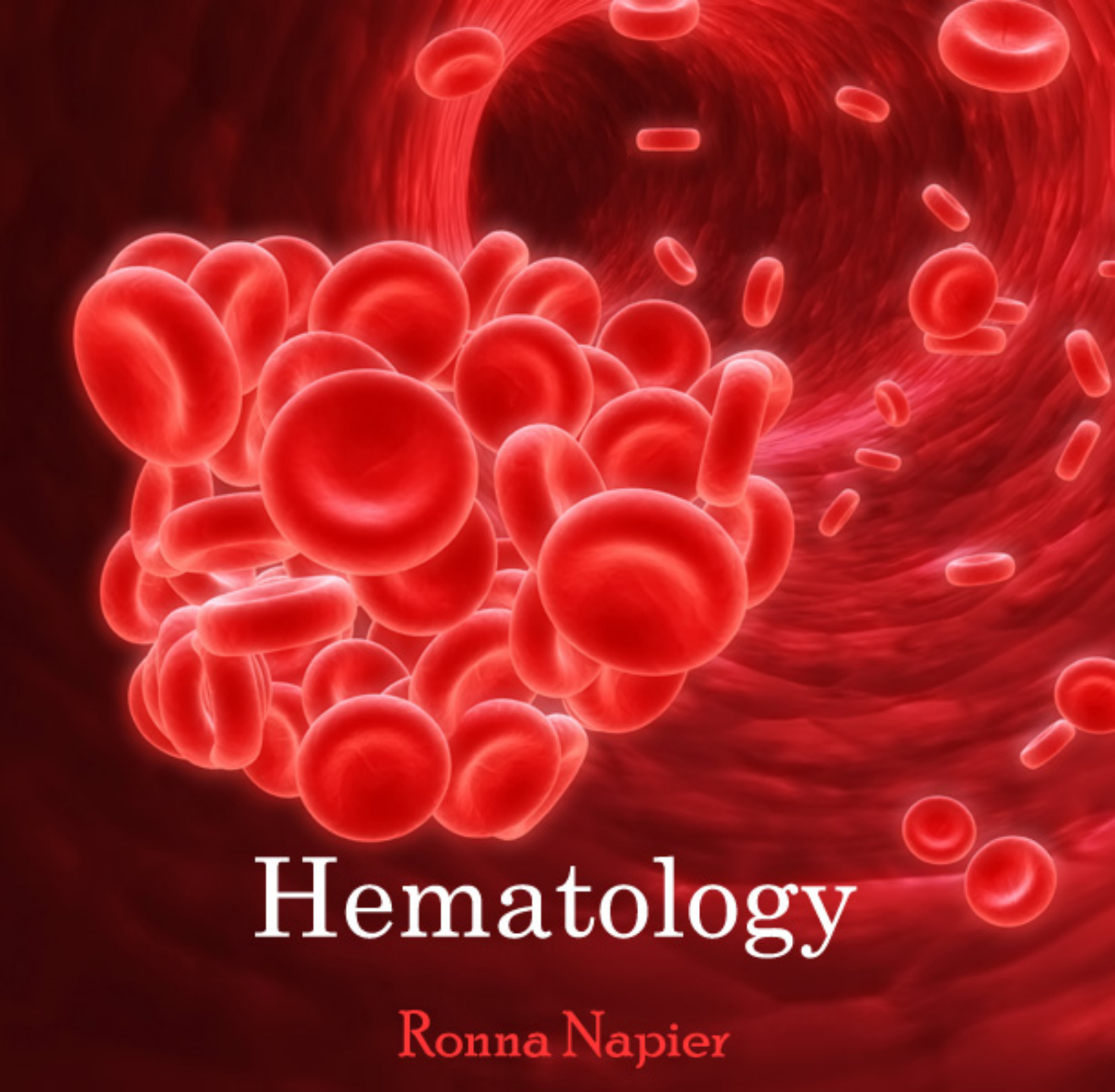

Hematology

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Hematologist	
Occupation	
Names	Medical Specialist
Type	Specialty
Activity sectors	Medicine
Description	
	Doctor of Medicine
Education required	Medical residency Fellowship (medicine)
Fields of employment	Hospitals, Clinics

Hematology, also spelled **haematology** (from the Greek *αἷμα haima* "blood" and -λογία), is the branch of internal medicine, physiology, pathology, clinical laboratory work, and pediatrics that is concerned with the study of blood, the blood-forming organs, and blood diseases. Hematology includes the study of etiology, diagnosis, treatment, prognosis, and prevention of blood diseases. The laboratory work that goes into the study of blood is frequently performed by a medical technologist. Hematologists physicians also very frequently do further study in oncology - the medical treatment of cancer.

Blood diseases affect the production of blood and its components, such as blood cells, hemoglobin, blood proteins, the mechanism of coagulation, etc.

Physicians specialized in hematology are known as *hematologists*. Their routine work mainly includes the care and treatment of patients with hematological diseases, although some may also work at the hematology laboratory viewing blood films and bone marrow slides under the microscope, interpreting various hematological test results. In some institutions, hematologists also manage the hematology laboratory. Physicians who work in hematology laboratories, and most commonly manage them, are pathologists specialized in the diagnosis of hematological diseases, referred to as **hematopathologists**. Hematologists and hematopathologists generally work in

conjunction to formulate a diagnosis and deliver the most appropriate therapy if needed. Hematology is a distinct subspecialty of internal medicine, separate from but overlapping with the subspecialty of medical oncology. Hematologists may specialize further or have special interests, for example in:

- treating bleeding disorders such as hemophilia and idiopathic thrombocytopenic purpura
- treating hematological malignacies such as lymphoma and leukemia
- treating hemoglobinopathies
- in the science of blood transfusion and the work of a blood bank
- in bone marrow and stem cell transplantation

only some blood disorders can be cured.

Hematology as basic medical science

- Blood
  - Venous blood
  - Venipuncture
  - Hematopoiesis
  - Blood tests
  - Cord blood
- Red blood cells
  - Erythropoiesis
  - Erythropoietin
  - Iron metabolism
  - Hemoglobin
  - Glycolysis
  - Pentose phosphate pathway
- Reticuloendothelial system
  - Bone marrow
  - Spleen
  - Liver
- Lymphatic system
- Blood transfusion
  - Blood plasma
  - Blood bank
  - Blood donors
  - Blood groups
- Hemostasis
  - Coagulation
  - Vitamin K
- Complement system
  - Immunoglobulins

abnormality of the hemoglobin molecule or of the rate of hemoglobin synthesis)

- **Anemias** (lack of red blood cells or hemoglobin)
- **Hematological malignancies**
- **Coagulopathies** (disorders of bleeding and coagulation)
- ...Sickle Cell Anemia

Treatments

Treatments include:

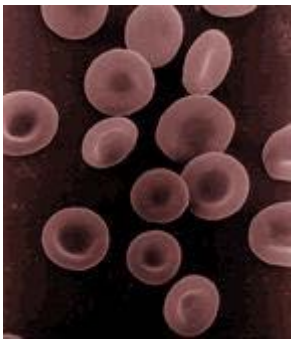
- Diet advice
- Oral medication - tablets or liquid medicines
- Anticoagulation therapy
- Intramuscular injections (for example, Vitamin B12 injections)
- Blood transfusion (for anemia)
- Venesection also known as therepeutic phlebotomy (for iron overload or polycythemia)
- Bone marrow transplant (for example, for leukemia)
- All kinds of anti-cancer chemotherapy
- Radiotherapy (for example, for cancer)

Alphabetical lists

- Hematologists
- Blood disorders
- Hematology topics

Chapter 1

Red Blood Cell



Human red blood cells (6-8μm)

Red blood cells (also referred to as **erythrocytes**) are the most common type of blood cell and the vertebrate organism's principal means of delivering oxygen (O_2) to the body tissues via the blood flow through the circulatory system. They take up oxygen in the lungs or gills and release it while squeezing through the body's capillaries.

These cells' cytoplasm is rich in hemoglobin, an iron-containing biomolecule that can bind oxygen and is responsible for the blood's red color.

In humans, mature red blood cells are flexible biconcave disks that lack a cell nucleus and most organelles. 2.4 million new erythrocytes are produced per second. The cells develop in the bone marrow and circulate for about 100–120 days in the body before their components are recycled by macrophages. Each circulation takes about 20 seconds. Approximately a quarter of the cells in the human body are red blood cells.

Red blood cells are also known as **RBCs**, **red blood corpuscles** (an archaic term), **haematids**, **erythroid cells** or **erythrocytes** (from Greek *erythros* for "red" and *kytos* for "hollow", with *cyte* translated as "cell" in modern usage). Packed red blood cells, which are made from whole blood with the plasma removed, are used in transfusion medicine.

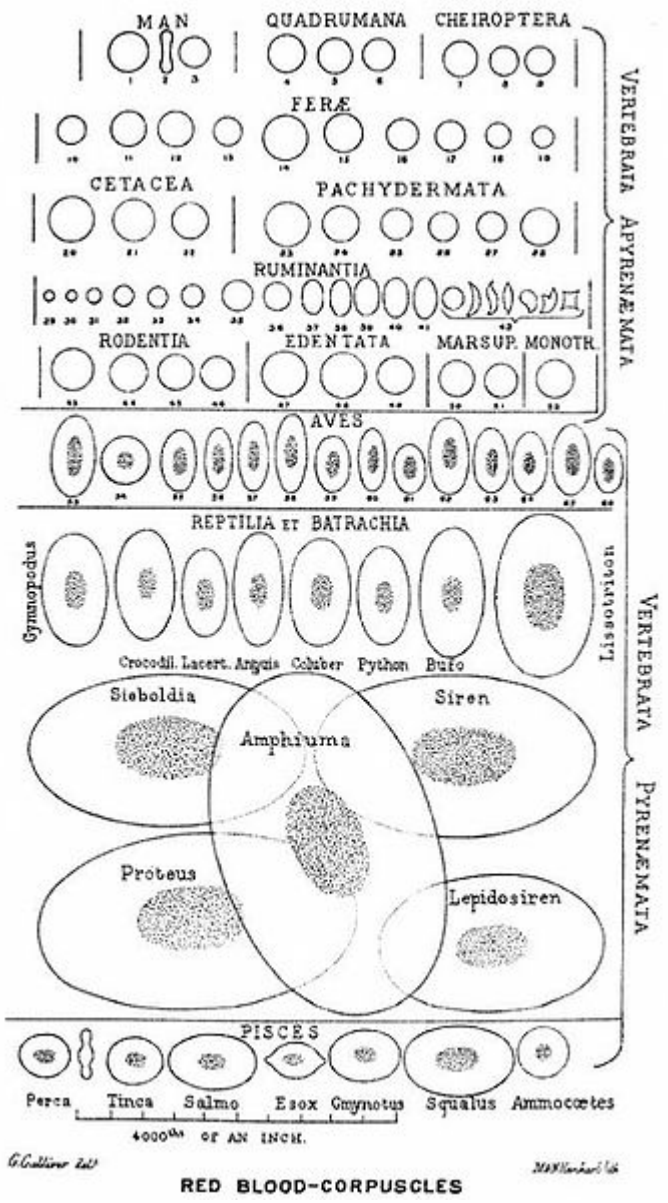
History

The first person to describe red blood cells was the young Dutch biologist Jan Swammerdam, who had used an early microscope in 1658 to study the blood of a frog. Unaware of this work, Anton van Leeuwenhoek provided another microscopic description in 1674, this time providing a more precise description of red blood cells, even approximating their size, "25,000 times smaller than a fine grain of sand".

In 1901 Karl Landsteiner published his discovery of the three main blood groups—A, B, and C (which he later renamed to O). Landsteiner described the regular patterns in which reactions occurred when serum was mixed with red blood cells, thus identifying compatible and conflicting combinations between these blood groups. A year later Alfred von Decastello and Adriano Sturli, two colleagues of Landsteiner, identified a fourth blood group—AB.

In 1959, by use of X-ray crystallography, Dr. Max Perutz was able to unravel the structure of hemoglobin, the red blood cell protein that carries oxygen.

Vertebrate erythrocytes



There is an immense size variation in vertebrate erythrocytes, as well as a correlation between cell and nucleus size. Mammalian erythrocytes, which do not contain nuclei, are considerably smaller than those of most other vertebrates.

Erythrocytes consist mainly of hemoglobin, a complex metalloprotein containing heme groups whose iron atoms temporarily bind to oxygen molecules (O_2) in the lungs or gills and release them throughout the body. Oxygen can easily diffuse through the red blood cell's cell membrane. Hemoglobin in the erythrocytes also carries some of the waste product carbon dioxide back from the tissues; most waste carbon dioxide, however, is transported back to the pulmonary capillaries of the lungs as bicarbonate (HCO_3^-)

dissolved in the blood plasma. Myoglobin, a compound related to hemoglobin, acts to store oxygen in muscle cells.

The color of erythrocytes is due to the heme group of hemoglobin. The blood plasma alone is straw-colored, but the red blood cells change color depending on the state of the hemoglobin: when combined with oxygen the resulting oxyhemoglobin is scarlet, and when oxygen has been released the resulting deoxyhemoglobin is of a dark red burgundy color, appearing bluish through the vessel wall and skin. Pulse oximetry takes advantage of this color change to directly measure the arterial blood oxygen saturation using colorimetric techniques.

The sequestration of oxygen carrying proteins inside specialized cells (rather than having them dissolved in body fluid) was an important step in the evolution of vertebrates as it allows for less viscous blood, higher concentrations of oxygen, and better diffusion of oxygen from the blood to the tissues. The size of erythrocytes varies widely among vertebrate species; erythrocyte width is on average about 25% larger than capillary diameter and it has been hypothesized that this improves the oxygen transfer from erythrocytes to tissues.

The only known vertebrates without erythrocytes are the crocodile icefishes (family Channichthyidae); they live in very oxygen rich cold water and transport oxygen freely dissolved in their blood. While they don't use hemoglobin anymore, remnants of hemoglobin genes can be found in their genome.

Nucleus

Erythrocytes in mammals are *anucleate* when mature, meaning that they lack a cell nucleus. In comparison, the erythrocytes of other vertebrates have nuclei; the only known exceptions are salamanders of the *Batrachoseps* genus and fish of the *Maurolicus* genus with closely related species.

Secondary functions

When erythrocytes undergo shear stress in constricted vessels, they release ATP which causes the vessel walls to relax and dilate so as to promote normal blood flow.

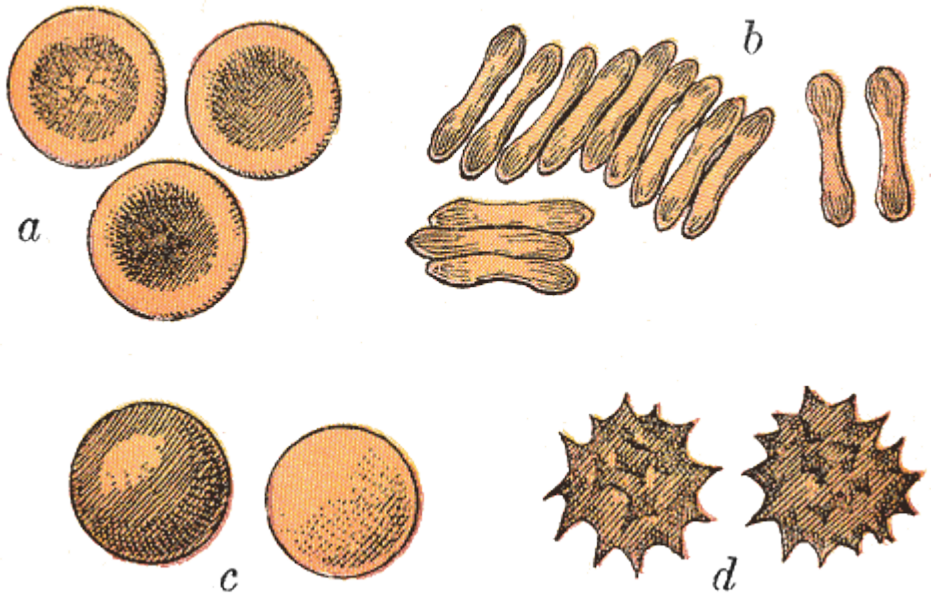
When their hemoglobin molecules are deoxygenated, erythrocytes release S-nitrosothiols which also acts to dilate vessels, thus directing more blood to areas of the body depleted of oxygen.

It has been recently demonstrated that erythrocytes can also synthesize nitric oxide enzymatically, using L-arginine as substrate, just like endothelial cells. Exposure of erythrocytes to physiological levels of shear stress activates nitric oxide synthase and export of nitric oxide, which may contribute to the regulation of vascular tonus.

Erythrocytes can also produce hydrogen sulfide, a signalling gas that acts to relax vessel walls. It is believed that the cardioprotective effects of garlic are due to erythrocytes converting its sulfur compounds into hydrogen sulfide.

Erythrocytes also play a part in the body's immune response: when lysed by pathogens such as bacteria, their hemoglobin releases free radicals which break down the pathogen's cell wall and membrane, killing it.

Mammalian erythrocytes



Typical mammalian erythrocytes: (a) seen from surface; (b) in profile, forming rouleaux; (c) rendered spherical by water; (d) rendered crenate by salt. (c) and (d) do not normally occur in the body.

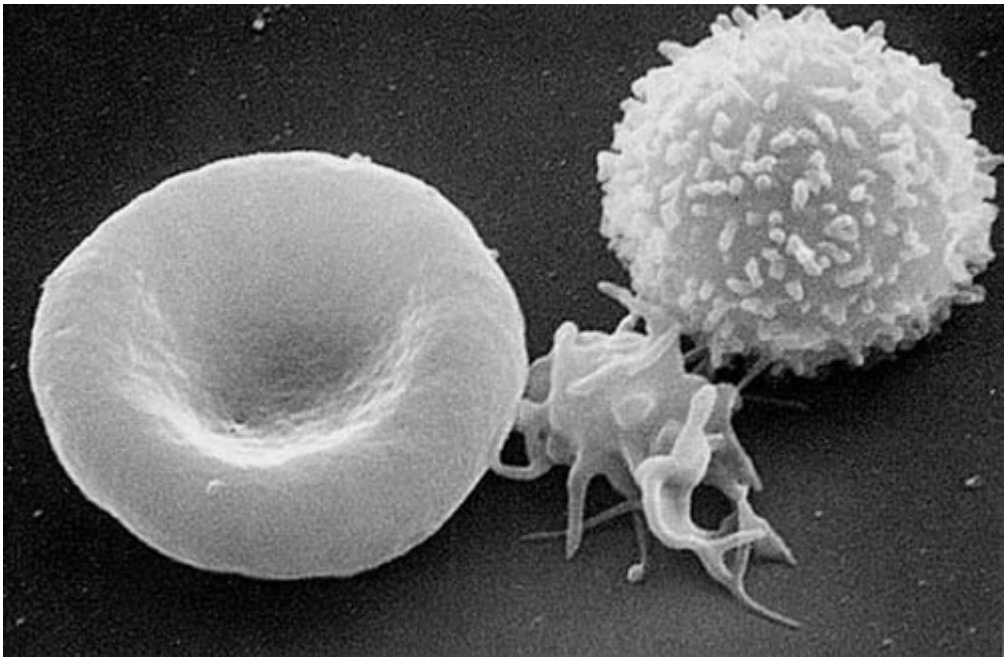
Mammalian erythrocytes are unique among the vertebrates as they are non-nucleated cells in their mature form. These cells have nuclei during early phases of erythropoiesis, but extrude them during development as they mature in order to provide more space for hemoglobin. In mammals, erythrocytes also lose all other cellular organelles such as their mitochondria, golgi apparatus and endoplasmic reticulum. As a result of not containing mitochondria, these cells use none of the oxygen they transport; instead they produce the energy carrier ATP by lactic acid fermentation of glucose. Because of the lack of nuclei and organelles, mature red blood cells do not contain DNA and cannot synthesize any RNA, and consequently cannot divide and have limited repair capabilities.

