenotype.
3. Cytogenetics
4. DNA analysis may be useful in showing a monoclonal rearrangement of either Ig (for B-cells) or T-cell receptor (TCR) genes (for T-cells).

**CHRONIC LYMPHOCYTIC LEUKEMA (CLL)**

CLL is a low grade clonal LPD characterized by progressive accumulation of usually well-differentiated CD5⁺ lymphocytes in the marrow with an accompanying peripheral lymphocytosis. Involvement of LN, spleen and liver invariably occurs sometimes during the disease course. The etiology is unknown. There is seven-fold increased risk of CLL in the close relatives of the patient.  CLL is the most common of the chronic lymphoid leukemia, accounting for 60% of cases, and it is the most common in the West representing about 25% of all leukemias in adults > 50 years.

**Clinical features of CLL**
1. Asymptomatic; most cases are diagnosed when routine blood test is performed.
2. Lymphadenopathy: Symmetrical enlargement of cervical, axillary or inguinal LNs is usually discrete and non-tender.
3. Features of anemia & thrombocytopenia may be present.
4. Splenomegaly and less commonly hepatomegaly are common in intermediate & later stages.
5. Early bacterial infections predominate but with advanced disease viral and fungal infections such as candidiasis and herpes zoster are also seen.

**Laboratory findings**
- *Lymphocytosis*: the absolute lymphocyte count is > 5 × 10⁹/L. The predominant cells are small lymphocytes with compact dark-staining round nuclei, scanty cytoplasm, and little variation in size. The CLL lymphocytes are fragile and are frequently disrupted during the preparation of smears, which produces characteristic *smudge cells*.
- *Anemia and Thrombocytopenia* are seen in later stages due to BM failure, or hypersplenism or autoimmune process.
- *BM examination: BMA* shows lymphocyte infiltration >30 % of all nucleated BM cells. *BM biopsy* reveals early interstitial and late diffuse pattern of involvement.
**Immunophenotype** shows pan-B-cell markers (CD19+ & CD22+) with CD5+ & CD23+, weak expression of surface membrane immunoglobulin with weak or negative FMC7 and CD79b.

**Karyotype.** The most common cytogenetic abnormalities are deletion of 13q14 which is associated with good prognosis. Trisomy 12, deletion at 11q23 and structural abnormalities of 17p involving the p53 gene have bad prognosis.

**Staging of CLL**
It is useful to stage patients at presentation both for prognosis and for deciding on therapy. The stage is determined by several variables such as peripheral lymphocyte count, BM lymphocyte percentage, presence or absence of lymphadenopathy, hepatosplenomegaly. The presence of anemia <10 gm/dL and/or thrombocytopenia <100,000/µL indicates advanced disease stage.

**Course & prognosis**
Many patients, in early stage, never need therapy. Survival ranges from 12 years for early stage to < 3 years for advanced stage. CLL may transform to:
- CLL/PL or frank prolymphocytic leukemia (PL) that is resistant to treatment
- Richter's transformation (Immunoblastic lymphoma, localized high grade NHL)

**PROLYMPHOCYTIC LEUKEMIA (PLL)**
The prolymphocyte is around twice the size of a CLL lymphocyte and has a larger central nucleolus. PLL typically presents with splenomegaly without lymphadenopathy and with a high and rapidly rising lymphocyte count. Diagnosis is made by the appearance of > 55% prolymphocytes in blood film. Response to treatment is poor.

**HAIRY CELL LEUKEMIA (HCL)**
HCL patients typically present with infections, anemia or splenomegaly. Lymphadenopathy is very uncommon. Pancytopenia is usual. The blood film reveals a variable number of unusually large lymphocytes with villous cytoplasmic projections. BM biopsy; shows a characteristic appearance of mild fibrosis and a loose diffuse cellular infiltrate.

**PLASMA CELL NEOPLASMS (PCNs)**
PCNs originate from a clone of B cells that differentiates into plasma cells and secretes a single complete and/or partial immunoglobulin (Ig). These disorders are also called monoclonal gammopathies, due to the presence of usually excessive amounts of serum Igs, referred to as an M-protein or paraprotein. However, the presence of an M-protein is not necessarily an indication of an overt B-cell malignancy as it is fairly common in otherwise normal elderly persons.

The plasma cell neoplasms can be divided into many variants:

1. **Multiple Myeloma (MM)**
MM is the most common of the malignant plasma cell dyscrasias. It is a clonal neoplastic proliferation characterized by plasma cell accumulation in the BM or plasmacytoma, the presence of monoclonal protein in the serum and/or urine and related tissue damage that is usually associated with multifocal lytic lesions throughout the skeletal system.

   The etiology of the disease is unknown. Dysregulation or increased expression of cyclin D is an early unifying event. IL 6 is a potent growth factor for myeloma cells and is often active by autocrine mechanism.

   Hyperploidy is present in about half of the tumors whereas non-hyperploid cases have a high incidence of translocations involving the Ig heavy-chain gene (IGH) on chromosome 14.
Monoallelic loss of 13q is frequent in both categories and all these genetic abnormalities are also seen in MGUS. The characteristic immunophenotype is CD38\textsuperscript{high}, CD138\textsuperscript{high} and CD45\textsuperscript{low}.

The most common M component is IgG (60%), followed by IgA (20% to 25%). In the remaining 15% to 20% of cases, the plasma cells produce only κ or λ light chains. Because of their low molecular weight, the free light chains are rapidly excreted in the urine, where they are termed \textit{Bence-Jones proteins} (BJP). Even more commonly, malignant plasma cells produce both serum M-proteins & BJP in urine. About (3%) of patients have \textit{non-secretory myeloma cells} with no paraprotein in serum or urine.