Mammalian erythrocytes are typically shaped as biconcave disks: flattened and depressed in the center, with a dumbbell-shaped cross section, and a torus-shaped rim on the edge of the disk. This distinctive biconcave shape optimises the flow properties of blood in the large vessels, such as maximization of laminar flow and minimization of platelet scatter, which suppresses their atherogenic activity in those large vessels. However, there are

some exceptions concerning shape in the artiodactyl order (even-toed ungulates including cattle, deer, and their relatives), which displays a wide variety of bizarre erythrocyte morphologies: small and highly ovaloid cells in llamas and camels (family Camelidae), tiny spherical cells in mouse deer (family Tragulidae), and cells which assume fusiform, lanceolate, crescentic, and irregularly polygonal and other angular forms in red deer and wapiti (family Cervidae). Members of this order have clearly evolved a mode of red blood cell development substantially different from the mammalian norm. Overall, mammalian erythrocytes are remarkably flexible and deformable so as to squeeze through tiny capillaries, as well as to maximize their apposing surface by assuming a cigar shape, where they efficiently release their oxygen load.

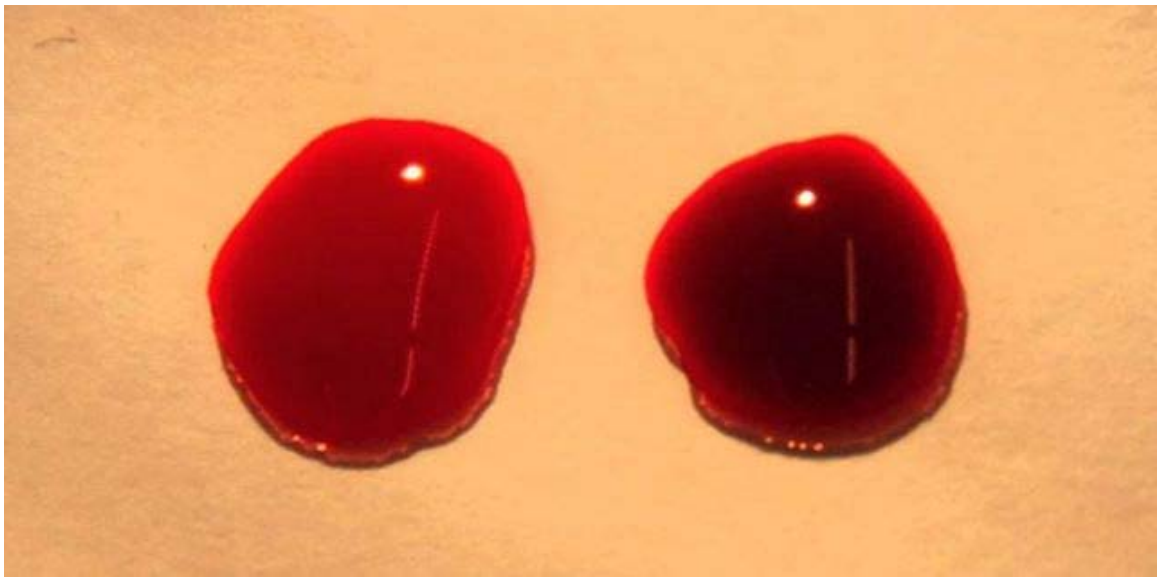
In large blood vessels, red blood cells sometimes occur as a stack, flat side next to flat side. This is known as *rouleaux formation*, and it occurs more often if the levels of certain serum proteins are elevated, as for instance during inflammation.

The spleen acts as a reservoir of red blood cells, but this effect is somewhat limited in humans. In some other mammals such as dogs and horses, the spleen sequesters large numbers of red blood cells which are dumped into the blood during times of exertion stress, yielding a higher oxygen transport capacity.

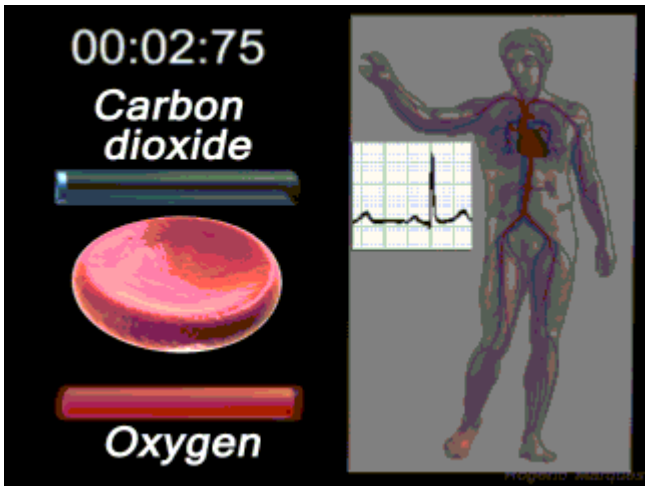


Scanning electron micrograph of blood cells. From left to right: human erythrocyte, thrombocyte (platelet), leukocyte.

Human erythrocytes



Two drops of blood are shown with a bright red oxygenated drop on the left and a deoxygenated drop on the right.



A typical human red blood cell cycle in the circulatory system. This image shows the red blood cell deform as it enters capillaries, as well as changing color as it alternates in states of oxygenation along the circulatory system.

A typical human erythrocyte has a disk diameter of 6–8 μm and a thickness of 2 μm, being much smaller than most other human cells. These cells have a volume of about 90 fL with a surface of about 136 μm^2, and can swell up to a sphere shape containing 150 fL, without membrane distension.

Adult humans have roughly 2–3 × 10^{13} (20-30 trillion) red blood cells at any given time, comprising approximately one quarter of the total human body cell number (women have

about 4 to 5 million erythrocytes per microliter (cubic millimeter) of blood and men about 5 to 6 million; people living at high altitudes with low oxygen tension will have more). Red blood cells are thus much more common than the other blood particles: there are about 4,000–11,000 white blood cells and about 150,000–400,000 platelets in each microliter of human blood.

Human red blood cells take on average 20 seconds to complete one cycle of circulation. As red blood cells contain no nucleus, protein biosynthesis is currently assumed to be absent in these cells, although a recent study indicates the presence of all the necessary biomachinery in human red blood cells for protein biosynthesis.

The blood's red color is due to the spectral properties of the hemic iron ions in hemoglobin. Each human red blood cell contains approximately 270 million of these hemoglobin biomolecules, each carrying four heme groups; hemoglobin comprises about a third of the total cell volume. This protein is responsible for the transport of more than 98% of the oxygen (the remaining oxygen is carried dissolved in the blood plasma). The red blood cells of an average adult human male store collectively about 2.5 grams of iron, representing about 65% of the total iron contained in the body.

Life cycle

Human erythrocytes are produced through a process named erythropoiesis, developing from committed stem cells to mature erythrocytes in about 7 days. When matured, these cells live in blood circulation for about 100 to 120 days. At the end of their lifespan, they become senescent, and are removed from circulation.

Erythropoiesis

Erythropoiesis is the development process in which new erythrocytes are produced, through which each cell matures in about 7 days. Through this process erythrocytes are continuously produced in the red bone marrow of large bones, at a rate of about 2 million per second in a healthy adult. (In the embryo, the liver is the main site of red blood cell production.) The production can be stimulated by the hormone erythropoietin (EPO), synthesised by the kidney. Just before and after leaving the bone marrow, the developing cells are known as reticulocytes; these comprise about 1% of circulating red blood cells.

Functional lifetime

This phase lasts about 100–120 days, during which the erythrocytes are continually moving by the blood flow push (in arteries), pull (in veins) and squeezing through microvessels such as capillaries as they compress against each other in order to move.

Senescence

The aging erythrocyte undergoes changes in its plasma membrane, making it susceptible to selective recognition by macrophages and subsequent phagocytosis in the

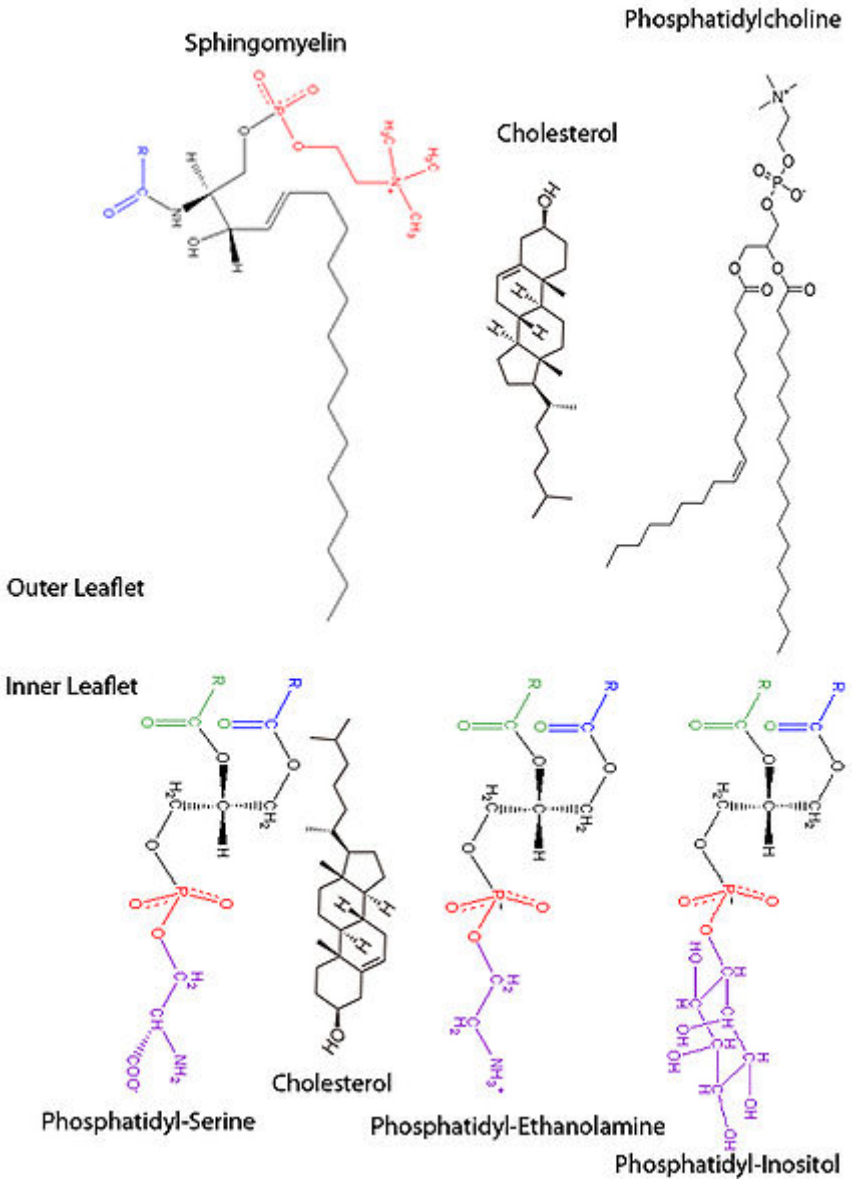
reticuloendothelial system (spleen, liver and bone marrow), thus removing old and defective cells and continually purging the blood. This process is termed eryptosis, or erythrocyte programmed cell death. This process normally occurs at the same rate of production by erythropoiesis, balancing the total circulating red blood cell count. Eryptosis is increased in a wide variety of diseases including sepsis, haemolytic uremic syndrome, malaria, sickle cell anemia, beta-thalassemia, glucose-6-phosphate dehydrogenase deficiency, phosphate depletion, iron deficiency and Wilson's disease. Eryptosis can be elicited by osmotic shock, oxidative stress, energy depletion as well as a wide variety of endogenous mediators and xenobiotics. Excessive eryptosis is observed in erythrocytes lacking the cGMP-dependent protein kinase type I or the AMP-activated protein kinase AMPK. Inhibitors of eryptosis include erythropoietin, nitric oxide, catecholamines and high concentrations of urea.

Much of the resulting important breakdown products are recirculated in the body. The heme constituent of hemoglobin are broken down into Fe^{3+} and biliverdin. The biliverdin is reduced to bilirubin, which is released into the plasma and recirculated to the liver bound to albumin. The iron is released into the plasma to be recirculated by a carrier protein called transferrin. Almost all erythrocytes are removed in this manner from the circulation before they are old enough to hemolyze. Hemolyzed hemoglobin is bound to a protein in plasma called haptoglobin which is not excreted by the kidney.

Membrane composition

The membrane of the red blood cell plays many roles that aid in regulating their surface deformability, flexibility, adhesion to other cells and immune recognition. These functions are highly dependent on its composition, which defines its properties. The red blood cell membrane is composed of 3 layers: the glycocalyx on the exterior, which is rich in carbohydrates; the lipid bilayer which contains many transmembrane proteins, besides its lipidic main constituents; and the membrane skeleton, a structural network of proteins located on the inner surface of the lipid bilayer. In human erythrocytes, like in most mammal erythrocytes, half of the membrane mass is represented by proteins and the other half are lipids, namely phospholipids and cholesterol.

Membrane lipids



The most common erythrocyte cell membrane lipids, schematically disposed as they are distributed on the bilayer. Relative abundances are not at scale.

The erythrocyte cell membrane comprises a typical lipid bilayer, similar to what can be found in virtually all human cells. Simply put, this lipid bilayer is composed of cholesterol and phospholipids in equal proportions by weight. The lipid composition is important as it defines many physical properties such as membrane permeability and fluidity. Additionally, the activity of many membrane proteins is regulated by interactions with lipids in the bilayer.

Unlike cholesterol which is evenly distributed between the inner and outer leaflets, the 5 major phospholipids are asymmetrically disposed, as shown below:

Outer monolayer

- Phosphatidylcholine (PC);
- Sphingomyelin (SM).

Inner monolayer

- Phosphatidylethanolamine (PE);
- Phosphoinositol (PI) (small amounts).
- Phosphatidylserine (PS);

This asymmetric phospholipid distribution among the bilayer is the result of the function of several energy-dependent and energy-independent phospholipid transport proteins. Proteins called "Flippases" move phospholipids from the outer to the inner monolayer while others called "floppases" do the opposite operation, against a concentration gradient in an energy dependent manner. Additionally, there are also "scramblase" proteins that move phospholipids in both directions at the same time, down their concentration gradients in an energy independent manner. There is still considerable debate ongoing regarding the identity of these membrane maintenance proteins in the red cell membrane.

The maintenance of an asymmetric phospholipid distribution in the bilayer (such as an exclusive localization of PS and PIs in the inner monolayer) is critical for the cell integrity and function due to several reasons:

- Macrophages recognize and phagocytose red cells that expose PS at their outer surface. Thus the confinement of PS in the inner monolayer is essential if the cell is to survive its frequent encounters with macrophages of the reticuloendothelial system, especially in the spleen.

- Premature destruction of thallassemic and sickle red cells has been linked to disruptions of lipid asymmetry leading to exposure of PS on the outer monolayer.

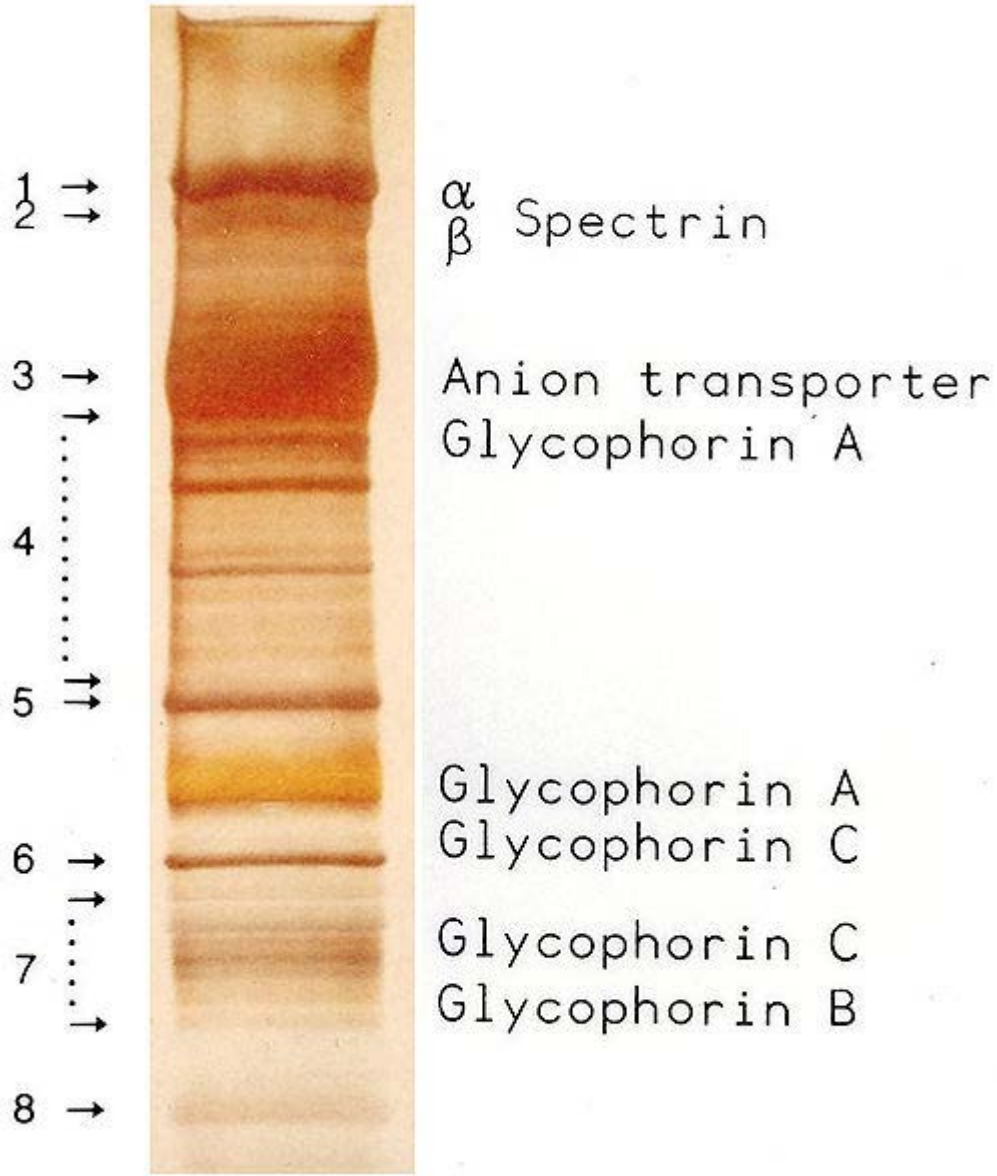
- An exposure of PS can potentiate adhesion of red cells to vascular endothelial cells, effectively preventing normal transit through the microvasculature. Thus it is important that PS is maintained only in the inner leaflet of the bilayer to ensure normal blood flow in microcirculation.