\textit{Gross pathologic features of MM}

- Multiple myeloma presents most often as multifocal destructive bone lesions throughout the skeletal system. The affected bones are; vertebral column (65%), ribs (45%), skull (40%), pelvis (30%), and femur (25%). There are often pathological fractures and vertebral collapse.
- These focal lesions generally begin in the medullary cavity, erode the cancellous bone, and progressively destroy the cortical bone. The osteolytic lesions are caused by osteoclast activation resulting from high serum level of RANKL (receptor activator of nuclear factor-κB ligand), produced by plasma cells and BM stroma, which binds to RANK receptors on the osteoclast surface, which promotes the differentiation and activation of osteoclasts.

\textit{Microscopic features of MM}

- BM examination reveals an increased number of clonal plasma cells.
- The neoplastic cells can resemble normal mature plasma cells, but they more often show abnormal features, such as prominent nucleoli or abnormal cytoplasmic inclusions containing immunoglobulin.
- Plasma cell infiltrations of soft tissues (plasmacytoma) can be encountered in the spleen, liver, skin, kidneys, lungs, and lymph nodes early or with disease progression.
- Terminally, a leukemic picture may emerge (plasma cell leukemia or acute leukemia).
- \textit{Myeloma nephrosis} refers to renal involvement; it is a distinctive feature of MM.
  a. Proteinaceous casts are prominent in the distal convoluted tubules and collecting ducts. Most of these casts are made up of BJPs.
  b. Some casts have tinctorial properties of amyloid.
  c. Multinucleate giant cells created by the fusion of infiltrating macrophages usually surround the casts.
  d. Very often the epithelial cells lining the cast-filled tubules become necrotic or atrophic because of the toxic actions of the Bence-Jones proteins.
  e. Pyelonephritis can also occur as a result of the increased susceptibility to bacterial infections. Less commonly, interstitial infiltrates of abnormal plasma cells are seen.
- \textit{Metastatic calcification} stemming from bone resorption and hypercalcemia may be encountered.

\textit{The clinical manifestations of the plasma cell dyscrasias result from:}

- A. The destructive effect of the infiltrating neoplastic cells in various tissues and,
- B. The abnormal immunoglobulins secreted by the tumors.

1. \textit{Bone pain especially backache.}
2. \textit{Features of Anemia} results from marrow replacement as well as from inhibition of hematopoiesis by tumor cells.
3. \textit{Recurrent bacterial infections} are serious clinical problems. They result from severe suppression of normal Ig secretion, abnormal cell-mediated immunity and neutropenia.
4. **Features of renal failure and/or hypercalcemia**: Renal insufficiency occurs in 50% of patients as a result of proteinaceous deposit from heavy BJ proteinuria, hypercalcemia, uric acid, amyloid and pyelonephritis.

5. **Bleeding tendency**: Myeloma protein may interfere with platelet function and coagulation factors; thrombocytopenia occurs in advanced disease.

6. **Amyloidosis** develops in 5% to 10% of patients.

7. **Hyperviscosity syndrome** may occur in 2% of MM cases due to excessive production and aggregation of myeloma proteins. Purpura, hemorrhages, visual failure, CNS symptoms, neuropathies and heart failure may be present but these are much more characteristic of LPL.

**Diagnosis of Multiple myeloma:**

Diagnosis can be made with reasonable certainty if two of the following three criteria are met:

1. BM clonal plasma cells >10% of all nucleated marrow cells.
2. A paraprotein in serum and/or urine and,
3. Complications related to organ or tissue infiltrations such as bone disease (osteolytic bone lesions or osteoporosis), renal impairment, anemia, hypercalcemia, hyperviscosity, amyloidosis or recurrent infection.

*Note: If the serum paraprotein > 30g/L and/or BM clonal plasma cells are >10% but there is no evidence of tissue damage the disease is termed asymptomatic or smouldering myeloma.*

- Electrophoresis of the serum and urine is an important diagnostic tool. In 97% of cases a monoclonal spike of complete Ig or Ig light chain can be detected in the serum and/or urine.
- Anemia is usually normochromic normocytic or macrocytic. Rouleaux formation is marked. Neutropenia and thrombocytopenia occur in advanced disease.
- Few plasma cells may appear in the blood film in 15% of cases.
- High ESR and C-reactive protein.
- Radiological; the diagnosis is strongly suspected when the characteristic focal, osteolytic punched-out lesions in the bone are present (in 60% of cases) especially when located in the vertebrae or calvarium. Generalized osteoporosis (20%) can also be seen and no bone lesions in (20%). In addition, pathological fractures or vertebral collapse are common.
- S. Calcium increased in 45% of patients. Typically, the S. alkaline phosphatase is normal (except following pathological fractures).
- S. Creatinine is raised in 20% of cases.
- S. Albumin decreases with advanced disease.
- S. β₂-microglobulin is often raised (level < 4 mg/L imply a relatively good prognosis).

**Prognosis**

Multiple myeloma is a progressive disease. Patients with serum β₂-microglobulin > 5.5 mg/L and serum albumin level < 35 g/L have poor survival as do those with frequent circulating plasma cells. The median survival with non-intensive chemotherapy is 3 - 4 years.

2. **Monoclonal Gammopathy of Undetermined Significance (MGUS)**

A serum paraprotein may sometimes be detected in asymptomatic individuals without any evidence of MM or other underlying disease and is termed MGUS. It has a high prevalence (3.2% and 5.8% in individuals over 50 and 70 years of age, respectively), making this the most common plasma cell dyscrasia. There is no related organ damage or tissue impairment
(such as bone lesions or renal impairment). The proportion of plasma cells in the BM is normal (<4%) or only slightly increased (<10%). S. paraproteins are <30 g/L, and there is no BJP in urine. Also there should be no evidence of other B-lineage lymphoproliferative disorder. However, patients with MGUS develop a well-defined plasma cell neoplasm (e.g MM) at a rate of 1% per year. Moreover, MGUS often show the same chromosomal translocations that are found in full-blown MM. Thus, the diagnosis of MGUS should be made with caution and only after careful exclusion of all other forms of monoclonal gammopathies, particularly multiple myeloma.

3. **Localized Plasmacytomas** (solitary plasmacytoma)
   These are isolated plasma cell tumors involving the skeleton or the soft tissues. *Extraosseous* lesions occur mainly in mucosa of the upper respiratory tract, GIT or the skin. The associated paraprotein disappears following radiotherapy to the primary lesion. Most of those with solitary skeletal plasmacytomas develop full-blown MM over a period of 5 to 10 years.

4. **Plasma cell leukemia** (PCL)
   PCL occurs either as a late complication of MM or as a primary disease characterized by the presence of ≥20% plasma cells in the peripheral blood. The outlook is poor.
**Bleeding Disorders: (Hemorrhagic Diatheses)**

Excessive bleeding can result from:
1. Increased fragility of vessels.
2. Platelet deficiency or dysfunction.
3. Derangement of coagulation.
4. Combinations of these.

**Tests used to evaluate different aspects of hemostasis are the following:**

- **Bleeding time:** This measures the time taken for a standardized skin puncture to stop bleeding and provides an in vivo assessment of platelet response to limited vascular injury. The reference range depends on the actual method employed and varies from 2 to 9 minutes. Prolongation generally indicates a defect in platelet numbers or function.
- **Platelet counts:** These are obtained on anticoagulated blood using an electronic particle counter. The reference range is 150 to 400 × 10^3/µL.
- **Prothrombin time (PT):** This assay tests the extrinsic and common coagulation pathways. A prolonged PT can result from deficiency or dysfunction of: factor VII, factors X, V, prothrombin, or fibrinogen.
- **Partial thromboplastin time (PTT):** This assay tests the intrinsic and common clotting pathways. Prolongation of the PTT can be due to deficiency or dysfunction of: factors VIII, IX, XI, or XII, factors X, V, prothrombin, or fibrinogen.

**Bleeding Disorders Caused By Vessel Wall Abnormalities:**

Disorders within this category, sometimes called nonthrombocytopenic purpuras, are relatively common but do not usually cause serious bleeding problems. Most often, they induce small hemorrhages (petechiae and purpura) in the skin or mucous membranes, particularly the gingivae. The platelet count, bleeding time, and results of the coagulation tests (PT, PTT) are usually normal.

The varied clinical conditions in which hemorrhages can be related to abnormalities in the vessel wall include the following:

- Many infections induce petechial and purpuric hemorrhages, but especially implicated are meningococcemia, other forms of septicemia, infective endocarditis, and several of the rickettsioses. The involved mechanism is presumably microbial damage to the microvasculature (vasculitis) or disseminated intravascular coagulation (DIC).
- Drug reactions sometimes induce cutaneous petechiae and purpura without causing thrombocytopenia. In many instances, the vascular injury is mediated by drug-induced antibodies and deposition of immune complexes in the vessel walls, leading to hypersensitivity (leukocytoclastic) vasculitis.
- Scurvy, Cushing syndrome and Ehlers-Danlos syndrome are associated with microvascular bleeding resulting from impaired formation of collagens needed for support of vessel walls.
Henoch-Schönlein purpura is a systemic hypersensitivity disease of unknown cause characterized by a purpuric rash, colicky abdominal pain (presumably due to focal hemorrhages into the gastrointestinal tract), polyarthralgia, and acute glomerulonephritis. All these changes result from the deposition of circulating immune complexes within vessels throughout the body and within the glomerular mesangial regions. It is an Ig A-mediated vasculitis.

Hereditary hemorrhagic telangiectasia is an autosomal dominant disorder characterized by dilated, tortuous blood vessels with thin walls that bleed readily.

Bleeding Related to Reduced Platelet Number:
Thrombocytopenia: Reduction in platelet number constitutes an important cause of generalized bleeding. A count below 100,000 platelets/L is generally considered to constitute thrombocytopenia. However, spontaneous bleeding does not become evident until platelet counts fall below 20,000 platelets/L. Platelet counts in the range of 20,000 to 50,000 platelets/L can aggravate post-traumatic bleeding. Bleeding resulting from thrombocytopenia is associated with a normal PT and PTT. Spontaneous bleeding associated with thrombocytopenia most often involves small vessels. Common sites for such hemorrhages are the skin and the mucous membranes of the gastrointestinal and genitourinary tracts. The many causes of thrombocytopenia can be classified into the four major categories:

- Decreased production of platelets: This can accompany generalized diseases of bone marrow such as aplastic anemia and leukemias or result from diseases that affect the megakaryocytes somewhat selectively. In vitamin B12 or folic acid deficiency, there is poor development and accelerated destruction of megakaryocytes within the bone marrow (ineffective megakaryopoiesis) because DNA synthesis is impaired.
- Decreased platelet survival: This important cause of thrombocytopenia can have an immunologic or nonimmunologic etiology.
  - In the immune conditions: platelet destruction is caused by circulating antiplatelet antibodies or, less often, immune complexes. The antiplatelet antibodies can be directed against a self-antigen on the platelets (autoantibodies) or against platelet antigens that differ among different individuals (alloantibodies). Alloimmune thrombocytopenias arise when an individual is exposed to platelets of another person, as may occur after blood transfusion or during pregnancy. In the latter case, neonatal or even fetal thrombocytopenia occurs by a mechanism analogous to erythroblastosis fetalis.
  - Nonimmunologic destruction of platelets: may be caused by:
    - Mechanical injury: in a manner analogous to red cell destruction in microangiopathic hemolytic anemia. The underlying conditions are also similar, including prosthetic heart valves and diffuse narrowing of the microvessels (e.g., malignant hypertension).
    - Sequestration: Thrombocytopenia, usually moderate in severity, may develop in any patient with marked splenomegaly, a condition sometimes referred to as hypersplenism.
The spleen normally sequesters 30% to 40% of the body's platelets, which remain in equilibrium with the circulating pool. When necessary, hypersplenic thrombocytopenia can be ameliorated by splenectomy.