- Both PS and phosphatidylinositol-4,5-bisphosphate (PIP2) can regulate membrane mechanical function, due to their interactions with skeletal proteins such as spectrin and protein 4.1R. Recent studies have shown that binding of spectrin to PS promotes membrane mechanical stability. PIP2 enhances the binding of

protein band 4.1R to glycophorin C but decreases its interaction with protein band 3, and thereby may modulate the linkage of the bilayer to the membrane skeleton.

The presence of specialized structures named "lipid rafts" in the erythrocyte membrane have been described by recent studies. These are structures enriched in cholesterol and sphingolipids associated with specific membrane proteins, namely flotillins, stomatins (band 7), G-proteins, and β-adrenergic receptors. Lipid rafts that have been implicated in cell signaling events in nonerythroid cells have been shown in erythroid cells to mediate β2-adregenic receptor signaling and increase cAMP levels, and thus regulating entry of malarial parasites into normal red cells.

Membrane proteins

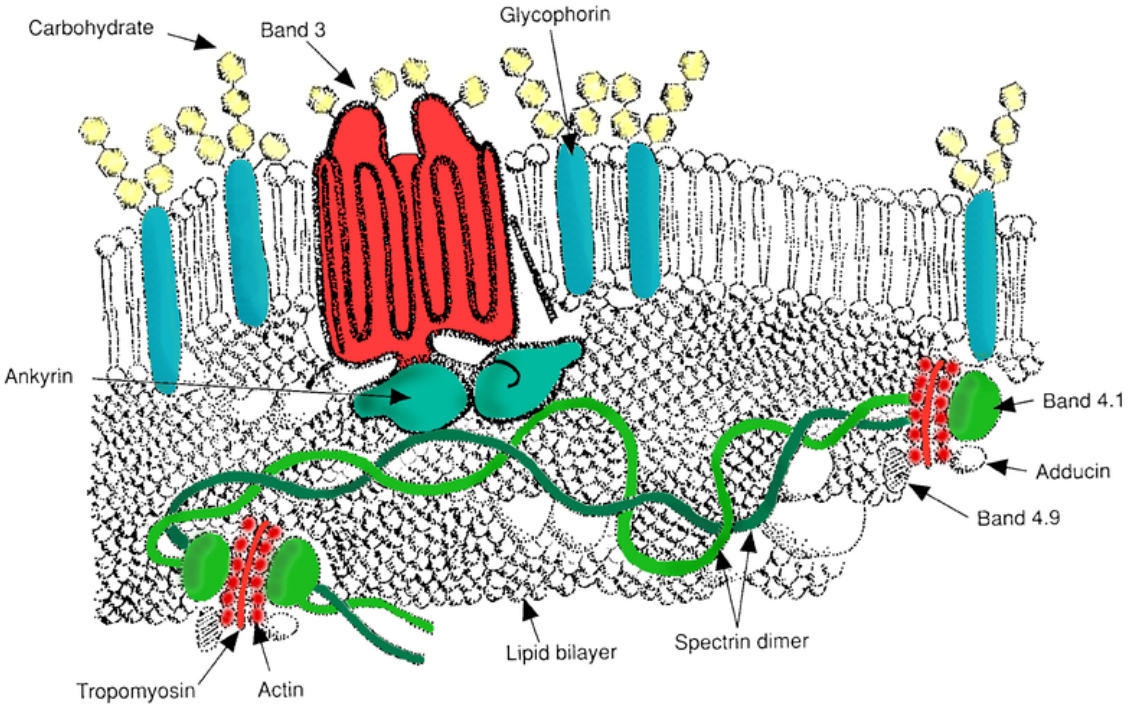


Red blood cell membrane proteins separated by SDS-Page and silverstained

The proteins of the membrane skeleton are responsible for the deformability, flexibility and durability of the red blood cell, enabling it to squeeze through capillaries less than half the diameter of the erythrocyte (7-8 μm) and recovering the discoid shape as soon as these cells stop receiving compressive forces, in a similar fashion to an object made of rubber.

There are currently more than 50 known membrane proteins, which can exist in a few hundred up to a million copies per erythrocyte. Approximately 25 of these membrane proteins carry the various blood group antigens, such as the A, B and Rh antigens, among many others. These membrane proteins can perform a wide diversity of functions, such as transporting ions and molecules across the red cell membrane, adhesion and interaction with other cells such as endothelial cells, as signaling receptors, as well as other currently unknown functions. The blood types of humans are due to variations in surface glycoproteins of erythrocytes. Disorders of the proteins in these membranes are associated with many disorders, such as hereditary spherocytosis, hereditary elliptocytosis, hereditary stomatocytosis, and paroxysmal nocturnal hemoglobinuria.

The red blood cell membrane proteins organized according to their function:



Red Blood Cell membrane major proteins

Transport

- Band 3 - Anion transporter, also an important structural component of the erythrocyte cell membrane, makes up to 25% of the cell membrane surface, each

red cell contains approximately one million copies. Defines the Diego Blood Group;
- Aquaporin 1 - water transporter, defines the Colton Blood Group;
- Glut1 - glucose and L-dehydroascorbic acid transporter;
- Kidd antigen protein - urea transporter;
- RhAG - gas transporter, probably of carbon dioxide, defines Rh Blood Group and the associated unusual blood group phenotype Rh_{null};
- Na^+/K^+ - ATPase;
- Ca^{2+} - ATPase;
- Na^+ K^+ $2Cl^-$ - cotransporter;
- Na^+-Cl^- - cotransporter;
- Na-H exchanger;
- K-Cl - cotransporter;
- Gardos Channel.

Cell adhesion

- ICAM-4 - interacts with integrins;
- BCAM - a glycoprotein that defines the Lutheran blood group and also known as Lu or laminin-binding protein.

Structural role - The following membrane proteins establish linkages with skeletal proteins and may play an important role in regulating cohesion between the lipid bilayer and membrane skeleton, likely enabling the red cell to maintain its favorable membrane surface area by preventing the membrane from collapsing (vesiculating).

- Ankyrin-based macromolecular complex - proteins linking the bilayer to the membrane skeleton through the interaction of their cytoplasmic domains with Ankyrin.
    - Band 3 - also assembles various glycolytic enzymes, the presumptive CO_2 transporter, and carbonic anhydrase into a macromolecular complex termed a "metabolon," which may play a key role in regulating red cell metabolism and ion and gas transport function);
    - RhAG - also involved in transport, defines associated unusual blood group phenotype Rh_{mod}.

- Protein 4.1R-based macromolecular complex - proteins interacting with Protein 4.1R.
    - Protein 4.1R - weak expression of Gerbich antigens;
    - Glycophorin C and D - glycoprotein, defines Gerbich Blood Group;
    - XK - defines the Kell Blood Group and the Mcleod unusual phenotype (lack of Kx antigen and greatly reduced expression of Kell antigens);
    - RhD/RhCE - defines Rh Blood Group and the associated unusual blood group phenotype Rh_{null};
    - Duffy protein - has been proposed to be associated with chemokine clearance;

- Adducin - interaction with band 3;
- Dematin- interaction with the Glut1 glucose transporter.

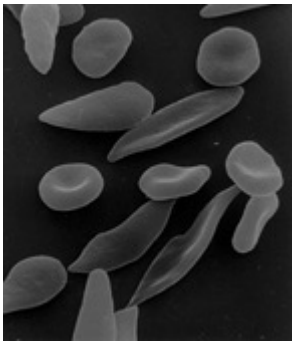
Separation and blood doping

Red blood cells can be obtained from whole blood by centrifugation, which separates the cells from the blood plasma. During plasma donation, the red blood cells are pumped back into the body right away and the plasma is collected. Some athletes have tried to improve their performance by blood doping: first about 1 litre of their blood is extracted, then the red blood cells are isolated, frozen and stored, to be reinjected shortly before the competition. (Red blood cells can be conserved for 5 weeks at −79 °C.) This practice is hard to detect but may endanger the human cardiovascular system which is not equipped to deal with blood of the resulting higher viscosity.

Artificially grown red blood cells

In 2008 it was reported that human embryonic stem cells had been successfully coaxed into becoming erythrocytes in the lab. The difficult step was to induce the cells to eject their nucleus; this was achieved by growing the cells on stromal cells from the bone marrow. It is hoped that these artificial erythrocytes can eventually be used for blood transfusions.

Diseases and diagnostic tools



Affected by Sickle-cell disease, red blood cells alter shape and threaten to damage internal organs.

Blood diseases involving the red blood cells include:

- Anemias (or anaemias) are diseases characterized by low oxygen transport capacity of the blood, because of low red cell count or some abnormality of the red blood cells or the hemoglobin.

- Iron deficiency anemia is the most common anemia; it occurs when the dietary intake or absorption of iron is insufficient, and hemoglobin, which contains iron, cannot be formed

- Sickle-cell disease is a genetic disease that results in abnormal hemoglobin molecules. When these release their oxygen load in the tissues, they become insoluble, leading to mis-shaped red blood cells. These sickle shaped red cells are less deformable and viscoelastic meaning that they have become rigid and can cause blood vessel blockage, pain, strokes, and other tissue damage.

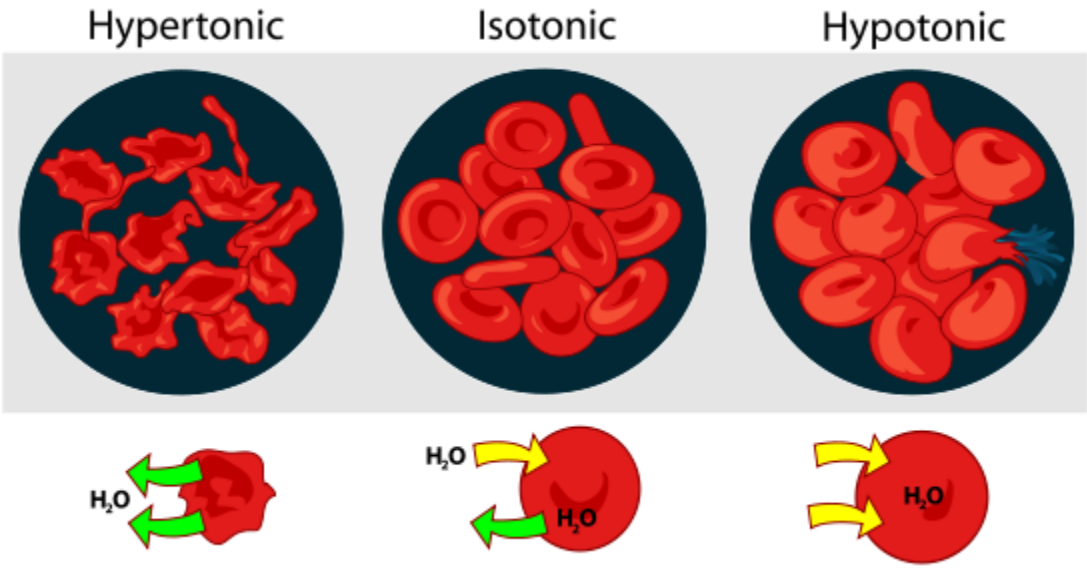
- Thalassemia is a genetic disease that results in the production of an abnormal ratio of hemoglobin subunits.

- Spherocytosis is a genetic disease that causes a defect in the red blood cell's cytoskeleton, causing the red blood cells to be small, sphere-shaped, and fragile instead of donut-shaped and flexible.

- Pernicious anemia is an autoimmune disease wherein the body lacks intrinsic factor, required to absorb vitamin B_{12} from food. Vitamin B_{12} is needed for the production of hemoglobin.

- Aplastic anemia is caused by the inability of the bone marrow to produce blood cells.

- Pure red cell aplasia is caused by the inability of the bone marrow to produce only red blood cells.



Effect of osmotic pressure on blood cells

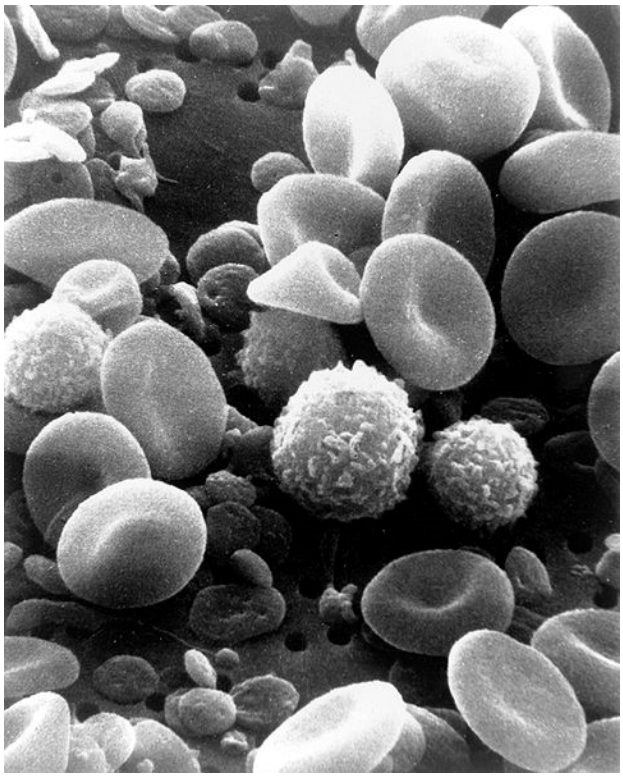
- Hemolysis is the general term for excessive breakdown of red blood cells. It can have several causes and can result in hemolytic anemia.
    - The malaria parasite spends part of its life-cycle in red blood cells, feeds on their hemoglobin and then breaks them apart, causing fever. Both sickle-cell disease and thalassemia are more common in malaria areas, because these mutations convey some protection against the parasite.
- Polycythemias (or erythrocytoses) are diseases characterized by a surplus of red blood cells. The increased viscosity of the blood can cause a number of symptoms.
    - In polycythemia vera the increased number of red blood cells results from an abnormality in the bone marrow.
- Several microangiopathic diseases, including disseminated intravascular coagulation and thrombotic microangiopathies, present with pathognomonic (diagnostic) red blood cell fragments called schistocytes. These pathologies generate fibrin strands that sever red blood cells as they try to move past a thrombus.
- Inherited hemolytic anemias caused by abnormalities of the erythrocyte membrane comprise an important group of inherited disorders. These disorders are characterized by clinical and biochemical heterogeneity and also genetic heterogeneity, as evidenced by recent molecular studies.
    - The Hereditary spherocytosis (HS) syndromes are a group of inherited disorders characterized by the presence of spherical-shaped erythrocytes on the peripheral blood smear. HS is found worldwide. It is the most common inherited anemia in individuals of northern European descent, affecting approximately 1 in 1000-2500 individuals depending on the diagnostic criteria. The primary defect in hereditary spherocytosis is a deficiency of membrane surface area. Decreased surface area may produced by two different mechanisms: 1) Defects of spectrin, ankyrin, or protein 4.2 lead to reduced density of the membrane skeleton, destabilizing the overlying lipid bilayer and releasing band 3-containing microvesicles. 2) Defects of band 3 lead to band 3 deficiency and loss of its lipid-stabilizing effect. This results in the loss of band 3-free microvesicles. Both pathways result in membrane loss, decreased surface area, and formation of spherocytes with decreased deformability. These deformed erythrocytes become trapped in the hostile environment of the spleen where splenic conditioning inflicts further membrane damage, amplifying the cycle of membrane injury.
    - Hereditary elliptocytosis
    - Hereditary pyropoikilocytosis
    - Hereditary stomatocytosis

- Hemolytic transfusion reaction is the destruction of donated red blood cells after a transfusion, mediated by host antibodies, often as a result of a blood type mismatch.

Several blood tests involve red blood cells, including the *RBC count* (the number of red blood cells per volume of blood), the hematocrit (percentage of blood volume occupied by red blood cells), and the erythrocyte sedimentation rate. The blood type needs to be determined to prepare for a blood transfusion or an organ transplantation.

Chapter 2

White Blood Cell



A scanning electron microscope image of normal circulating human blood. In addition to the irregularly shaped leukocytes, both red blood cells and many small disc-shaped platelets are visible.

White blood cells, or **leukocytes** (also spelled "leucocytes," "leuco-" being Greek for white), are cells of the immune system involved in defending the body against both infectious disease and foreign materials. Five different and diverse types of leukocytes exist, but they are all produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. Leukocytes are found throughout the body, including the blood and lymphatic system.

The number of WBCs in the blood is often an indicator of disease. There are normally between 4×10^9 and 1.1×10^{10} white blood cells in a litre of blood, making up approximately 1% of blood in a healthy adult. An increase in the number of leukocytes over the upper limits is called leukocytosis, and a decrease below the lower limit is called leukopenia. The physical properties of leukocytes, such as volume, conductivity, and granularity, may change due to activation, the presence of immature cells, or the presence of malignant leukocytes in leukemia.

Etymology

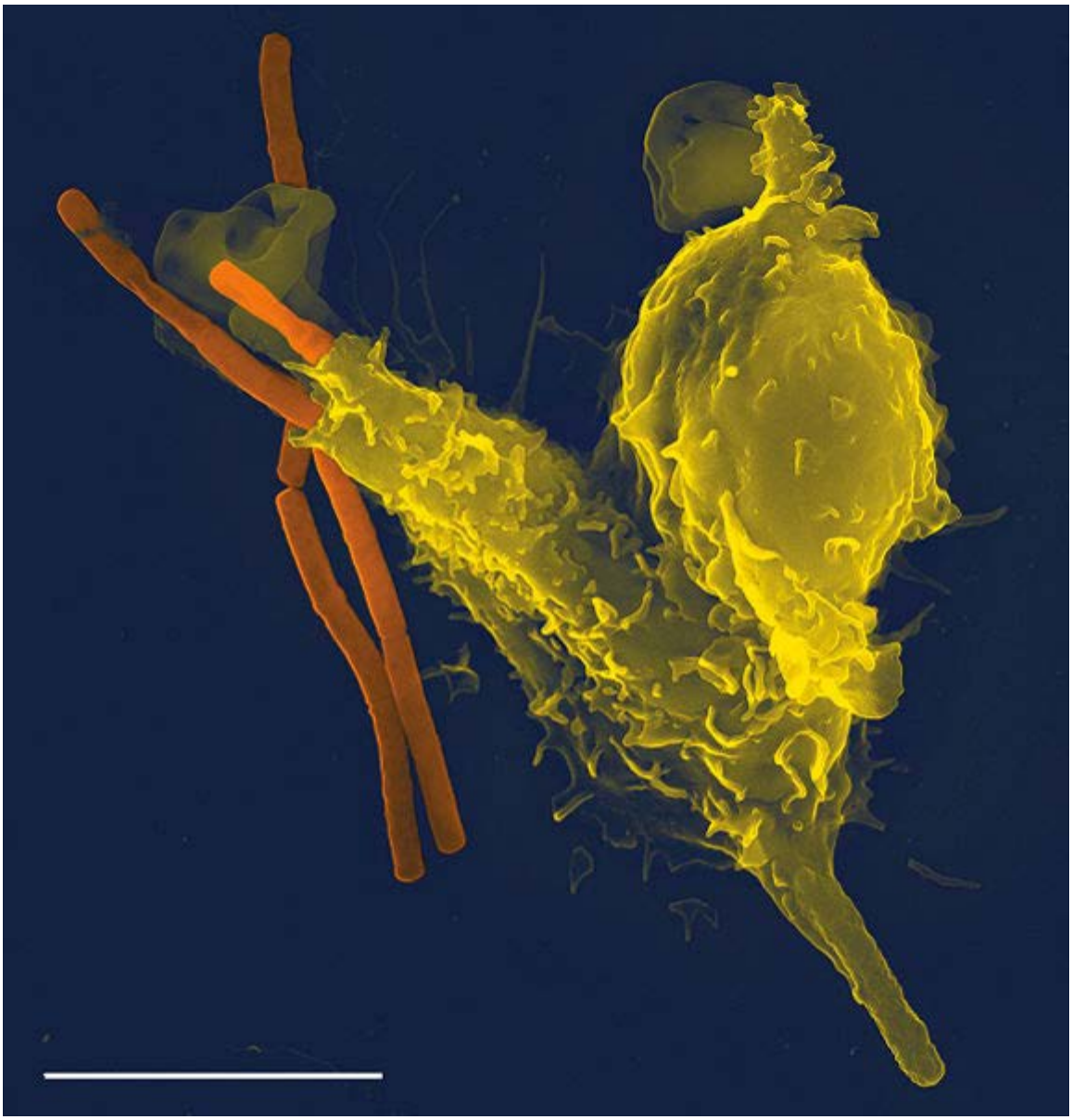
The name "white blood cell" derives from the fact that after centrifugation of a blood sample, the white cells are found in the *buffy coat*, a thin, typically white layer of nucleated cells between the sedimented red blood cells and the blood plasma. The scientific term *leukocyte* directly reflects this description, derived from Greek *λευκό* (white), and *κύτταρο* (cell). Blood plasma may sometimes be green if there are large amounts of neutrophils in the sample, due to the heme-containing enzyme myeloperoxidase that they produce.