- Dilutional: Massive transfusions can produce a dilutional thrombocytopenia. Blood stored for longer than 24 hours contains virtually no viable platelets; thus, plasma volume and red cell mass are reconstituted by transfusion, but the number of circulating platelets is relatively reduced.

**Immune Thrombocytopenic Purpura (ITP):** ITP can occur in:
- The setting of a variety of conditions and exposures (secondary ITP) or
- In the absence of any known risk factors (primary or idiopathic ITP).

There are two clinical subtypes of primary ITP: acute and chronic; both are autoimmune disorders in which platelet destruction results from the formation of antiplatelet autoantibodies.

**Chronic ITP:**
Pathogenesis: Chronic ITP is caused by the formation of autoantibodies against platelet membrane glycoproteins. Antibodies reactive with these membrane glycoproteins can be demonstrated in the plasma as well as bound to the platelet surface (platelet-associated immunoglobulins) in approximately 80% of patients. In the overwhelming majority of cases, the antiplatelet antibodies are of the IgG class. The mechanism of platelet destruction is as follows: Opsonized platelets are rendered susceptible to phagocytosis by the cells of the mononuclear phagocyte system especially of the spleen. About 75% to 80% of patients are remarkably improved after splenectomy, indicating that the spleen is the major site of removal of sensitized platelets. Since it is also an important site of autoantibody synthesis, the beneficial effects of splenectomy may in part derive from removal of the source of autoantibodies.

Acute ITP: Like chronic ITP, this condition is caused by antiplatelet autoantibodies, but its clinical features and course are distinct. Acute ITP is a disease of childhood occurring with equal frequency in both sexes.

Drug-induced immune thrombocytopenia: An immunological mechanism has been demonstrated as the cause of many drug-induced thrombocytopenias. Quinine, quinidine and heparin are particularly common causes. An antibody-drug-protein complex is deposited on the platelet surface. If complement is attached and the sequence goes to completion, the platelet may be lysed directly. Otherwise, it is removed by reticuloendothelial cells because of opsonization with immunoglobulin and / or the C3 component of complement. The platelet count is often less than 10 x 10^9/L, and the bone
marrow shows normal or increased numbers of megakaryocytes. Drug dependent antibodies against platelets may be demonstrated in the sera of some patients.

**Bleeding Disorders Related To Defective Platelet Functions:** Qualitative defects of platelet function can be congenital or acquired. Several congenital disorders characterized by prolonged bleeding time and normal platelet count have been described. Congenital disorders of platelet function can be classified into three groups on the basis of the specific functional abnormality:
1. Defects of adhesion.
2. Defects of aggregation.
3. Disorders of platelet secretion (release reaction).
Acquired defects of platelet function:
- Ingestion of aspirin and other nonsteroidal anti-inflammatory drugs which significantly prolongs the bleeding time.
- Aspirin: Is a potent, irreversible inhibitor of the enzyme cyclooxygenase.
- Uremia: Several abnormalities of platelet function are found.

**Hemorrhagic Diatheses Related To Abnormalities In Clotting Factors:** A deficiency of every clotting factor has been reported to be the cause of a bleeding disorder, with the exception of factor XII deficiency, which does not cause bleeding. The bleeding in factor deficiencies differs from platelet deficiencies in that spontaneous petechiae or purpura are uncommon. Rather, the bleeding is manifested by large post-traumatic ecchymoses or hematomas, or prolonged bleeding after a laceration or any form of surgical procedure. Bleeding into the gastrointestinal and urinary tracts, and particularly into weight-bearing joints, is common. Hereditary deficiencies have been identified for each of the clotting factors. Deficiencies of factor VIII (hemophilia A) and of factor IX (Christmas disease, or hemophilia B) are transmitted as sex-linked recessive disorders. Most others follow autosomal patterns of transmission. These hereditary disorders typically involve a single clotting factor.

**Deficiencies of Factor VIII-vWF Complex:** Hemophilia A and von Willebrand disease, two of the most common inherited disorders of bleeding, are caused by qualitative or quantitative defects involving the factor VIII-vWF complex. Plasma factor VIII-vWF is a complex made up of two separate proteins (factor VIII and vWF). Factor VIII; is an intrinsic pathway component required for activation of factor X. Deficiency of factor VIII gives rise to hemophilia A. Circulating factor VIII is noncovalently associated with very large vWF multimers. The most important function of vWF in vivo is to promote the adhesion of platelets to subendothelial matrix. The two components of the factor VIII-vWF complex are encoded by separate genes and synthesized in different cells. vWF is produced by endothelial cells and megakaryocytes and can be demonstrated in platelet α-
granules. Endothelial cells are the major source of subendothelial and plasma vWF. vWF gene is located on chromosome 12. Factor VIII is made in several tissues; sinusoidal endothelial cells and Kupffer cells in the liver and glomerular and tubular epithelial cells in the kidney appear to be particularly important sites of synthesis. Factor VIII gene is located on X chromosome.

**Von Willebrand Disease:** With an estimated frequency of 1%, von Willebrand disease is believed to be one of the most common inherited disorders of bleeding in humans. Clinically, it is characterized by spontaneous bleeding from mucous membranes, excessive bleeding from wounds, menorrhagia. In this disorder there is either a reduced level or abnormal function of VWF resulting from a point mutation or major deletion. Patients with von Willebrand disease have defects in platelet function despite a normal platelet count.