Types

There are several different types of white blood cells. They all have many things in common, but are all distinct in form and function. A major distinguishing feature of some leukocytes is the presence of granules; white blood cells are often characterized as granulocytes or agranulocytes:

- **Granulocytes** (polymorphonuclear leukocytes): leukocytes characterised by the presence of differently staining granules in their cytoplasm when viewed under light microscopy. These granules are membrane-bound enzymes which primarily act in the digestion of endocytosed particles. There are three types of granulocytes: neutrophils, basophils, and eosinophils, which are named according to their staining properties.
- **Agranulocytes** (mononuclear leucocytes): leukocytes characterized by the apparent absence of granules in their cytoplasm. Although the name implies a lack of granules these cells do contain non-specific azurophilic granules, which are lysosomes. The cells include lymphocytes, monocytes, and macrophages.

Neutrophil



Neutrophil engulfing anthrax bacteria.

Neutrophils defend against bacterial or fungal infection and other very small inflammatory processes that are usually first responders to microbial infection; their activity and death in large numbers forms pus. They are commonly referred to as polymorphonuclear (PMN) leukocytes, although technically PMN refers to all granulocytes. They have a multilobed nucleus which may appear like multiple nuclei, hence the name polymorphonuclear leukocyte. The cytoplasm may look transparent because of fine granules that are pale lilac. Neutrophils are very active in phagocytosing bacteria and are present in large amount in the pus of wounds. These cells are not able to renew their lysosomes used in digesting microbes and die after having phagocytosed a few pathogens. Most common cell seen in acute inflammation, comes in and kill foreign

substance.They make up 60-70% of total leukocyte count.The life span of neutrophil is about 8 days.

Eosinophil

Eosinophils primarily deal with parasitic infections and an increase in them may indicate such. Eosinophils are also the predominant inflammatory cells in allergic reactions. The most important causes of eosinophilia include allergies such as asthma, hay fever, and hives; and also parasitic infections. Generally their nucleus is bi-lobed. The cytoplasm is full of granules which assume a characteristic pink-orange color with eosin stain.

Basophil

Basophils are chiefly responsible for allergic and antigen response by releasing the chemical histamine causing vasodilation. The nucleus is bi- or tri-lobed, but it is hard to see because of the number of coarse granules which hide it. They are characterized by their large blue granules.

Lymphocyte

Lymphocytes are much more common in the lymphatic system. Lymphocytes are distinguished by having a deeply staining nucleus which may be eccentric in location, and a relatively small amount of cytoplasm. The blood has three types of lymphocytes:

- B cells: B cells make antibodies that bind to pathogens to enable their destruction. (B cells not only make antibodies that bind to pathogens, but after an attack, some B cells will retain the ability to produce an antibody to serve as a 'memory' system.)
- T cells:
    - CD4+ (helper) T cells co-ordinate the immune response and are important in the defense against intracellular bacteria. In acute HIV infection, these T cells are the main index to identify the individual's immune system activity. Research has shown that CD8+ cells are also another index to identify human's immune activity.
    - CD8+ cytotoxic T cells are able to kill virus-infected and tumor cells.
    - γδ T cells possess an alternative T cell receptor as opposed to CD4+ and CD8+ αβ T cells and share characteristics of helper T cells, cytotoxic T cells and natural killer cells.
- Natural killer cells: Natural killer cells are able to kill cells of the body which are displaying a signal to kill them, as they have been infected by a virus or have become cancerous.

Monocyte

Monocytes share the "vacuum cleaner" (phagocytosis) function of neutrophils, but are much longer lived as they have an additional role: they present pieces of pathogens to T

cells so that the pathogens may be recognized again and killed, or so that an antibody response may be mounted. Monocytes eventually leave the bloodstream to become tissue macrophages which remove dead cell debris as well as attacking microorganisms. Neither of these can be dealt with effectively by the neutrophils. Unlike neutrophils, monocytes are able to replace their lysosomal contents and are thought to have a much longer active life. They have the kidney shaped nucleus and are typically agranulated. They also possess abundant cytoplasm.

Once monocytes move from the bloodstream out into the body tissues, they undergo changes (differentiate) allowing phagocytosis and are then known as macrophages.

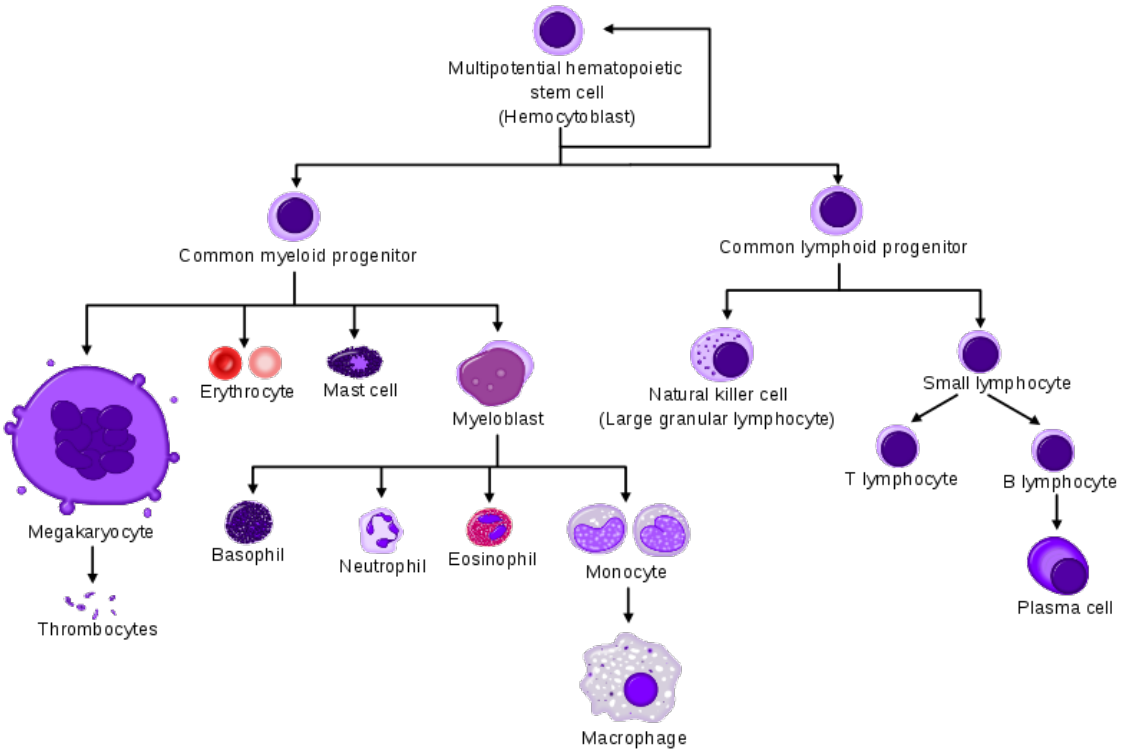
Medication causing leukopenia

Some medications can have an impact on the number and function of white blood cells. Leukopenia is the reduction in the number of white blood cells, which may affect the overall white cell count or one of the specific populations of white blood cells. For example, if the number of neutrophils is low, the condition is known as neutropenia. Likewise, low lymphocyte levels are termed lymphopenia. Medications which can cause leukopenia include clozapine, an antipsychotic medication with a rare adverse effect leading to the total absence of all granulocytes (neutrophils, basophils, eosinophils). Other medications include immunosuppressive drugs, such as sirolimus, mycophenolate mofetil, tacrolimus, and cyclosporine. Interferons used to treat multiple sclerosis, like Rebif, Avonex, and Betaseron, can also cause leukopenia.

Fixed leukocytes

Some leukocytes migrate into the tissues of the body to take up a permanent residence at that location rather than remaining in the blood. Often these cells have specific names depending upon which tissue they settle in, such as fixed macrophages in the liver which become known as Kupffer cells. These cells still serve a role in the immune system.

- Histiocytes
- Dendritic cells (Although these will often migrate to local lymph nodes upon ingesting antigens)
- Mast cells
- Microglia



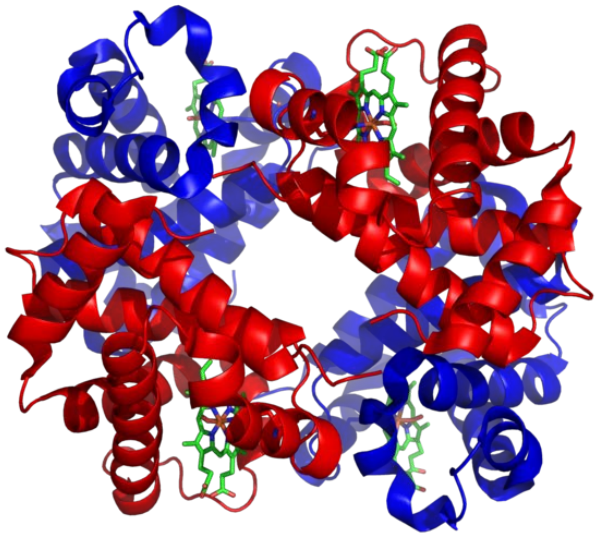
HSC=Hematopoietic stem cell, Progenitor=Progenitor cell, L-blast=Lymphoblast, Lymphocyte, Mo-blast=Monoblast, Monocyte, Myeloblast, Pro-M=Promyelocyte, Myelocyte ,Meta-M=Metamyelocyte, Neutrophil, Eosinophil, Basophil, Pro-E=Proerythroblast, Baso-E=Basophilic erythroblast, poly-E=Polychromatic erythroblast, Ortho-E=Orthochromatic erythroblast , Erythrocyte , Promegakaryocyte , Megakaryocyte , Platlet

Chapter 3

Hemoglobin

Hemoglobin, human, adult

(heterotetramer, $(\alpha\beta)_2$)



Structure of human hemoglobin. The protein's α and β subunits are in red and blue, and the iron-containing heme groups in green. From PDB 1GZX *Proteopedia Hemoglobin*

-

Protein type	metalloprotein, globulin
Function	oxygen-transport

Cofactor(s)	heme (4)

-

Subunit Name	Gene	Chromosomal Locus
Hb-α1	HBA1	Chr. 16 p13.3
Hb-α2	HBA2	Chr. 16 p13.3
Hb-β	HBB	Chr. 11 p15.5

Hemoglobin (also spelled **haemoglobin** and abbreviated **Hb** or **Hgb**) is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates (except the fish family Channichthyidae) and the tissues of some invertebrates. Hemoglobin in the blood is what transports oxygen from the lungs or gills to the rest of the body (i.e. the tissues) where it releases the oxygen for cell use, and collects carbon dioxide to bring it back to the lungs.

In mammals the protein makes up about 97% of the red blood cells' dry content, and around 35% of the total content (including water). Hemoglobin has an oxygen binding capacity of 1.34 ml O_2 per gram of hemoglobin, which increases the total blood oxygen capacity seventyfold.

Hemoglobin is involved in the transport of other gases: it carries some of the body's respiratory carbon dioxide (about 10% of the total) as carbaminohemoglobin, in which CO_2 is bound to the globin protein. The molecule also carries the important regulatory molecule nitric oxide bound to a globin protein thiol group, releasing it at the same time as oxygen.

Hemoglobin is also found outside red blood cells and their progenitor lines. Other cells that contain hemoglobin include the A9 dopaminergic neurons in the substantia nigra, macrophages, alveolar cells, and mesangial cells in the kidney. In these tissues, hemoglobin has a non-oxygen-carrying function as an antioxidant and a regulator of iron metabolism.

Hemoglobin and hemoglobin-like molecules are also found in many invertebrates, fungi, and plants. In these organisms, hemoglobins may carry oxygen, or they may act to transport and regulate other things such as carbon dioxide, nitric oxide, hydrogen sulfide and sulfide. A variant of the molecule, called leghemoglobin, is used to scavenge oxygen, to keep it from poisoning anaerobic systems, such as nitrogen-fixing nodules of leguminous plants.

Research history

The oxygen-carrying protein hemoglobin was discovered by Hünefeld in 1840. In 1851, Otto Funke published a series of articles in which he described growing hemoglobin crystals by successively diluting red blood cells with a solvent such as pure water, alcohol or ether, followed by slow evaporation of the solvent from the resulting protein solution. Hemoglobin's reversible oxygenation was described a few years later by Felix Hoppe-Seyler.

In 1959 Max Perutz determined the molecular structure of hemoglobin by X-ray crystallography. This work resulted in his sharing with John Kendrew the 1962 Nobel Prize in Chemistry.

The role of hemoglobin in the blood was elucidated by physiologist Claude Bernard. The name *hemoglobin* is derived from the words *heme* and *globin*, reflecting the fact that each subunit of hemoglobin is a globular protein with an embedded heme (or haem) group. Each heme group contains one iron atom, that can bind one oxygen molecule through ion-induced dipole forces. The most common type of hemoglobin in mammals contains four such subunits.

Genetics

Hemoglobin consists mostly of protein (the "globin" chains), and these proteins, in turn, are composed of sequences of amino acids. These sequences are linear, in the manner of letters in a written sentence or beads on a string. In all proteins, it is the variation in the type of amino acids in the protein sequence of amino acids, which determine the protein's chemical properties and function. This is true of hemoglobin, where the sequence of amino acids may affect crucial functions such as the protein's affinity for oxygen.

There is more than one hemoglobin gene. The amino acid sequences of the globin proteins in hemoglobins usually differ between species, although the differences grow with the evolutionary distance between species. For example, the most common hemoglobin sequences in humans and chimpanzees are nearly identical, differing by only one amino acid in both the alpha and the beta globin protein chains. These differences grow larger between less closely related species.

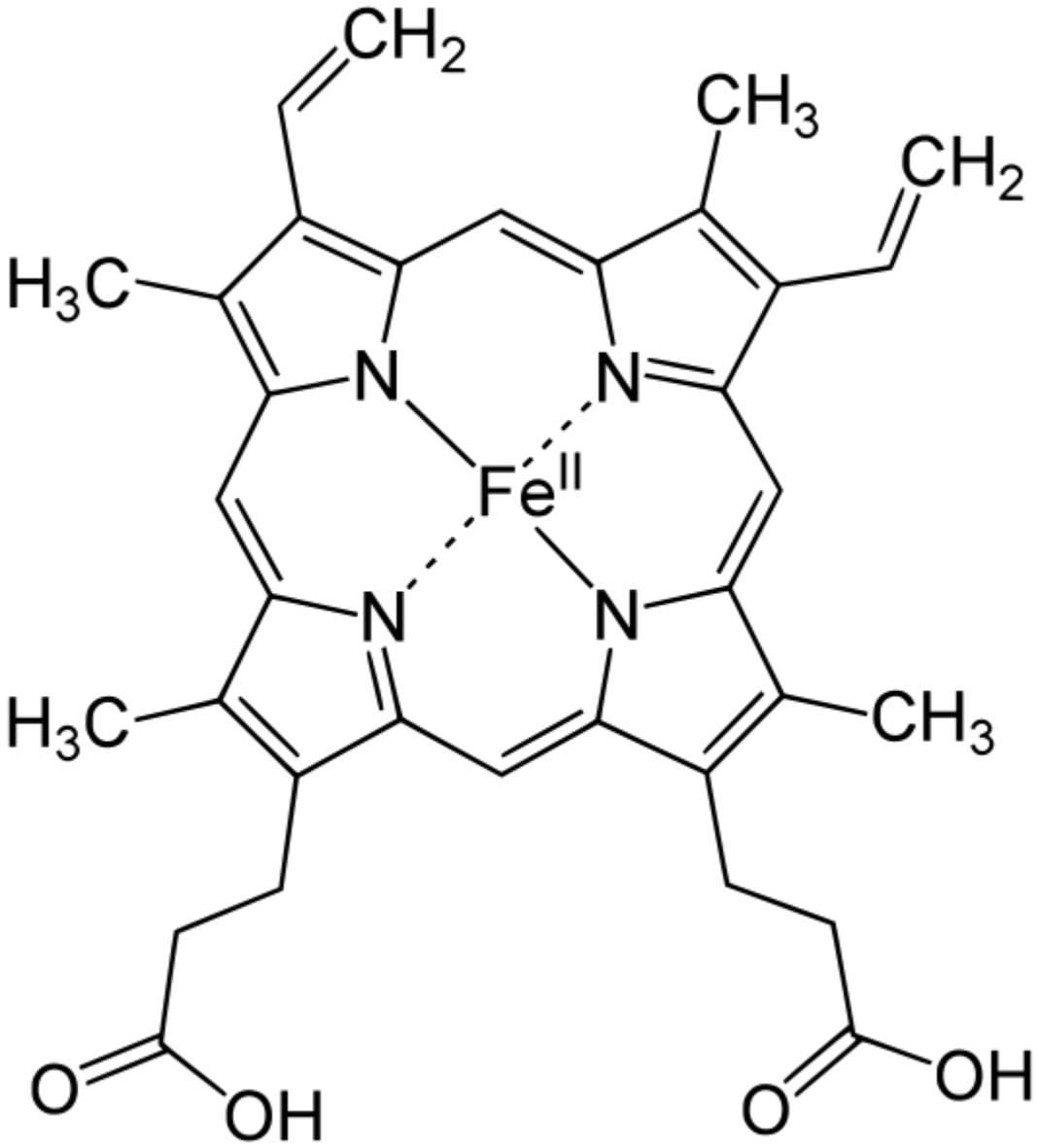
Even within a species, different variants of hemoglobin always exist, although one sequence is usually a "most common" one in each species. Mutations in the genes for the hemoglobin protein in a species result in hemoglobin variants. Many of these mutant forms of hemoglobin cause no disease. Some of these mutant forms of hemoglobin, however, cause a group of hereditary diseases termed the *hemoglobinopathies*. The best known hemoglobinopathy is sickle-cell disease, which was the first human disease whose mechanism was understood at the molecular level. A (mostly) separate set of diseases called thalassemias involves underproduction of normal and sometimes abnormal hemoglobins, through problems and mutations in globin gene regulation. All these diseases produce anemia.