**Lab findings:**
Patients with von Willebrand disease typically have:
• A prolonged bleeding time.
• A normal platelet count.
• The plasma level of active vWF is reduced.
(Because vWF stabilizes factor VIII by binding to it, a deficiency of vWF gives rise to a secondary decrease in factor VIII levels); this may be reflected by a prolongation of the PTT in von Willebrand disease types 1 and 3. In most cases, it is transmitted as an autosomal dominant disorder, but several rare autosomal recessive variants have been identified. Because a severe deficiency of vWF has a marked affect on the stability of factor VIII, some of the bleeding characteristics resemble those seen in hemophilia.

**Hemophilia A (Factor VIII Deficiency):**
Hemophilia A is the most common hereditary disease associated with serious bleeding. It is caused by a reduction in the amount or activity of factor VIII. Hemophilia A is inherited as an X-linked recessive trait, and thus occurs in males and in homozygous females. However, excessive bleeding has been described in heterozygous females, presumably due to extremely unfavorable lyonization (inactivation of the normal X chromosome in most of the cells). Approximately 30% of patients have no family history; their disease is presumably caused by new mutations. Hemophilia A exhibits a wide range of clinical severity that correlates well with the level of factor VIII activity.
• Those with less than 1% of normal activity develop severe disease.
• Levels between 2% and 5% of normal are associated with moderate disease.
• Patients with 6% to 50% of activity develop mild disease.
The variable degrees of factor VIII deficiency are largely explained by heterogeneity in the causative mutations. Several genetic lesions (deletions, nonsense mutations that create stop codons, splicing errors) have been documented.
Lab findings:
Patients with hemophilia A typically have:
• A normal bleeding time.
• A normal platelet count, and a normal PT.
• A prolonged PTT.
(These tests point to an abnormality of the intrinsic coagulation pathway).
Factor VIII-specific assays are required for diagnosis.

Hemophilia B (Christmas Disease, Factor IX Deficiency):
Severe factor IX deficiency produces a disorder clinically indistinguishable from factor VIII deficiency (hemophilia A). This should not be surprising, given that factor VIII and IX function together to activate factor X. Wide spectrums of mutations involving the factor IX gene are found in hemophilia B. Like hemophilia A, it is inherited as an X-linked recessive trait and shows variable clinical severity. In about 14% of these patients, factor IX is present but nonfunctional.

Lab findings:
Patients with hemophilia B typically have:
• A normal bleeding time.
• A normal platelet count, and a normal PT.
• A prolonged PTT.
Factor IX-specific assays are required for diagnosis.

Disseminated Intravascular Coagulation (DIC):
DIC is an acute, subacute, or chronic thrombohemorrhagic disorder occurring as a secondary complication in a variety of diseases.
• It is characterized by activation of the coagulation sequence that leads to the formation of microthrombi throughout the microcirculation of the body, often in a quixotically uneven distribution.
• Sometimes the coagulopathy is localized to a specific organ or tissue.
• As a consequence of the thrombotic diathesis, there is consumption of platelets, fibrin, and coagulation factors and, secondarily, activation of fibrinolytic mechanisms. Thus, DIC can present with signs and symptoms relating to:
  ▪ Tissue hypoxia and infarction caused by the myriad microthrombi or
  ▪ A hemorrhagic disorder related to depletion of the elements required for hemostasis (hence, the term "consumption coagulopathy" is sometimes used to describe DIC). Activation of the fibrinolytic mechanism aggravates the hemorrhagic diathesis.
Etiology and Pathogenesis: At the outset, it must be emphasized that DIC is not a primary disease. It is a coagulopathy that occurs in the course of a variety of clinical conditions. Two major mechanisms trigger DIC:
1. Release of tissue factor or thromboplastic substances into the circulation: Tissue thromboplastic substances can be derived from a variety of sources, such as the placenta in obstetric complications and the granules of leukemic cells in acute promyelocytic leukemia. Mucus released from certain adenocarcinomas can also act as a thromboplastic substance by directly activating factor X, independent of factor VII. In gram-negative sepsis (an important cause of DIC), bacterial endotoxins cause activated monocytes to release interleukin-1 and TNF, both of which increase the expression of tissue factor on endothelial cell membranes and simultaneously decrease the expression of thrombomodulin. The net result is a shift in balance toward procoagulation.

2. Widespread injury to the endothelial cells: The other major trigger, can initiate DIC by causing release of tissue factor, promoting platelet aggregation, and activating the intrinsic coagulation pathway. TNF is an extremely important mediator of endothelial cell inflammation and injury in septic shock. Even subtle endothelial injury can unleash procoagulant activity by enhancing membrane expression of tissue factor. Widespread endothelial injury may be produced by deposition of antigen-antibody complexes (e.g., systemic lupus erythematosus), temperature extremes (e.g., heat stroke, burns), or microorganisms (e.g., meningococci, rickettsiae).

The initiating factors in these conditions are often multiple and interrelated. The consequences of DIC are twofold:

- There is widespread deposition of fibrin within the microcirculation. This can lead to:
  - Ischemia of the more severely affected or more vulnerable organs
  - A hemolytic anemia resulting from fragmentation of red cells as they squeeze through the narrowed microvasculature (microangiopathic hemolytic anemia).
  - A hemorrhagic diathesis can dominate the clinical picture. This results from consumption of platelets and clotting factors as well as activation of plasminogen. Plasmin can not only cleave fibrin, but also digest factors V and VIII, thereby reducing their concentration further.

Morphology: In general, thrombi are found in the following sites in decreasing order of frequency: brain, heart, lungs, kidneys, adrenals, spleen, and liver.

However, no tissue is spared, and thrombi are occasionally found in only one or several organs without affecting others.

**Acquired disorders** are usually characterized by multiple clotting abnormalities.

**Vitamin K deficiency:** Results in impaired synthesis of factors II, VII, IX, and X and protein C.

**Severe parenchymal liver disease:** Can be associated with a hemorrhagic diathesis.

**Disseminated intravascular coagulation:** Produces a deficiency of multiple coagulation factors.
HEMATOPATHOLOGY

TRANSFUSION MEDICINE

Blood transfusion refers to the 'Safe' transfer of blood or blood components from a donor to a recipient.

PRINCIPLES
- Blood donation should always be voluntary.
- Never give transfusion unnecessarily.
- Blood transfusion should follow components policy.

BLOOD DONATION
- Donor must be fit & healthy.
- It should not harm the donor.
- It should not transmit any disease to the recipient.

**Before blood donation the donor should be subjected to:**
1. Detailed Medical history (Questionnaire Form)
2. Limited physical examination

**Questionnaire form:**

1. Name of the donor
2. Sex
3. Age 18-65 year
4. Weight > 50 Kg (vasovagal reactions become more common in those who weigh < 50 kg, as the standard donation represents a greater proportion of their total blood volume).
5. Occupation **Exclusion of any donor returning to occupations such as** fire fighter or driving bus, plane or train, heavy machine or crane operator, scaffolding, , etc. because delayed faint would be dangerous
6. Last donation Not less than 2 months
7. Frequency of donation 2-3 times/y (Max 3 times/yr for females and 4times/yr for males
8. History of blood transfusion defer 6 months
9. Major surgery defer 6 months
10. History of heart disease, active pulmonary disease (active T.B), diabetics, hypertension, hyperthyroidism. Those with one of the above diseases are generally deferred from donation.
11. History of blood diseases such as leukemia, lymphoma, thalassaemia major, sickle cell anemia and polycythemia should be deferred from donation.

12. History of abnormal bleeding tendency should also be deferred.

13. History of epilepsy is generally a cause of deferral.

14. History of infectious diseases

15. AIDS patients, AIDS contacts, homosexuals, drug abusers, those with multiple partners, hemophiliacs receiving products of human origin all should be indefinitely deferred.

16. Hepatitis: history of jaundice or viral hepatitis A: deferred one year. Hepatitis B (HBs Ag +) or C is deferred permanently.

17. Malaria: those infected are not accepted as blood donors.

18. Brucellosis: deferred for 2 years from last febrile episode.

19. EBV infected patients are deferred for 2 years.

20. Syphilis: patients with this disease are considered as permanent deferral.

21. Drugs: patients on certain drugs (anticoagulants, antihypertensive, insulin) are not accepted.

22. Pregnancy: not allowed. Accepted 3-6 ms postpartum to protect the donor from iron deficiency.

23. Donor consent: written consent.

Physical Examination

This should be simple & brief and include

1. General appearance
2. Temp: Not more than 37°C
3. Pulse: 60-100 beats/ min
5. Weight: At least 50 Kg
6. Hb level: more than 13.5 g/dl for males & 12.5 g/dl for females

Anticoagulants:

ACD (Acid citrate dextrose)
Shelf life of blood 21 days (Now used only in automated plasmapheresis).

CPD (Citrate phosphate dextrose)
Shelf life of blood 28 days

CPD-A (Plus Adenine)
Shelf life of blood 35 days (used now)
Blood donation is taken by an aseptic technique into plastic bags designed to hold 450 ml ± 45 ml of blood, mixed with 63 ml of anticoagulant. The ratio of anticoagulant to blood must be maintained at the optimal level of 1:7. The citrate anticoagulates the blood by combining with the blood calcium.

**Standard routine cross matching is done by:**

- **Saline tube**: Mixing donor cells & recipient serum, leave the tube at room temp (18-25°C)
- **Albumin tube**: by adding albumin to the mixture of the donor cells & recipient serum at 37°C to detect warm- reacting antibodies
- **Indirect antiglobulin test**: at 37°C to detect antibodies in the recipient serum that coat or cause sensitization of the donor red cells

**Mandatory tests on blood units:**
1. ABO &Rh grouping
2. Test for HIV Ab
3. Test for HBs Ag
4. Test for HCV Ab
5. Test for syphilis
6. Screening for atypical antibodies.

**BLOOD TRANSFUSION**
Before giving blood to the patient we should do **Compatibility testing, which includes:**
1. ABO & Rh typing of the donor and the recipient blood
2. Screening of the donor & the recipient sera for unexpected antibodies
3. Cross matching the donor & the recipient blood by cross matching the donor cells & the recipient serum.

**Objectives of cross matching are:**
1. Assurance of the ABO compatibility
2. Recognition of clinically significant antibodies
The limits for infusions:

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<thead>
<tr>
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<th>Start infusion</th>
<th>Complete infusion</th>
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<tbody>
<tr>
<td>Whole blood or red cells</td>
<td>Within 30 minutes of removing pack from refrigerator</td>
<td>Within 4 hours* (or less in high ambient temperature)</td>
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<tr>
<td>Platelet concentrates</td>
<td>Immediately</td>
<td>Within 20 minutes</td>
</tr>
<tr>
<td>Fresh frozen plasma and cryoprecipitate</td>
<td>As soon as possible</td>
<td>Within 20 minutes</td>
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*If a unit is not completed within 4 hours:
Discontinue its use and
Dispose of the remainder through the clinical waste system.

The Whole blood or Packed red cells should be kept refrigerated at 2-6 °C
The upper limit of 6°C is essential to minimize the growth of any bacterial contamination in the unit of blood.
The lower limit of 2°C is essential to prevent hemolysis, which can cause fatal bleeding problems or renal failure.