Variations in hemoglobin amino acid sequences, as with other proteins, may be adaptive. For example, recent studies have suggested genetic variants in deer mice that help explain how deer mice that live in the mountains are able to survive in the thin air that accompanies high altitudes. A researcher from the University of Nebraska-Lincoln found mutations in four different genes that can account for differences between deer mice that live in lowland prairies versus the mountains. After examining wild mice captured from both highlands and lowlands, it was found that: the genes of the two breeds are "virtually identical–except for those that govern the oxygen-carrying capacity of their hemoglobin". "The genetic difference enables highland mice to make more efficient use of their oxygen", since less is available at higher altitudes, such as those in the mountains. Mammoth hemoglobin featured mutations that allowed for oxygen delivery at lower temperatures, thus enabling mammoths to migrate to higher latitudes during the Pleistocene.

Synthesis

Hemoglobin (Hb) is synthesized in a complex series of steps. The heme part is synthesized in a series of steps in the mitochondria and the cytosol of immature red blood cells, while the globin protein parts are synthesized by ribosomes in the cytosol. Production of Hb continues in the cell throughout its early development from the proerythroblast to the reticulocyte in the bone marrow. At this point, the nucleus is lost in mammalian red blood cells, but not in birds and many other species. Even after the loss of the nucleus in mammals, residual ribosomal RNA allows further synthesis of Hb until the reticulocyte loses its RNA soon after entering the vasculature (this hemoglobin-synthetic RNA in fact gives the reticulocyte its reticulated appearance and name).

Structure



Heme b group

Hemoglobin has a quaternary structure characteristic of many multi-subunit globular proteins. Most of the amino acids in hemoglobin form alpha helices, connected by short non-helical segments. Hydrogen bonds stabilize the helical sections inside this protein, causing attractions within the molecule, folding each polypeptide chain into a specific shape. Hemoglobin's quaternary structure comes from its four subunits in roughly a tetrahedral arrangement.

In most vertebrates, the hemoglobin molecule is an assembly of four globular protein subunits. Each subunit is composed of a protein chain tightly associated with a non-

protein heme group. Each protein chain arranges into a set of alpha-helix structural segments connected together in a globin fold arrangement, so called because this arrangement is the same folding motif used in other heme/globin proteins such as myoglobin. This folding pattern contains a pocket that strongly binds the heme group.

A heme group consists of an iron (Fe) ion (charged atom) held in a heterocyclic ring, known as a porphyrin. This porphyrin ring consists of four pyrrole molecules cyclically linked together (by methene bridges) with the iron ion bound in the center. The iron ion, which is the site of oxygen binding, coordinates with the four nitrogens in the center of the ring, which all lie in one plane. The iron is bound strongly (covalently) to the globular protein via the imidazole ring of the F8 histidine residue (also known as the proximal histidine) below the porphyrin ring. A sixth position can reversibly bind oxygen by a coordinate covalent bond, completing the octahedral group of six ligands. Oxygen binds in an "end-on bent" geometry where one oxygen atom binds Fe and the other protrudes at an angle. When oxygen is not bound, a very weakly bonded water molecule fills the site, forming a distorted octahedron.

Even though carbon dioxide is carried by hemoglobin, it does not compete with oxygen for the iron-binding positions, but is actually bound to the protein chains of the structure.

The iron ion may be either in the Fe^{2+} or in the Fe^{3+} state, but ferrihemoglobin (methemoglobin) (Fe^{3+}) cannot bind oxygen. In binding, oxygen temporarily and reversibly oxidizes (Fe^{2+}) to (Fe^{3+}) while oxygen temporally turns into superoxide, thus iron must exist in the +2 oxidation state to bind oxygen. If superoxide ion associated to Fe^{3+} is protonated the hemoglobin iron will remain oxidized and incapable to bind oxygen. In such cases, the enzyme methemoglobin reductase will be able to eventually reactivate methemoglobin by reducing the iron center.

In adult humans, the most common hemoglobin type is a tetramer (which contains 4 subunit proteins) called **hemoglobin A**, consisting of two α and two β subunits non-covalently bound, each made of 141 and 146 amino acid residues, respectively. This is denoted as $\alpha_2\beta_2$. The subunits are structurally similar and about the same size. Each subunit has a molecular weight of about 17,000 daltons, for a total molecular weight of the tetramer of about 68,000 daltons (64,458 g/mol). Thus, 1 g/dL = 0.01551 mmol/L. Hemoglobin A is the most intensively studied of the hemoglobin molecules.

In human infants, the hemoglobin molecule is made up of 2 α chains and 2 gamma chains. The gamma chains are gradually replaced by β chains as the infant grows.

The four polypeptide chains are bound to each other by salt bridges, hydrogen bonds, and the hydrophobic effect. There are two kinds of contacts between the α and β chains: $\alpha_1\beta_1$ and $\alpha_1\beta_2$.

In general, hemoglobin can be saturated with oxygen molecules (oxyhemoglobin), or desaturated with oxygen molecules (deoxyhemoglobin). *Oxyhemoglobin* is formed during physiological respiration when oxygen binds to the heme component of the protein

hemoglobin in red blood cells. This process occurs in the pulmonary capillaries adjacent to the alveoli of the lungs. The oxygen then travels through the blood stream to be dropped off at cells where it is utilized in aerobic glycolysis and in the production of ATP by the process of oxidative phosphorylation. It does not, however, help to counteract a decrease in blood pH. Ventilation, or breathing, may reverse this condition by removal of carbon dioxide, thus causing a shift up in pH.

Hemoglobin exists in two forms, a **taut form** (T) and a **relaxed form** (R). Various factors such as low pH, high CO_2 and high 2,3 BPG at the level of the tissues favor the taut form, which has low oxygen affinity and releases oxygen in the tissues. The opposite of these aforementioned factors at the level of the lung capillaries favors the relaxed form which can better bind oxygen.

Deoxyhemoglobin is the form of hemoglobin without the bound oxygen. The absorption spectra of oxyhemoglobin and deoxyhemoglobin differ. The oxyhemoglobin has significantly lower absorption of the 660 nm wavelength than deoxyhemoglobin, while at 940 nm its absorption is slightly higher. This difference is used for measurement of the amount of oxygen in patient's blood by an instrument called pulse oximeter.

Iron's oxidation state in oxyhemoglobin

Assigning oxygenated hemoglobin's oxidation state is difficult because oxyhemoglobin ($Hb\text{-}O_2$), by experimental measurement, is diamagnetic (no net unpaired electrons), yet the low-energy electron configurations in both oxygen and iron are paramagnetic (suggesting at least one unpaired electron in the complex). The lowest-energy form of oxygen, and the lowest energy forms of the relevant oxidation states of iron, are these:

- Triplet oxygen, the lowest energy molecular oxygen species, has two unpaired electrons in antibonding π^* molecular orbitals.
- Iron(II) tends to exist in a high-spin configuration where unpaired electrons exist in E_g antibonding orbitals.
- Iron(III) has an odd number of electrons, and thus must have one or more unpaired electrons, in any energy state.

All of these structures are paramagnetic (have unpaired electrons), not diamagnetic. Thus, a non-intuitive (e.g., a higher-energy for at least one species) distribution of electrons in the combination of iron and oxygen must exist, in order to explain the observed diamagnetism and no unpaired electrons.

The three logical possibilities to produce diamagnetic (no net spin) $Hb\text{-}O_2$ are:

1. Low-spin Fe^{2+} binds to singlet oxygen. Both low-spin iron and singlet oxygen are diamagnetic. However, the singlet form of oxygen is the higher-energy form of the molecule.
2. Low-spin Fe^{3+} binds to $\cdot O_2^-$ (the superoxide ion) and the two unpaired electrons couple antiferromagnetically, giving diamagnetic properties.

3. Low-spin Fe^{4+} binds to peroxide, O_2^{2-}. Both are diamagnetic.

Direct experimental data:

- X-ray photoelectron spectroscopy suggests iron has an oxidation state of approximately 3.2
- infrared stretching frequencies of the O-O bond suggests a bond length fitting with superoxide (a bond order of about 1.6, with superoxide being 1.5).

Thus, the nearest formal oxidation state of iron in Hb-O_2 is the +3 state, with oxygen in the -1 state (as superoxide $\cdot O_2^-$). The diamagnetism in this configuration arises from the single unpaired electron on superoxide aligning antiferromagnetically from the single unpaired electron on iron, to give no net spin to the entire configuration, in accordance with diamagnetic oxyhemoglobin from experiment.

The second choice of the three logical possibilities above for diamagnetic oxyhemoglobin being found correct by experiment, is not surprising: singlet oxygen (possibility #1) and large separations of charge (possibility #3) are both unfavorably high-energy states. Iron's shift to a higher oxidation state in Hb-O_2 decreases the atom's size, and allows it into the plane of the porphyrin ring, pulling on the coordinated histidine residue and initiating the allosteric changes seen in the globulins.

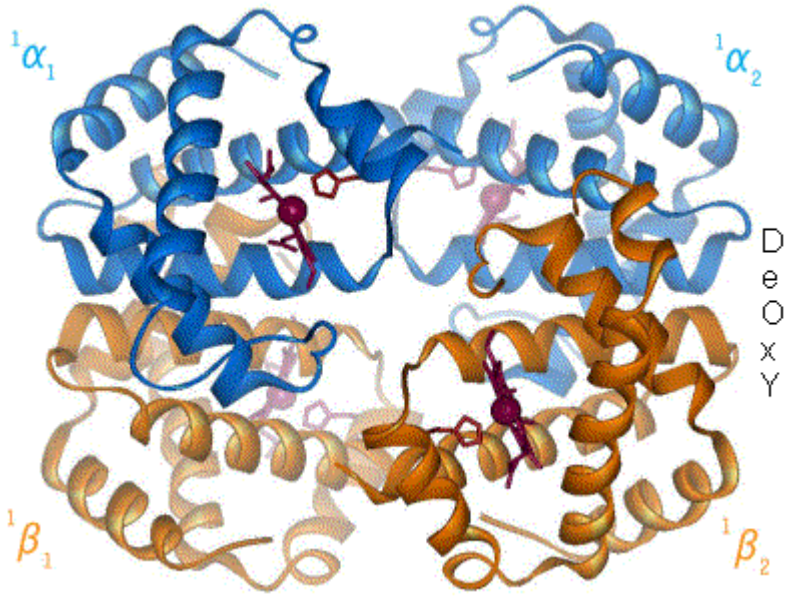
Early postulates by bio-inorganic chemists claimed that possibility #1 (above) was correct and that iron should exist in oxidation state II. This seemed particularly likely since the iron oxidation state III as methemoglobin, when **not** accompanied by superoxide $\cdot O_2^-$ to "hold" the oxidation electron, was known to render hemoglobin incapable of binding normal triplet O_2 as it occurs in the air. It was thus assumed that iron remained as Fe(II) when oxygen gas was bound in the lungs. The iron chemistry in this previous classical model was elegant, but the required presence of the required diamagnetic high-energy singlet oxygen was never explained. It was classically argued that the binding of an oxygen molecule placed high-spin iron(II) in an octahedral field of strong-field ligands; this change in field would increase the crystal field splitting energy, causing iron's electrons to pair into the low-spin configuration, which would be diamagnetic in Fe(II). This forced low-spin pairing is indeed thought to happen in iron when oxygen binds, but is not enough to explain iron's change in size. Extraction of an additional electron from iron by oxygen is required to explain both iron's smaller size and observed increased oxidation state, and oxygen's weaker bond.

It should be noted that the assignment of a whole-number oxidation state is a formalism, as the covalent bonds are not required to have perfect bond orders involving whole electron-transfer. Thus, all three models for paramagnetic Hb-O_2 may contribute to some small degree (by resonance) to the actual electronic configuration of Hb-O_2. However, the model of iron in Hb-O_2 being Fe(III) is more correct than the classical idea that it remains Fe(II).

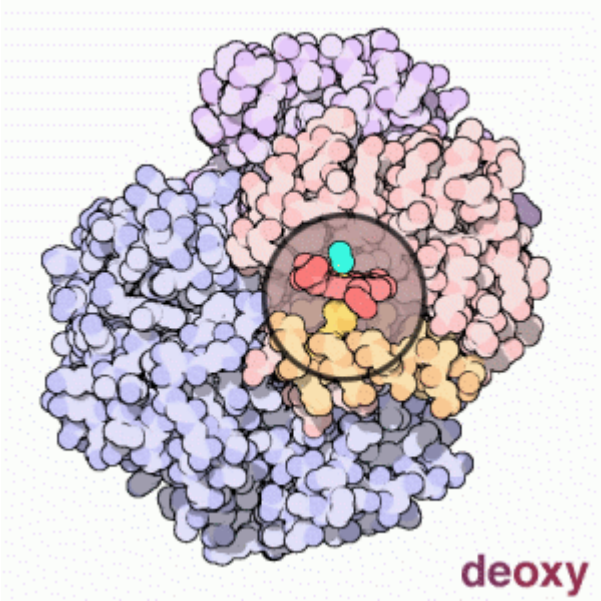
Binding for ligands other than oxygen

Besides the oxygen ligand, which binds to hemoglobin in a cooperative manner, hemoglobin ligands also include competitive inhibitors such as carbon monoxide (CO) and allosteric ligands such as carbon dioxide (CO_2) and nitric oxide (NO). The carbon dioxide is bound to amino groups of the globin proteins as carbaminohemoglobin, and is thought to account for about 10% of carbon dioxide transport in mammals. Nitric oxide is bound to specific thiol groups in the globin protein to form an S-nitrosothiol which dissociates into free nitric oxide and thiol again, as the hemoglobin releases oxygen from its heme site. This nitric oxide transport to peripheral tissues is hypothesised to assist oxygen transport in tissues, by releasing vasodilatory nitric oxide to tissues in which oxygen levels are low.

Cooperative



A schematic visual model of oxygen-binding process, showing all four monomers and hemes, and protein chains only as diagramatic coils, to facilitate visualization into the molecule. Oxygen is not shown in this model, but, for each of the iron atoms, it binds to the iron (red sphere) in the flat heme. For example, in the upper left of the four hemes shown, oxygen binds at the left of the iron atom shown in the upper left of diagram. This causes the iron atom to move backward into the heme which holds it (the iron moves upward as it binds oxygen, in this illustration), tugging the histidine residue (modeled as a red pentagon on the right of the iron) closer, as it does. This, in turn, pulls on the protein chain holding the histidine.



Another view of how binding and release of ligands induces a conformational (structural) change in hemoglobin. Only one of the four heme groups is shown, but more of the electron cloud of the protein chain is included in this diagram, as compared with above. The binding and release of oxygen (shown now in green) illustrates the structural differences between oxy- and deoxyhemoglobin, respectively. The histidine, which is pulled by motion of the iron atom, is shown here in yellow.

When oxygen binds to the iron complex, it causes the iron atom to move back toward the center of the plane of the porphyrin ring. At the same time, the imidazole side-chain of the histidine residue interacting at the other pole of the iron is pulled toward the porphyrin ring. This interaction forces the plane of the ring sideways toward the outside of the tetramer, and also induces a strain in the protein helix containing the histidine as it moves nearer to the iron atom. This strain is transmitted to the remaining three monomers in the tetramer, where it induces a similar conformational change in the other heme sites such that binding of oxygen to these sites becomes easier.

In the tetrameric form of normal adult hemoglobin, the binding of oxygen is, thus, a cooperative process. The binding affinity of hemoglobin for oxygen is increased by the oxygen saturation of the molecule, with the first oxygens bound influencing the shape of the binding sites for the next oxygens, in a way favorable for binding. This positive cooperative binding is achieved through steric conformational changes of the hemoglobin protein complex as discussed above; i.e., when one subunit protein in hemoglobin becomes oxygenated, a conformational or structural change in the whole complex is initiated, causing the other subunits to gain an increased affinity for oxygen. As a consequence, the oxygen binding curve of hemoglobin is sigmoidal, or *S*-shaped, as opposed to the normal hyperbolic curve associated with noncooperative binding.

The dynamic mechanism of the cooperativity in hemoglobin and its relation with the low-frequency resonance has been discussed.

Competitive

Hemoglobin's oxygen-binding capacity is decreased in the presence of carbon monoxide because both gases compete for the same binding sites on hemoglobin, carbon monoxide binding preferentially in place of oxygen.

The binding of oxygen is affected by molecules such as carbon monoxide (CO) (for example, from tobacco smoking, car exhaust, and incomplete combustion in furnaces). CO competes with oxygen at the heme binding site. Hemoglobin binding affinity for CO is 250 times greater than its affinity for oxygen, meaning that small amounts of CO dramatically reduce hemoglobin's ability to transport oxygen. When hemoglobin combines with CO, it forms a very bright red compound called carboxyhemoglobin, which may cause the skin of CO poisoning victims to appear pink in death, instead of white or blue. When inspired air contains CO levels as low as 0.02%, headache and nausea occur; if the CO concentration is increased to 0.1%, unconsciousness will follow. In heavy smokers, up to 20% of the oxygen-active sites can be blocked by CO.

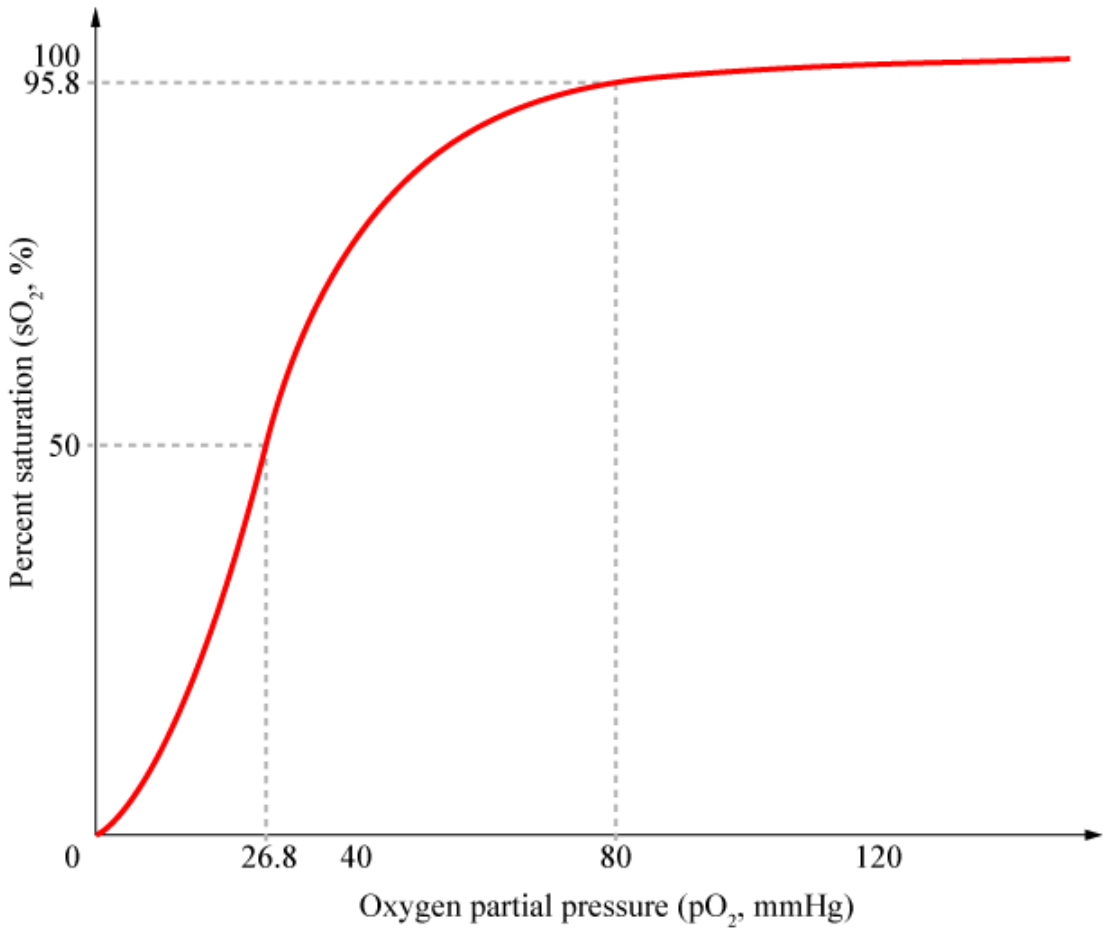
In similar fashion, hemoglobin also has competitive binding affinity for cyanide (CN^-), sulfur monoxide (SO), nitric oxide (NO), and sulfide(S^{2-}), including hydrogen sulfide (H_2S). All of these bind to iron in heme without changing its oxidation state, but they nevertheless inhibit oxygen-binding, causing grave toxicity.

The iron atom in the heme group must initially be in the ferrous (Fe^{2+}) oxidation state to support oxygen and other gases' binding and transport (it temporarily switches to ferric during the time oxygen is bound, as explained above). Initial oxidation to the ferric (Fe^{3+}) state without oxygen converts hemoglobin into "hem**i**globin" or methemoglobin (pronounced "MET-hemoglobin"), which cannot bind oxygen. Hemoglobin in normal red blood cells is protected by a reduction system to keep this from happening. Nitric oxide is capable of converting a small fraction of hemoglobin to methemoglobin in red blood cells. The latter reaction is a remnant activity of the more ancient nitric oxide dioxygenase function of globins.

Allosteric

Carbon *di*oxide occupies a different binding site on the hemoglobin. Carbon dioxide is more readily dissolved in deoxygenated blood, facilitating its removal from the body after the oxygen has been released to tissues undergoing metabolism. This increased affinity for carbon dioxide by the venous blood is known as the Haldane effect. Through the enzyme carbonic anhydrase, carbon dioxide reacts with water to give carbonic acid, which decomposes into bicarbonate and protons:

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+$$



The sigmoidal shape of hemoglobin's oxygen-dissociation curve results from cooperative binding of oxygen to hemoglobin.

Hence blood with high carbon dioxide levels is also lower in pH (more acidic). Hemoglobin can bind protons and carbon dioxide, which causes a conformational change in the protein and facilitates the release of oxygen. Protons bind at various places on the protein, while carbon dioxide binds at the α-amino group. Carbon dioxide binds to hemoglobin and forms carbaminohemoglobin. This decrease in hemoglobin's affinity for oxygen by the binding of carbon dioxide and acid is known as the Bohr effect (shifts the O_2-saturation curve to the *right*). Conversely, when the carbon dioxide levels in the blood decrease (i.e., in the lung capillaries), carbon dioxide and protons are released from hemoglobin, increasing the oxygen affinity of the protein.

It is necessary for hemoglobin to release the oxygen that it binds; if not, there is no point in binding it. The sigmoidal curve of hemoglobin makes it efficient in binding (taking up O_2 in lungs), and efficient in unloading (unloading O_2 in tissues).

In people acclimated to high altitudes, the concentration of 2,3-Bisphosphoglycerate (2,3-BPG) in the blood is increased, which allows these individuals to deliver a larger amount

of oxygen to tissues under conditions of lower oxygen tension. This phenomenon, where molecule Y affects the binding of molecule X to a transport molecule Z, is called a *heterotropic* allosteric effect.

A variant hemoglobin, called fetal hemoglobin (HbF, $\alpha_2\gamma_2$), is found in the developing fetus, and binds oxygen with greater affinity than adult hemoglobin. This means that the oxygen binding curve for fetal hemoglobin is left-shifted (i.e., a higher percentage of hemoglobin has oxygen bound to it at lower oxygen tension), in comparison to that of adult hemoglobin. As a result, fetal blood in the placenta is able to take oxygen from maternal blood.

Hemoglobin also carries nitric oxide in the globin part of the molecule. This improves oxygen delivery in the periphery and contributes to the control of respiration. NO binds reversibly to a specific cysteine residue in globin; the binding depends on the state (R or T) of the hemoglobin. The resulting S-nitrosylated hemoglobin influences various NO-related activities such as the control of vascular resistance, blood pressure and respiration. NO is not released in the cytoplasm of erythrocytes but transported by an anion exchanger called AE1 out of them.

A study was performed to examine the influence of the form of hemoglobin (Hb) on the partitioning of inhaled volatile organic compounds (VOCs) into [human and animal] blood. Benzene was the prototypic VOC used in the investigations for this research due to the similar properties it shares with many other VOCs. To be specific, this study analyses the influence of the water solubility of Hb on the partitioning coefficient (PC) of a VOC as compared to the influence of the "species" or form of Hb. The different forms of blood used include: human hemoglobin (HbA), rat Hb, and sickle-cell hemoglobin (HbS). Rat Hb contains little water and is in a quasi-crystalline form, found inside the red blood cells (RBC), meaning they are more hydrophobic than human Hb, which are water-soluble. Sickle-cell hemoglobin (HbS) is water-soluble, however it can become water-insoluble, forming hydrophobic polymers, when deoxygenated. The findings state that the benzene PC for rat Hb was much higher than human that for Hb; however, the tests that measured the PCs of the oxygenated and deoxygenated forms of HbA and HbS did not differ, indicating that the affinity of benzene was not affected by the water solubility of Hb.

Types in humans

Hemoglobin variants are a part of the normal embryonic and fetal development, but may also be pathologic mutant forms of hemoglobin in a population, caused by variations in genetics. Some well-known hemoglobin variants such as sickle-cell anemia are responsible for diseases, and are considered hemoglobinopathies. Other variants cause no detectable pathology, and are thus considered non-pathological variants.

In the embryo:

- Gower 1 ($\zeta_2\varepsilon_2$)

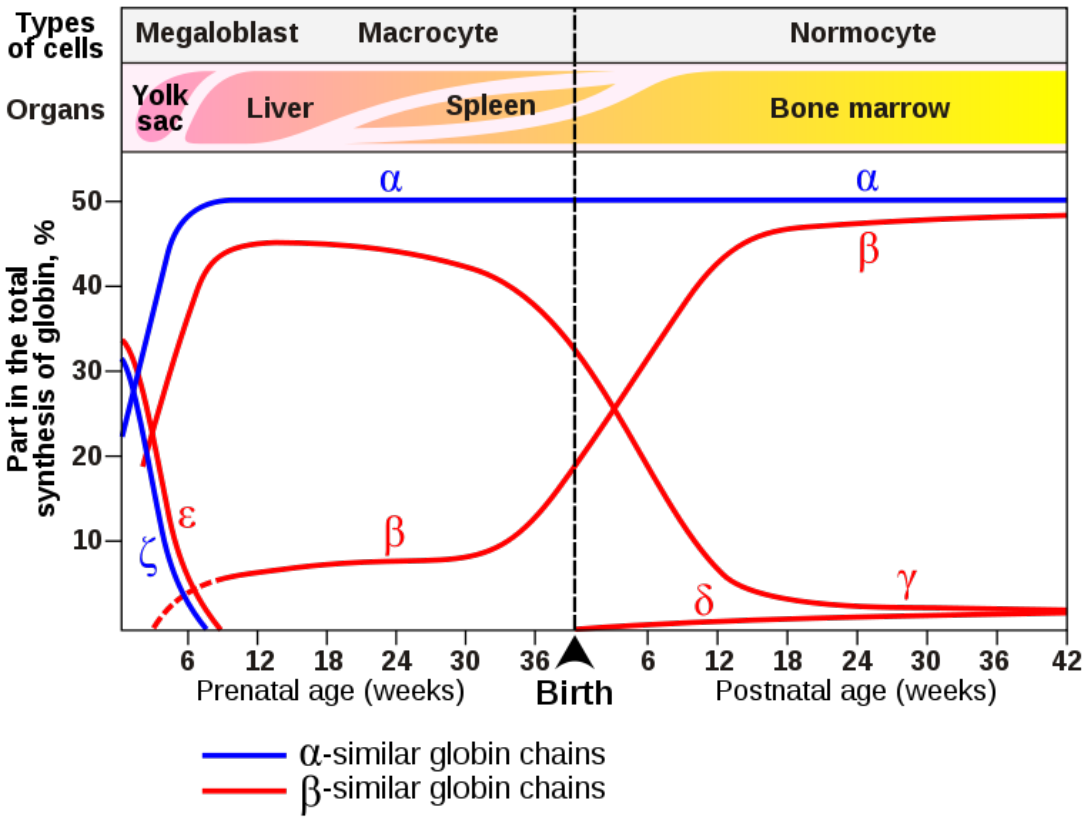
- Gower 2 ($\alpha_2\varepsilon_2$) (PDB 1A9W)
- Hemoglobin Portland ($\zeta_2\gamma_2$)

In the fetus:

- Hemoglobin F ($\alpha_2\gamma_2$) (PDB 1FDH)

In adults:

- Hemoglobin A ($\alpha_2\beta_2$) (PDB 1BZ0) - The most common with a normal amount over 95%
- Hemoglobin A_2 ($\alpha_2\delta_2$) - δ chain synthesis begins late in the third trimester and in adults, it has a normal range of 1.5-3.5%
- Hemoglobin F ($\alpha_2\gamma_2$) - In adults Hemoglobin F is restricted to a limited population of red cells called F-cells. However, the level of Hb F can be elevated in persons with sickle-cell disease and beta-thalassemia.



Gene expression of hemoglobin before and after birth. Also identifies the types of cells and organs in which the gene expression (data on *Wood W.G.*, (1976). **Br. Med. Bull. 32, 282.**)

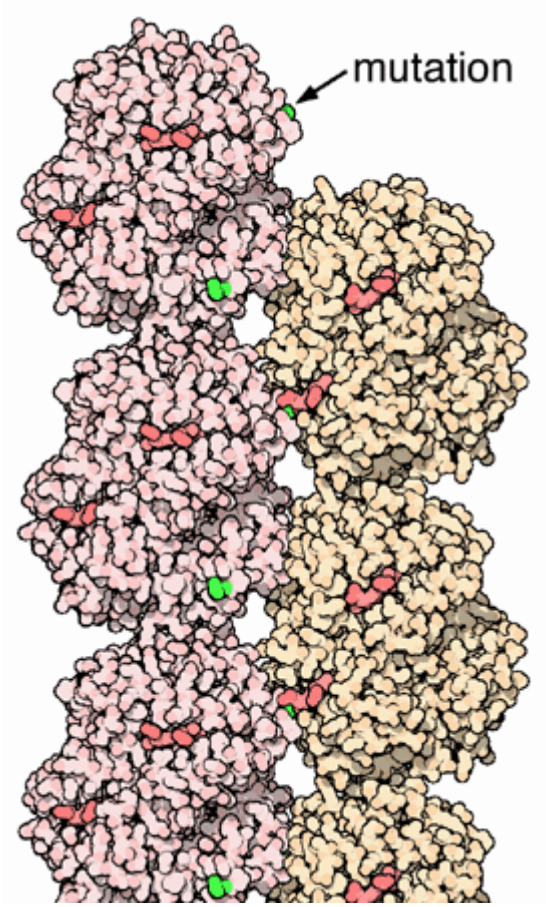
Variant forms that cause disease:

- Hemoglobin H (β_4) - A variant form of hemoglobin, formed by a tetramer of β chains, which may be present in variants of α thalassemia.
- Hemoglobin Barts (γ_4) - A variant form of hemoglobin, formed by a tetramer of γ chains, which may be present in variants of α thalassemia.
- Hemoglobin S ($\alpha_2\beta^S_2$) - A variant form of hemoglobin found in people with sickle cell disease. There is a variation in the β-chain gene, causing a change in the properties of hemoglobin, which results in sickling of red blood cells.
- Hemoglobin C ($\alpha_2\beta^C_2$) - Another variant due to a variation in the β-chain gene. This variant causes a mild chronic hemolytic anemia.
- Hemoglobin E ($\alpha_2\beta^E_2$) - Another variant due to a variation in the β-chain gene. This variant causes a mild chronic hemolytic anemia.
- Hemoglobin AS - A heterozygous form causing Sickle cell trait with one adult gene and one sickle cell disease gene
- Hemoglobin SC disease - Another heterozygous form with one sickle gene and another encoding Hemoglobin C.

Degradation in vertebrate animals

When red cells reach the end of their life due to aging or defects, they are broken down, the hemoglobin molecule is broken up and the iron gets recycled. When the porphyrin ring is broken up, the fragments are normally secreted in the bile by the liver. This process also produces one molecule of carbon monoxide for every molecule of heme degraded. This is one of the few natural sources of carbon monoxide production in the human body, and is responsible for the normal blood levels of carbon monoxide even in people breathing pure air. The other major final product of heme degradation is bilirubin. Increased levels of this chemical are detected in the blood if red cells are being destroyed more rapidly than usual. Improperly degraded hemoglobin protein or hemoglobin that has been released from the blood cells too rapidly can clog small blood vessels, especially the delicate blood filtering vessels of the kidneys, causing kidney damage.

Role in disease



In sickle cell hemoglobin (HbS) glutamic acid in position 6 (in beta chain) is mutated to valine. This change allows the deoxygenated form of the hemoglobin to stick to each other.

Hemoglobin deficiency can be caused either by decreased amount of hemoglobin molecules, as in anemia, or by decreased ability of each molecule to bind oxygen at the same partial pressure of oxygen. Hemoglobinopathies (genetic defects resulting in abnormal structure of the hemoglobin molecule) may cause both. In any case, hemoglobin deficiency decreases blood oxygen-carrying capacity. Hemoglobin deficiency is, in general, strictly distinguished from hypoxemia, defined as decreased partial pressure of oxygen in blood, although both are causes of hypoxia (insufficient oxygen supply to tissues).

Other common causes of low hemoglobin include loss of blood, nutritional deficiency, bone marrow problems, chemotherapy, kidney failure, or abnormal hemoglobin (such as that of sickle-cell disease).

High hemoglobin levels may be caused by exposure to high altitudes, smoking, dehydration, or tumors.

The ability of each hemoglobin molecule to carry oxygen is normally modified by altered blood pH or CO_2, causing an altered oxygen-hemoglobin dissociation curve. However, it can also be pathologically altered in, e.g., carbon monoxide poisoning.

Decrease of hemoglobin, with or without an absolute decrease of red blood cells, leads to symptoms of anemia. Anemia has many different causes, although iron deficiency and its resultant iron deficiency anemia are the most common causes in the Western world. As absence of iron decreases heme synthesis, red blood cells in iron deficiency anemia are *hypochromic* (lacking the red hemoglobin pigment) and *microcytic* (smaller than normal). Other anemias are rarer. In hemolysis (accelerated breakdown of red blood cells), associated jaundice is caused by the hemoglobin metabolite bilirubin, and the circulating hemoglobin can cause renal failure.

Some mutations in the globin chain are associated with the hemoglobinopathies, such as sickle-cell disease and thalassemia. Other mutations, as discussed at the beginning, are benign and are referred to merely as hemoglobin variants.

There is a group of genetic disorders, known as the *porphyrias* that are characterized by errors in metabolic pathways of heme synthesis. King George III of the United Kingdom was probably the most famous porphyria sufferer.

To a small extent, hemoglobin A slowly combines with glucose at the terminal valine (an alpha aminoacid) of each β chain. The resulting molecule is often referred to as Hb A_{1c}. As the concentration of glucose in the blood increases, the percentage of Hb A that turns into Hb A_{1c} increases. In diabetics whose glucose usually runs high, the percent Hb A_{1c} also runs high. Because of the slow rate of Hb A combination with glucose, the Hb A_{1c} percentage is representative of glucose level in the blood averaged over a longer time (the half-life of red blood cells, which is typically 50–55 days).

Glycosylated hemoglobin is the form of hemoglobin to which glucose is bound. The binding of glucose to amino acids in the hemoglobin takes place spontaneously (without the help of an enzyme) in many proteins, and is not known to serve a useful purpose. However, the binding to hemoglobin does serve as a record for average blood glucose levels over the life time of red cells, which is approximately 120 days. The levels of glycosylated hemoglobin are therefore measured in order to monitor the long-term control of the chronic disease of type 2 diabetes mellitus (T2DM). Poor control of T2DM results in high levels of glycosylated hemoglobin in the red blood cells. The normal reference range is approximately 4–5.9 %. Though difficult to obtain, values less than 7 % are recommended for people with T2DM. Levels greater than 9 % are associated with poor control of the glycosylated hemoglobin, and levels greater than 12 % are associated with very poor control. Diabetics who keep their glycosylated hemoglobin levels close to 7 % have a much better chance of avoiding the complications that may accompany diabetes (than those whose levels are 8 % or higher).

Elevated levels of hemoglobin are associated with increased numbers or sizes of red blood cells, called polycythemia. This elevation may be caused by congenital heart

disease, cor pulmonale, pulmonary fibrosis, too much erythropoietin, or polycythemia vera.

Elevation in levels of hemoglobin were found in one study of the yogic practice of Yoga Nidra (yogic sleep) for half an hour daily.

Diagnostic uses

Hemoglobin concentration measurement is among the most commonly performed blood tests, usually as part of a complete blood count. For example it is typically tested before or after blood donation. Results are reported in g/L, g/dL or mol/L. 1 g/dL equals about 0.6206 mmol/L. Normal levels are:

- Men: 13.8 to 18.0 g/dL (138 to 182 g/L, or 8.56 to 11.3 mmol/L)
- Women: 12.1 to 15.1 g/dL (121 to 151 g/L, or 7.51 to 9.37 mmol/L)
- Children: 11 to 16 g/dL (111 to 160 g/L, or 6.83 to 9.93 mmol/L)
- Pregnant women: 11 to 12 g/dL (110 to 120 g/L, or 6.83 to 7.45 mmol/L)

Normal values of hemoglobin in the 1st and 3rd trimesters of pregnant women must be at least 11 g/dL and at least 10.5 g/dL during the 2nd trimester.

If the concentration is below normal, this is called anemia. Anemias are classified by the size of red blood cells, the cells that contain hemoglobin in vertebrates. The anemia is called "microcytic" if red cells are small, "macrocytic" if they are large, and "normocytic" otherwise.

Hematocrit, the proportion of blood volume occupied by red blood cells, is typically about three times the hemoglobin level. For example, if the hemoglobin is measured at 17, that compares with a hematocrit of 51.

Long-term control of blood sugar concentration can be measured by the concentration of Hb A_{1c}. Measuring it directly would require many samples because blood sugar levels vary widely through the day. Hb A_{1c} is the product of the irreversible reaction of hemoglobin A with glucose. A higher glucose concentration results in more Hb A_{1c}. Because the reaction is slow, the Hb A_{1c} proportion represents glucose level in blood averaged over the half-life of red blood cells, is typically 50–55 days. An Hb A_{1c} proportion of 6.0% or less show good long-term glucose control, while values above 7.0% are elevated. This test is especially useful for diabetics.