Complications of blood transfusion
Incidence of transfusion reaction is about 2-5%. It is mostly of mild degree.
Most of the cases are due to (clerical or administrative error).
Laboratory error, nursing service, anesthesia service, and clinical staff errors.

Fatal complications are uncommon (1 in 100,000 to 1 in 500,000 patients transfused), mainly due to improper patient identification (the major cause of transfusion deaths).
Complications can be divided broadly into:
1. Immunological complications
2. Nonimmunological complications

IMMUNOLOGICAL COMPLICATIONS:

1. Sensitization to red cells antigens
Because the ABO & Rh D antigens are the only Ags matched between donor and recipient, there is a possibility of sensitization to other red cells antigens.

   In clinical practice this sensitization could lead to:
   A. Hemolytic disease of the newborn if the recipient is a female
   B. Difficulties in compatibility testing if the recipient required further transfusion
   C. Hemolytic transfusion reaction

2. Hemolytic transfusion reaction
This reaction is caused by premature destruction, almost always of the donor cells by antibodies present in the recipient plasma.

The hemolytic transfusion reaction could be: Immediate or Delayed
Immediate Transfusion reaction:
- This is the most dangerous type
- Usually caused by ABO incompatibility

The antibodies are IgM in type that bind to the red cells and cause complement activation leading to **intravascular lysis** of the red cells with production of the anaphylatoxins the C3a & C5a liberated during complement activation. The C3a & C5a will cause smooth muscle contraction, platelets aggregation, increased capillary permeability, release of vasoactive amines and hydrolases from mast cells and granulocytes

**Sign & Symptoms**
- Occur within minutes to I hour from the start of transfusion
- Heat in the vein
- Throbbing headache
- Flushing of the face
- Chest tightness
- Nausea
- Lumber pain
- Hypotension & tachycardia
- DIC, hemoglobinuria, acute renal failure, collapse & death in severe cases.

Less commonly the haemolysis is extra-vascular caused by removal of C3b & IgG coated red cells by the macrophages in the liver and spleen. Symptoms are usually less rapid in onset occur usually after 1 hour with fever, jaundice and unexplained decrease in Hb. Renal failure is rare.

**Management of Hemolytic Transfusion Reaction**
- Stop transfusion immediately. Keep the IV line.
- Give physiological saline to maintain the blood pressure >100 mg Hg.
- Give diuretics to maintain urinary flow > 100 ml/hour
- Collect blood sample from site a way from the site of infusion in 3 tubes
  1. EDTA sample – for CBP.
  2. Citrated sample-for coagulation studies
  3. Clotted sample - for serological studies (Blood grouping, Coombs test, repeat antibodies screening for the recipient, repeat the compatibility testing)
- Collect the next urine sample and 24 hr urine post transfusion check for Hburia
- Check the label and the number on the blood unit and check the cross match form for any error.

**Tests to be done in the lab**
- Check the ABO & Rh group of the recipient and the donor samples again
- Examine the post transfusion sample for hemolysis & check the donor unit for hemolysis
- Do Coombs test on recipient post transfusion sample
- Repeat cross match with both pre- & post-transfusion samples
- Screen pre- & post-transfusion samples and donor plasma for antibodies
- Check the Hb
- Coagulation screening test for the possibility of DIC
• Bacteriological evaluation: inspect the donor unit hemolysis or clot. Blood from the giving set and the blood unit should be cultured
• Biochemical studies: test for hemoglobinemia and for bilirubin
• Check the urine for hemoglobinuria.

**Delayed Transfusion reaction**
This is manifested usually 7-10 days after transfusion and is caused by antibodies, which are present in low titer and are not detected at time of cross matching. So this reaction is neither predictable nor preventable. The antibodies are caused by sensitization due to previous pregnancy or transfusion.

**Signs and symptoms**: fever, jaundice and lowering of Hb.

3. **Febrile reaction due to WBC & platelets Antigens:**
   • Most common immunological reaction
   • Seen in patients having multiple blood transfusion or pregnancy
   • Caused by Ab to HLA Ags, WBC & platelets specific Ags (Usually WBC)
   • The onset of the reaction is delayed 30-90 min after start of transfusion
   • The main symptom is fever

**Management**
• Slow the transfusion
• Give antipyretic
• No need to terminate the transfusion
• If symptom recur in patients require repeated transfusions we should check the patient for WBC or Platelets Abs & if these are present we should use WBC depleted blood (by using WBC filter).

4. **Reaction to platelets Ag (Post-transfusion Purpura)**
   • Seen in women with history of multiple pregnancies or in those with history of multiple transfusion
   • Caused by Abs to platelets Ag (PI)
   • The reaction occurs 7-10 days after transfusion
   • The main feature is purpura due to thrombocytopenia (caused by destruction of the platelets by the Abs)
   • It is usually self limiting

5. **Reaction due to plasma protein antibodies**
   • Majority are due to Anti IgA antibodies
   • Main symptom is urticaria
   • Treatment is by antihistamine
   • Rarely more severe anaphylactic reaction occur which should be treated urgently with adrenaline and any next transfusion should be IgA deficient blood
Important notes:

- Severe reactions most commonly present during the first 15 minutes of a transfusion.
- All patients and, in particular, unconscious patients should be monitored during this period and for the first 15 minutes of each subsequent unit.

NON-IMMUNOLOGICAL COMPLICATIONS:

1. Reaction due to bacterial pyrogens or bacteria:
   Although rare complication, it has very high mortality rate characterized by sudden onset of high fever, shock and bleeding due to DIC. Blood may be contaminated by cold-growing organisms (pseudomonas or colon-aerogenes group). These microorganisms utilize citrate as the primary source of carbon, which leads to citrate depletion and hence clotting of blood. Visual inspection of the blood units may reveal clots and indicate the presence of contamination.
   The infusion of large number of gram-negative microorganisms results in a serious reaction i.e. endotoxic shock. The latter is accompanied by fever, marked hypotension, pain, vomiting and the development of profound shock. The reaction may start with shaking chills following a latent period of 30 minutes or more. As little as 10 ml of blood may contain sufficient microorganisms to produce the reaction.

Management
- Do direct examination and culture of the blood from the patient and the blood unit
- Give antibiotic IV

This complication could be prevented by
- Ensuring aseptic technique in the preparation of blood bags & anticoagulant
- Aseptic condition in blood donation
- Bags should not be opened for sampling and the unit should be transfused within 24 hr if any open method has been used
- Blood should be kept in accurately controlled refrigerator at 2-6 C
- Avoid leaving blood at room temp.
- Inspect all blood units for signs of contamination as clotting or haemolysis.

2. Circulatory overload
   Transfusion generally increases blood volume except in those who are actively bleeding. This increase in blood volume may be dangerous in the elderly with a compromised cardiovascular function, pregnancy and in those with severe anemia

Prevention
- Blood should be given slowly over 4 hr.
- Give diuretics at the start of transfusion-No more than 2 units should be given within 24 hr.
- Blood should be given during the daytime and the patient should be followed carefully
If signs and symptoms of overload and pulmonary edema occur
- Transfusion should be stopped
- Patient propped upright
- Give diuretics IV

3. **Thrombophlebitis**; this is a complication of indwelling venous cannulae and is not specifically related to blood transfusion.

4. **Air embolism**; this is now a rare complication of transfusion therapy due to the introduction of plastic bags, which provide a closed system. Only large volumes of air, and not the entry of a few bubbles, result in a clinically significant air embolism. Symptoms include pain, cough, and sudden onset of dyspnoea. The treatment includes clamping off the administrating tube.

5. **Hemosiderosis**; each unit of blood contain approximately 200 mg of iron. Repeated transfusions over many years, in the absence of blood loss, cause deposition of iron initially in the reticuloendothelial system. After 50 units in adults, and lesser amount in children, the liver, myocardium and endocrine glands are damaged. This is a major problem in thalassemia major and other severe chronic refractory anemias, and this could be prevented by giving chelating agent.

6. **Complications of massive transfusion**
These tend to occur in cases of replacement the total blood volume within 24 hr (For adult about 10 units/24 hr). **This could lead to:**
1. **Dilution of platelets**. As blood stored more than 48 hr has no functional platelets. Transfusion of 8-10 units of blood to an adult will lead to thrombocytopenia (low platelets). It follows that any patient receiving many blood units should be monitored through platelets count & judged on his clinical condition. Some give one platelets unit for every 4 blood units. Others give platelets transfusion if platelets count becomes less than 100,000 /cmm if there is bleeding or surgical intervention
2. **Dilution of coagulation factors**
This occurs if blood stored more than 14 days is given. Blood stored less than 14 days has adequate level of most of the coagulation factors except factor V & VIII, as they are the most labile factors.
3. **Metabolic changes**
   a. **Citrate toxicity**. This is not a problem except in a very rapid transfusion (unit every 5 minutes).
   b. **Hyperkalemia & hypocalcemia**. These are usually transient & rapidly corrected.

7. **Transmission of Infection**:
A. **Bacterial diseases**
   **Syphilis**
   - **The agent** is Treponema Pallidum
   - **Donor is infective** during the early spirochetemia phase i.e. before the development of the antibodies
   - **Blood products implicated**: fresh blood & components
   - **Viability in blood**: the bacteria are unlikely to survive more than 3 days at 4-6 C, so transmission of syphilis by blood is a rare complication.
• It is more likely to be transmitted by platelets concentrate because of its storage at room temp and its short shelf life.
• If blood is taken from seropositive donor (Showing positive serological tests for syphilis) this cause passive transmission of the antibodies to the recipient and the recipient become seropositive for 4-10 days

**Prevention**
• Mandatory screening of all donor units by VDRL or TPHA
• Exclusion of high-risk group.

**Brucellosis**
• **The agent** is Brucella abortus
• **Viability** in stored blood: months
• **Incubation period**: 6 days- 4 months
• Reports of transfusion related brucellosis: mainly in children, splenectomized or immunocompromized.

**Prevention**: defer infected patient for 2 years after cure

## B. Protozoal diseases

### Malaria
• **The gent** is Plasmodia Species (vivax, ovale, malariae, falciparum)
• **Viability**: viable in stored blood at 4°C at least 1 week; in case of P. falciparum up to 2 weeks
• **Blood product implicated**: products containing red cells
• **Incubation period**: vivax & falciparum 1 week to 1 month; malariae: months

**Prevention**
In endemic areas: prophylactic treatment of donors with chloroquine 48 h before donation or single dose of chloroquine to the recipient 24 before transfusion.

## C. Viral diseases

### AIDS (Acquired immune deficiency syndrome)
• **The agent** is Human immunodeficiency virus HIV type I & II
• **Blood product implicated**: whole blood (cellular & plasma blood components)
• Incubation period: mean incubation period is 4.5 yr

**Prevention**
1. Education through the media
2. Self-exclusion of high risk group
3. Screening all donors for HIV antibodies

### Hepatitis Viruses

**Post transfusion hepatitis** could be caused by the following viruses

1. *Hepatitis viruses (A, B, C)*
2. *Cytomegalovirus (CMV)*
3. *Epstien-Bar virus (EBV)*

**Prevention**
Tests to screen for Hepatitis B (HBsAg)
Tests to screen for HCV
Exclusion of high risk group