The functional magnetic resonance imaging (fMRI) machine uses the signal from deoxyhemoglobin, which is sensitive to magnetic fields since it is paramagnetic.

Analogues in non-vertebrate organisms

A variety of oxygen-transport and -binding proteins exist in organisms throughout the animal and plant kingdoms. Organisms including bacteria, protozoans, and fungi all have

hemoglobin-like proteins whose known and predicted roles include the reversible binding of gaseous ligands. Since many of these proteins contain globins and the heme moiety (iron in a flat porphyrin support), they are often called hemoglobins, even if their overall tertiary structure is very different from that of vertebrate hemoglobin. In particular, the distinction of "myoglobin" and hemoglobin in lower animals is often impossible, because some of these organisms do not contain muscles. Or, they may have a recognizable separate circulatory system but not one that deals with oxygen transport (for example, many insects and other arthropods). In all these groups, heme/globin-containing molecules (even monomeric globin ones) that deal with gas-binding are referred to as oxyhemoglobins. In addition to dealing with transport and sensing of oxygen, they may also deal with NO, CO_2, sulfide compounds, and even O_2 scavenging in environments that must be anaerobic. They may even deal with detoxification of chlorinated materials in a way analogous to heme-containing P450 enzymes and peroxidases.

The giant tube worm *Riftia pachyptila* showing red hemoglobin-containing plumes

The structure of hemoglobins varies across species. Hemoglobin occurs in all kingdoms of organisms, but not in all organisms. Primitive species such as bacteria, protozoa, algae, and plants often have single-globin hemoglobins. Many nematode worms, molluscs, and crustaceans contain very large multisubunit molecules, much larger than those in vertebrates. In particular, chimeric hemoglobins found in fungi and giant annelids may contain both globin and other types of proteins.

One of the most striking occurrences and uses of hemoglobin in organisms is in the giant tube worm (*Riftia pachyptila*, also called Vestimentifera), which can reach 2.4 meters

length and populates ocean volcanic vents. Instead of a digestive tract, these worms contain a population of bacteria constituting half the organism's weight. The bacteria react with H_2S from the vent and O_2 from the water to produce energy to make food from H_2O and CO_2. The worms end with a deep red fan-like structure ("plume"), which extends into the water and absorbs H_2S and O_2 for the bacteria, and CO_2 for use as synthetic raw material similar to photosynthetic plants. The structures are bright-red due to their containing several extraordinarily complex hemoglobins that have up to 144 globin chains, each including associated heme structures. These hemoglobins are remarkable for being able to carry oxygen in the presence of sulfide, and even to carry sulfide, without being completely "poisoned" or inhibited by it as hemoglobins in most other species are.

Other oxygen-binding proteins

Myoglobin: Found in the muscle tissue of many vertebrates, including humans, it gives muscle tissue a distinct red or dark gray color. It is very similar to hemoglobin in structure and sequence, but is not a tetramer; instead, it is a monomer that lacks cooperative binding. It is used to store oxygen rather than transport it.

Hemocyanin: The second most common oxygen-transporting protein found in nature, it is found in the blood of many arthropods and molluscs. Uses copper prosthetic groups instead of iron heme groups and is blue in color when oxygenated.

Hemerythrin: Some marine invertebrates and a few species of annelid use this iron-containing non-heme protein to carry oxygen in their blood. Appears pink/violet when oxygenated, clear when not.

Chlorocruorin: Found in many annelids, it is very similar to erythrocruorin, but the heme group is significantly different in structure. Appears green when deoxygenated and red when oxygenated.

Vanabins: Also known as **vanadium chromagens**, they are found in the blood of sea squirts. There were once hypothesized to use the rare metal vanadium as an oxygen binding prosthetic group. However, although they do contain vandadium by preference, they apparently bind little oxygen, and thus have some other function, which has not been elucidated (sea squirts also contain some hemoglobin). They may act as toxins.

Erythrocruorin: Found in many annelids, including earthworms, it is a giant free-floating blood protein containing many dozens—possibly hundreds—of iron- and heme-bearing protein subunits bound together into a single protein complex with a molecular mass greater than 3.5 million daltons.

Pinnaglobin: Only seen in the mollusc *Pinna squamosa*. Brown manganese-based porphyrin protein.

Leghemoglobin: In leguminous plants, such as alfalfa or soybeans, the nitrogen fixing bacteria in the roots are protected from oxygen by this iron heme containing oxygen-binding protein. The specific enzyme protected is nitrogenase, which is unable to reduce nitrogen gas in the presence of free oxygen.

Coboglobin: A synthetic cobalt-based porphyrin. Coboprotein would appear colorless when oxygenated, but yellow when in veins.

Presence in nonerythroid cells

Some nonerythroid cells (i.e., cells other than the red blood cell line) contain hemoglobin. In the brain, these include the A9 dopaminergic neurons in the substantia nigra, astrocytes in the cerebral cortex and hippocampus, and in all mature oligodendrocytes. It has been suggested that brain hemoglobin in these cell may enable the "storage of oxygen to provide a homeostatic mechanism in anoxic conditions, which is especially important for A9 DA neurons that have an elevated metabolism with a high requirement for energy production". It has been noted further that "A9 dopaminergic neurons may be at particular risk since in addition to their high mitochondrial activity they are under intense oxidative stress caused by the production of hydrogen peroxide via autoxidation and/or monoamine oxidase (MAO)-mediated deamination of dopamine and the subsequent reaction of accessible ferrous iron to generate highly toxic hydroxyl radicals". This may explain the risk of these cells for degeneration in Parkinson's disease. The presence of iron from hemoglobin in these cells also results in the post-mortem darkness of these cells, which is the origin of the Latin name, substantia *nigra*.

Outside the brain, hemoglobin has non-oxygen-carrying functions as an antioxidant and a regulator of iron metabolism in macrophages, alveolar cells, and mesangial cells in the kidney.

In history, art and music

The planet Mars

Historically, the color of blood was associated with rust, as ancient Romans associated the planet Mars with the god of war since Mars is orange-red. The color of Mars is due to iron-oxygen in the Martian soil, but the red in blood is not due to the iron in hemoglobin and its oxides, which is a common misconception. The red is due to the porphyrin moiety of hemoglobin to which the iron is bound, not the iron itself, although the ligation and redox state of the iron can influence the pi to pi* electronic transitions of the porphyrin and hence its optical characteristics.

Heart of Steel (Hemoglobin) (2005) by Julian Voss-Andreae. The images show the 5' (1.60 m) tall sculpture right after installation, after 10 days, and after several months of exposure to the elements.

Artist Julian Voss-Andreae created a sculpture called "Heart of Steel (Hemoglobin)" in 2005, based on the protein's backbone. The sculpture was made from glass and weathering steel. The intentional rusting of the initially shiny work of art mirrors hemoglobin's fundamental chemical reaction of oxygen binding to iron.

Rock band Placebo recorded a song called "Haemoglobin" with the lyrics "Haemoglobin is the key to a healthy heartbeat".

Chapter 4

Platelet

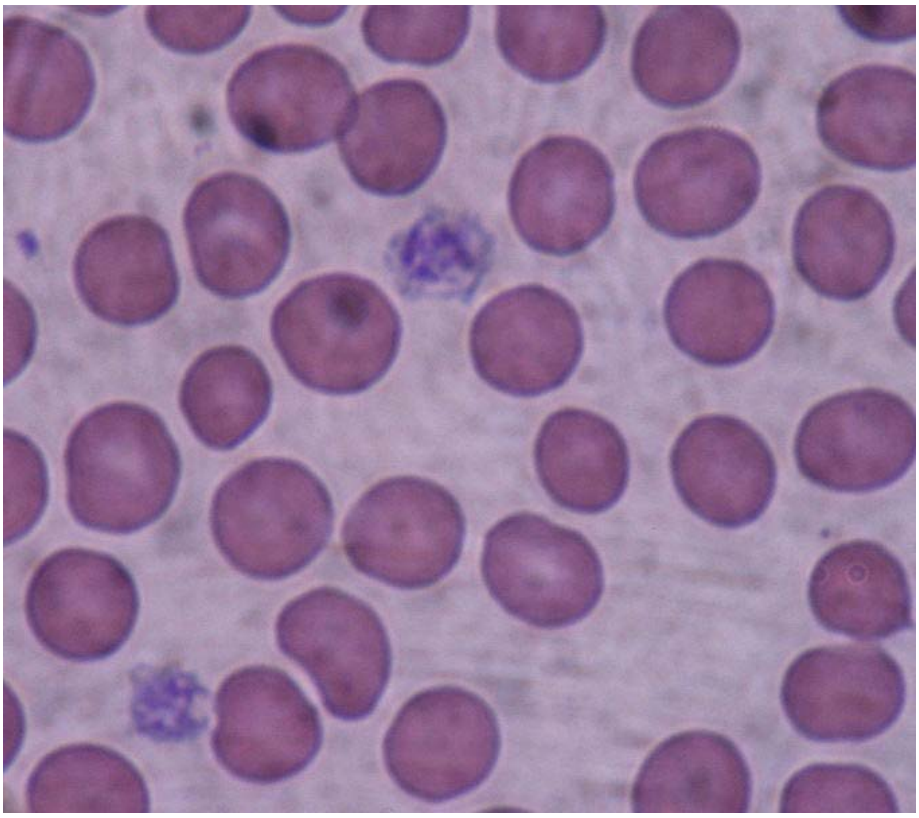


Image from a light microscope (40x) from a peripheral blood smear surrounded by red blood cells. One platelet can be seen in the upper left side of the image (purple) and is significantly smaller in size than the red blood cells (stained pink) and the two large platelets (stained purple).

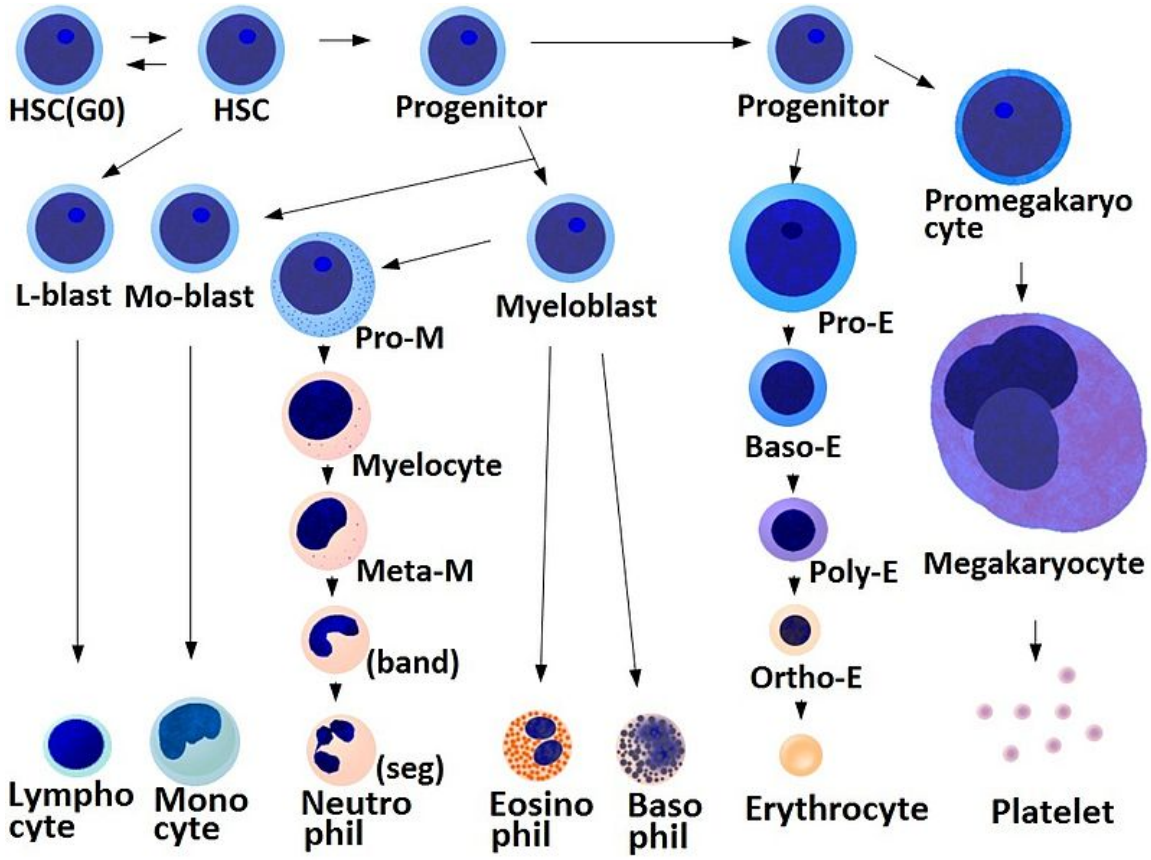
Platelets, or **thrombocytes** (from Greek θρόμβος, "clot" and κύτος, "cell"), are small, regularly-shaped clear cell fragments (i.e. cells that do not have a nucleus containing DNA), 2-3 µm in diameter, which are derived from fragmentation of precursor megakaryocytes. The average lifespan of a platelet is normally just 5 to 9 days. Platelets play a fundamental role in hemostasis and are a natural source of growth factors. They

circulate in the blood of mammals and are involved in hemostasis, leading to the formation of blood clots.

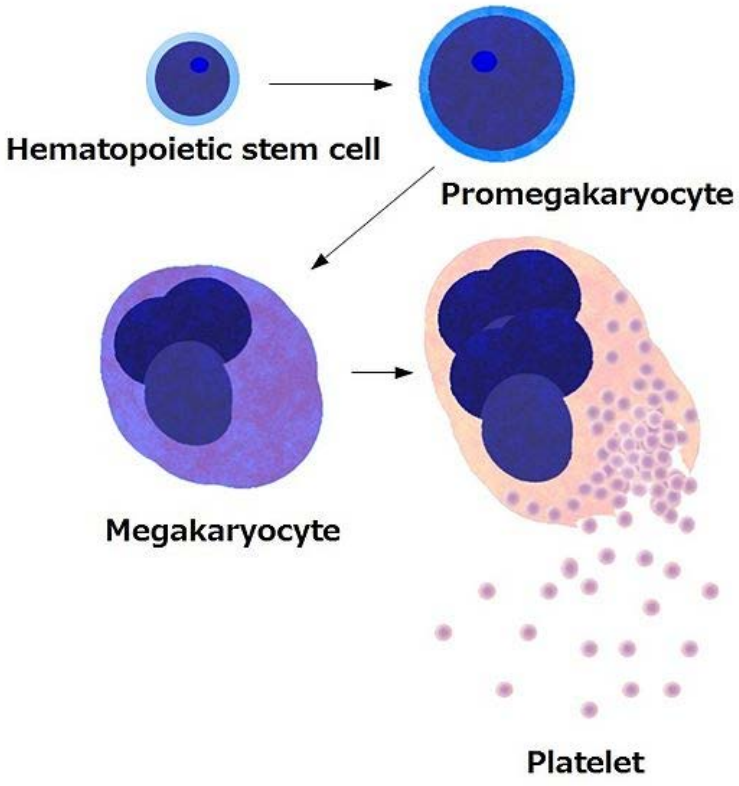
If the number of platelets is too low, excessive bleeding can occur. However, if the number of platelets is too high, blood clots can form (thrombosis), which may obstruct blood vessels and result in such events as a stroke, myocardial infarction, pulmonary embolism or the blockage of blood vessels to other parts of the body, such as the extremities of the arms or legs. An abnormality or disease of the platelets is called a thrombocytopathy, which could be either a low number of platelets (thrombocytopenia), a decrease in function of platelets (thrombasthenia), or an increase in the number of platelets (thrombocytosis). There are disorders that reduce the number of platelets, such as heparin-induced thrombocytopenia (HIT) or thrombotic thrombocytopenic purpura (TTP) that typically cause thromboses, or clots, instead of bleeding.

Platelets release a multitude of growth factors including Platelet-derived growth factor (PDGF), a potent chemotactic agent, and TGF beta, which stimulates the deposition of extracellular matrix. Both of these growth factors have been shown to play a significant role in the repair and regeneration of connective tissues. Other healing-associated growth factors produced by platelets include basic fibroblast growth factor, insulin-like growth factor 1, platelet-derived epidermal growth factor, and vascular endothelial growth factor. Local application of these factors in increased concentrations through Platelet-rich plasma (PRP) has been used as an adjunct to wound healing for several decades.

Kinetics



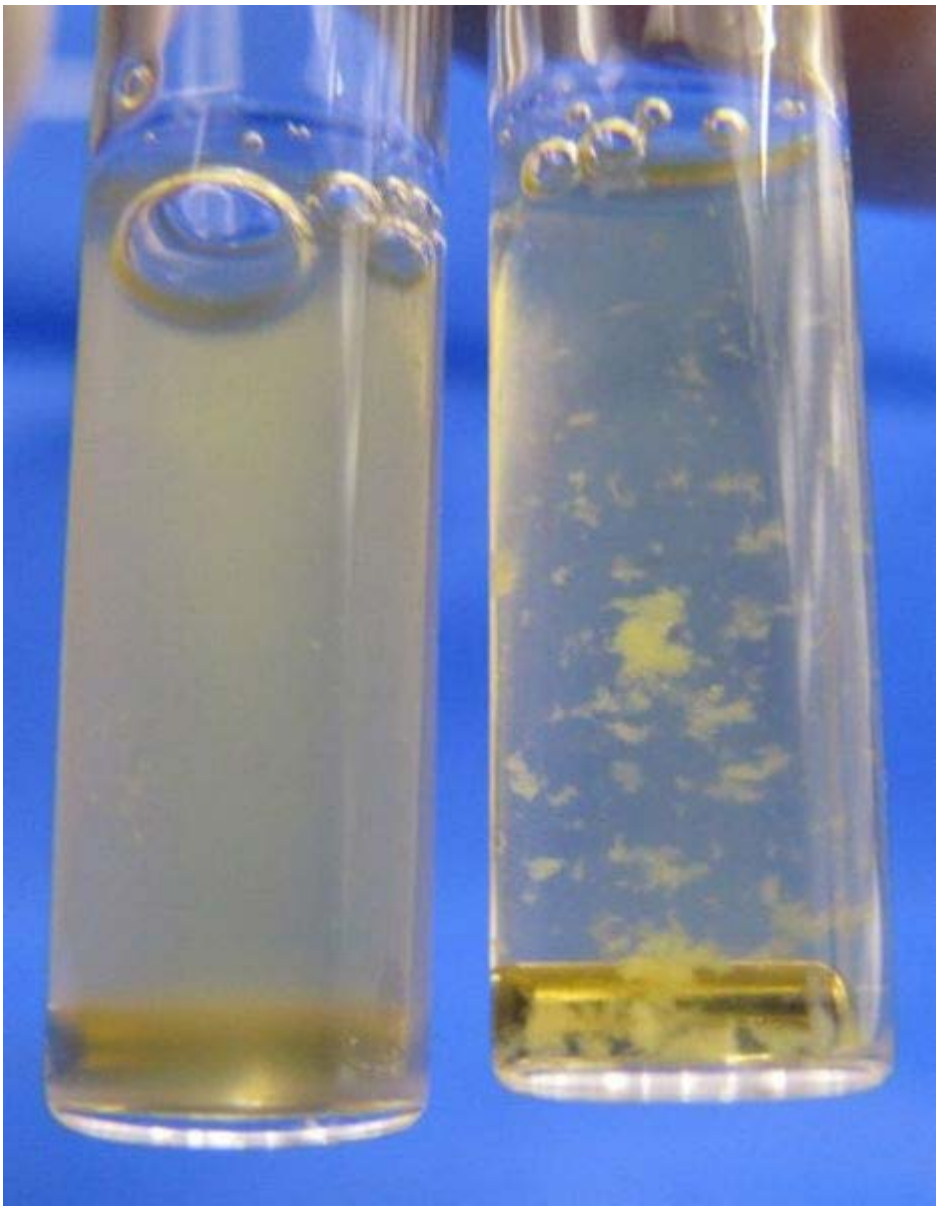
HSC=Hematopoietic stem cell, Progenitor=Progenitor cell, L-blast=Lymphoblast, Lymphocyte, Mo-blast=Monoblast, Monocyte, Myeloblast, Pro-M=Promyelocyte, Myelocyte, Meta-M=Metamyelocyte, Neutrophil, Eosinophil, Basophil, Pro-E=Proerythroblast, Baso-E=Basophilic erythroblast, poly-E=Polychromatic erythroblast ,Ortho-E=Orthochromatic erythroblast, Erythrocyte, Promegakaryocyte, Megakaryocyte , Platlet



Blood cell lineage

- Platelets are produced in blood cell formation (thrombopoiesis) in bone marrow, by budding off from megakaryocytes.
- The physiological range for platelets is 150-400 x 10^9 per liter.
- Around 1 x 10^{11} platelets are produced each day by an average healthy adult.
- The lifespan of circulating platelets is 5 to 9 days.
- Megakaryocyte and platelet production is regulated by thrombopoietin, a hormone usually produced by the liver and kidneys.
- Each megakaryocyte produces between 5,000 and 10,000 platelets.
- Old platelets are destroyed by phagocytosis in the spleen and by Kupffer cells in the liver.
- A reserve of platelets are stored in the spleen and are released when needed by sympathetically-induced splenic contraction.

Thrombus formation



Aggregation of platelets. Platelet rich human blood plasma (left vial) is a turbid liquid. Upon addition of ADP, platelets are activated and start to aggregate, forming white flakes (right vial).

The function of platelets is the maintenance of hemostasis. This is achieved primarily by the formation of thrombi, when damage to the endothelium of blood vessels occurs. On the converse, thrombus formation must be inhibited at times when there is no damage to the endothelium.

Activation

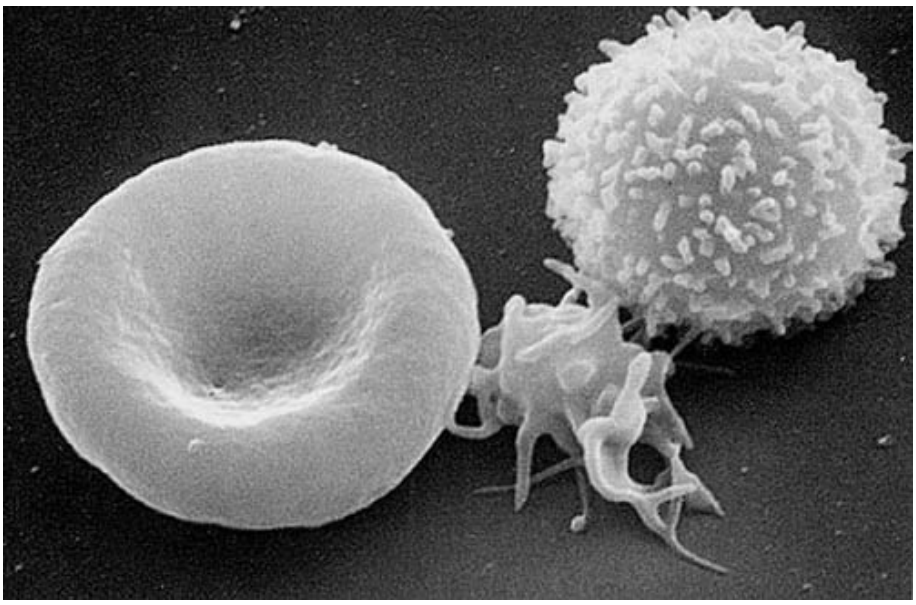
The inner surface of blood vessels is lined with a thin layer of endothelial cells that, in normal hemostasis, acts to inhibit platelet activation by producing nitric oxide, endothelial-ADPase, and PGI_2. Endothelial-ADPase clears away the platelet activator, ADP.

Endothelial cells produce a protein called von Willebrand factor (vWF), a cell adhesion ligand, which helps endothelial cells adhere to collagen in the basement membrane. Under physiological conditions, collagen is not exposed to the bloodstream. vWF is secreted constitutively into the plasma by the endothelial cells, and is stored in granules within the endothelial cell and in platelets.

When the endothelial layer is injured, collagen, vWF and tissue factor from the subendothelium is exposed to the bloodstream. When the platelets contact collagen or vWF, they are activated (e.g. to clump together). They are also activated by thrombin (formed with the help of tissue factor). They can also be activated by a negatively-charged surface, such as glass.

Platelet activation further results in the scramblase-mediated transport of negatively-charged phospholipids to the platelet surface. These phospholipids provide a catalytic surface (with the charge provided by phosphatidylserine and phosphatidylethanolamine) for the tenase and prothrombinase complexes. Calcium ions are essential for binding of these coagulation factors.

Shape change



Scanning electron micrograph of blood cells. From left to right: human erythrocyte, activated **thrombocyte** (platelet), leukocyte.

Activated platelets change in shape to become more spherical, and pseudopods form on their surface. Thus they assume a stellate shape.

Granule secretion

Platelets contain alpha and dense granules. Activated platelets excrete the contents of these granules into their canalicular systems and into surrounding blood. There are three types of granules:

- dense (or delta) granules (containing ADP or ATP, calcium, and serotonin)
- lambda granules - similar to lysosomes and contain several hydrolytic enzymes.
- Alpha granules (containing platelet factor 4, transforming growth factor-β1, platelet-derived growth factor, fibronectin, B-thromboglobulin, vWF, fibrinogen, and coagulation factors V and XIII).

Thromboxane A2 synthesis

Platelet activation initiates the arachidonic acid pathway to produce TXA_2. TXA_2 is involved in activating other platelets and its formation is inhibited by COX inhibitors, such as aspirin.

Adhesion and aggregation

Platelets aggregate, or clump together, using fibrinogen and vWF as a connecting agent. The most abundant platelet aggregation receptor is glycoprotein IIb/IIIa (gpIIb/IIIa); this is a calcium-dependent receptor for fibrinogen, fibronectin, vitronectin, thrombospondin, and von Willebrand factor (vWF). Other receptors include GPIb-V-IX complex (vWF) and GPVI (collagen).

Activated platelets will adhere, via glycoprotein (GP) Ia, to the collagen that is exposed by endothelial damage. Aggregation and adhesion act together to form the platelet plug. Myosin and actin filaments in platelets are stimulated to contract during aggregation, further reinforcing the plug.

Platelet aggregation is stimulated by ADP, thromboxane, and α2 receptor-activation, but inhibited by other inflammatory products like PGI2 and PGD2. Platelet aggregation is enhanced by exogenous administration of anabolic steroids.

Wound repair

The blood clot is only a temporary solution to stop bleeding; vessel repair is therefore needed. The aggregated platelets help this process by secreting chemicals that promote the invasion of fibroblasts from surrounding connective tissue into the wounded area to completely heal the wound or form a scar. The obstructing clot is slowly dissolved by the fibrinolytic enzyme, plasmin, and the platelets are cleared by phagocytosis.

Other functions

- Clot retraction
- Pro-coagulation
- Inflammation
- Cytokine signalling
- Phagocytosis

Cytokine signaling

In addition to being the chief cellular effector of hemostasis, platelets are rapidly deployed to sites of injury or infection, and potentially modulate inflammatory processes by interacting with leukocytes and by secreting cytokines, chemokines, and other inflammatory mediators . Platelets also secrete platelet-derived growth factor (PDGF).

Role in disease

High and low counts

A normal platelet count in a healthy individual is between 150,000 and 450,000 per µl (microlitre) of blood (150–450 x 10^9/L). Ninety-five percent of healthy people will have platelet counts in this range. Some will have statistically abnormal platelet counts while having no demonstrable abnormality. However, if it is either very low or very high, the likelihood of an abnormality being present is higher.

Both thrombocytopenia and thrombocytosis may present with coagulation problems. In general, low platelet counts increase bleeding risks; however there are exceptions. For example, immune heparin-induced thrombocytopenia and thrombocytosis (high counts) may lead to thrombosis, although this is mainly when the elevated count is due to myeloproliferative disorder.

Low platelet counts are, in general, not corrected by transfusion unless the patient is bleeding or the count has fallen below 5 x 10^9/L. Transfusion is contraindicated in thrombotic thrombocytopenic purpura (TTP), as it fuels the coagulopathy. In patients undergoing surgery, a level below 50 x 10^9/L is associated with abnormal surgical bleeding, and regional anaesthetic procedures such as epidurals are avoided for levels below 80-100.

Normal platelet counts are not a guarantee of adequate function. In some states, the platelets, while being adequate in number, are *dysfunctional*. For instance, aspirin irreversibly disrupts platelet function by inhibiting cyclooxygenase-1 (COX1), and hence normal hemostasis. The resulting platelets are unable to produce new cyclooxygenase because they have no DNA. Normal platelet function will not return until the use of aspirin has ceased and enough of the affected platelets have been replaced by new ones, which can take over a week. Ibuprofen, another NSAID, does not have such a long duration effect, with platelet function usually returning within 24 hours, and taking

ibuprofen before aspirin will prevent the irreversible effects of aspirin. Uremia, a consequence of renal failure, leads to platelet dysfunction that may be ameliorated by the administration of desmopressin.

Medications

Oral agents, often used to alter/suppress platelet function: aspirin, clopidogrel, cilostazol, ticlopidine.

Intravenous agents, often used to alter/suppress platelet function: abciximab, eptifibatide, tirofiban.

Diseases

Disorders leading to a reduced platelet count:

- Thrombocytopenia
  - Idiopathic thrombocytopenic purpura - also known as immune thrombocytopenic purpura (ITP)
  - Thrombotic thrombocytopenic purpura
  - Drug-induced thrombocytopenic purpura (for example heparin-induced thrombocytopenia (HIT))
- Gaucher's disease
- Aplastic anemia
- Onyalai

Alloimmune disorders

- Fetomaternal alloimmune thrombocytopenia
- Some transfusion reactions

Disorders leading to platelet dysfunction or reduced count:

- HELLP syndrome
- Hemolytic-uremic syndrome
- Chemotherapy
- Dengue

Disorders featuring an elevated count:

- Thrombocytosis, including essential thrombocytosis (elevated counts, either reactive or as an expression of myeloproliferative disease); may feature dysfunctional platelets

Disorders of platelet adhesion or aggregation:

- Bernard-Soulier syndrome
- Glanzmann's thrombasthenia
- Scott's syndrome
- von Willebrand disease
- Hermansky-Pudlak Syndrome
- Gray platelet syndrome

Disorders of platelet metabolism

- Decreased cyclooxygenase activity, induced or congenital
- Storage pool defects, acquired or congenital

Disorders that indirectly compromise platelet function:

- Haemophilia

Disorders in which platelets play a key role:

- Atherosclerosis
- Coronary artery disease, CAD and myocardial infarction, MI
- Cerebrovascular disease and Stroke, CVA (cerebrovascular accident)
- Peripheral artery occlusive disease (PAOD)
- Cancer
- Malaria

Condition	Prothrombin time	Partial thromboplastin time	Bleeding time	Platelet count
Vitamin K deficiency or warfarin	prolonged	prolonged	unaffected	unaffected
Disseminated intravascular coagulation	prolonged	prolonged	prolonged	decreased
Von Willebrand disease	unaffected	prolonged	prolonged	unaffected
Haemophilia	unaffected	prolonged	unaffected	unaffected
Aspirin	unaffected	unaffected	prolonged	unaffected
Thrombocytopenia	unaffected	unaffected	prolonged	decreased
Early Liver failure	prolonged	unaffected	unaffected	unaffected
End-stage Liver failure	prolonged	prolonged	prolonged	decreased
Uremia	unaffected	unaffected	prolonged	unaffected
Congenital afibrinogenemia	prolonged	prolonged	prolonged	unaffected
Factor V deficiency	prolonged	prolonged	unaffected	unaffected
Factor X deficiency as	prolonged	prolonged	unaffected	unaffected

seen in amyloid purpura				
Glanzmann's thrombasthenia	unaffected	unaffected	prolonged	unaffected
Bernard-Soulier syndrome	unaffected	unaffected	prolonged	decreased

Discovery

Brewer traced the history of the discovery of the platelet. Although red blood cells had been known since van Leeuwenhoek (1632–1723), it was the German anatomist Max Schultze (1825–1874) who first offered a description of the platelet in his newly-founded journal *Archiv für mikroscopische Anatomie*. He describes "spherules" to be much smaller than red blood cells that are occasionally clumped and may participate in collections of fibrous material. He recommends further study of the findings.

Giulio Bizzozero (1846–1901), building on Schultze's findings, used "living circulation" to study blood cells of amphibians microscopically *in vivo*. He is especially noted for discovering that platelets clump at the site of blood vessel injury, a process that precedes the formation of a blood clot. This observation confirmed the role of platelets in coagulation.

In transfusion medicine

Platelet concentrate.

Platelets are either isolated from collected units of whole blood and pooled to make a therapeutic dose or collected by apheresis, sometimes concurrently with plasma or red blood cells. The industry standard is for platelets to be tested for bacteria before transfusion to avoid septic reactions, which can be fatal. Recently the AABB Industry Standards for Blood Banks and Transfusion Services (5.1.5.1) has allowed for use of pathogen reduction technology as an alternative to bacterial screenings in platelets.

Pooled whole-blood platelets, sometimes called "random" platelets, are made primarily by two methods. In the US, a unit of whole blood is placed into a large centrifuge in what is referred to as a "soft spin." At these settings, the platelets remain suspended in the plasma. The platelet-rich plasma (PRP) is removed from the RBCs, then centrifuged at a

faster setting to harvest the platelets from the plasma. In other regions of the world, the unit of whole blood is centrifuged using settings that cause the platelets to become suspended in the "buffy coat" layer, which includes the platelets and the white blood cells. The "buffy coat" is isolated in a sterile bag, suspended in a small amount of red blood cells and plasma, then centrifuged again to separate the platelets and plasma from the red and white blood cells. Regardless of the initial method of preparation, multiple platelets may be combined into one container using a sterile connection device to manufacture a single product with the desired therapeutic dose.

Apheresis platelets are collected using a mechanical device that draws blood from the donor and centrifuges the collected blood to separate out the platelets and other components to be collected. The remaining blood is returned to the donor. The advantage to this method is that a single donation provides at least one therapeutic dose, as opposed to the multiple donations for whole-blood platelets. This means that a recipient is not exposed to as many different donors and has less risk of transfusion-transmitted disease and other complications. Sometimes a person such as a cancer patient that requires routine transfusions of platelets will receive repeated donations from a specific donor to further minimize the risk. Pathogen reduction of platelets using for example, riboflavin and UV light treatments can also be carried out to reduce the infectious load of pathogens contained in donated blood products, thereby reducing the risk of transmission of transfusion transmitted diseases.

Platelets are not cross-matched unless they contain a significant amount of red blood cells (RBCs), which results in a reddish-orange color to the product. This is usually associated with whole-blood platelets, as apheresis methods are more efficient than "soft spin" centrifugation at isolating the specific components of blood. An effort is usually made to issue type specific platelets, but this is not as critical as it is with RBCs.

Platelets collected by either method have a very short shelf life, typically five days. This results in frequent problems with short supply, as testing the donations often requires up to a full day. Since there are no effective preservative solutions for platelets, they lose potency quickly and are best when fresh.

Platelets are stored under constant agitation at 20-24 °C. Storage at room temperature provides an environment where any bacteria that are introduced to the blood component during the collection process may proliferate and subsequently cause bacteremia in the patient. Regulations are in place in the United States that require products to be tested for the presence of bacterial contamination before transfusion.

Platelets, either apheresis or random-donor platelets, can be processed through a volume reduction process. In this process, the platelets are spun in a centrifuge and the excess plasma is removed, leaving 10 to 100 ml of platelet concentrate. Volume-reduced platelets are normally transfused only to neonatal and pediatric patients when a large volume of plasma could overload the child's small circulatory system. The lower volume of plasma also reduces the chances of an adverse transfusion reaction to plasma proteins. Volume reduced platelets have a shelf life of only four hours.

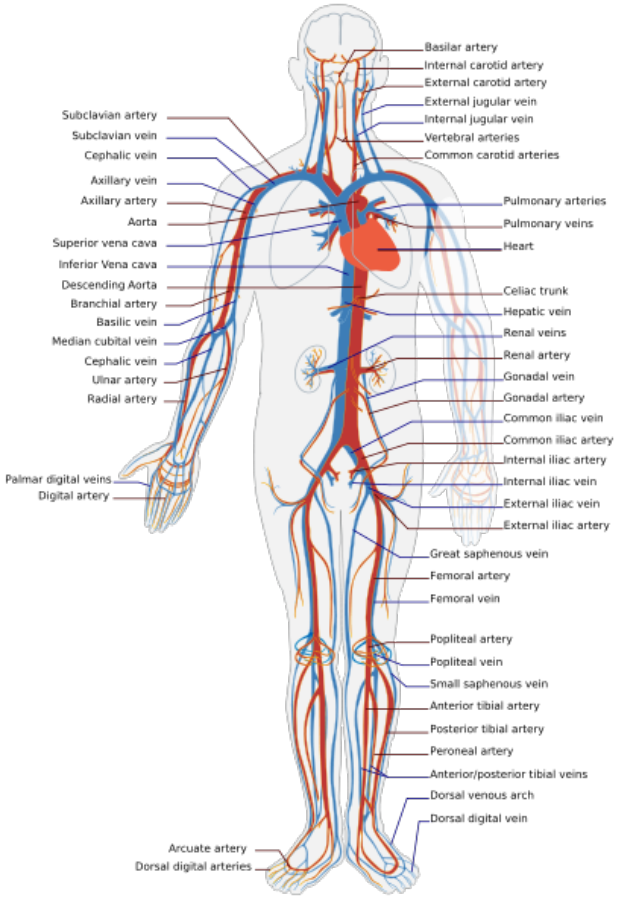
Other species

Nucleated thrombocytes of nonmammalian vertebrates differ from the mammalian thrombocytes not only in having a nucleus and resembling B lymphocytes, but also these nucleated thrombocytes do not aggregate in response to ADP, serotonin and adrenaline (although they do aggregate with thrombin).

Chapter 5

Blood Vessel

Blood vessel



Simple diagram of the human circulatory system.

Latin *vas sanguineum*

The **blood vessels** are the part of the circulatory system that transport blood throughout the body. There are three major types of blood vessels: the arteries, which carry the blood

away from the heart; the capillaries, which enable the actual exchange of water and chemicals between the blood and the tissues; and the veins, which carry blood from the capillaries back toward the heart.

Anatomy

The arteries and veins have different structures, veins having two layers and arteries having three.

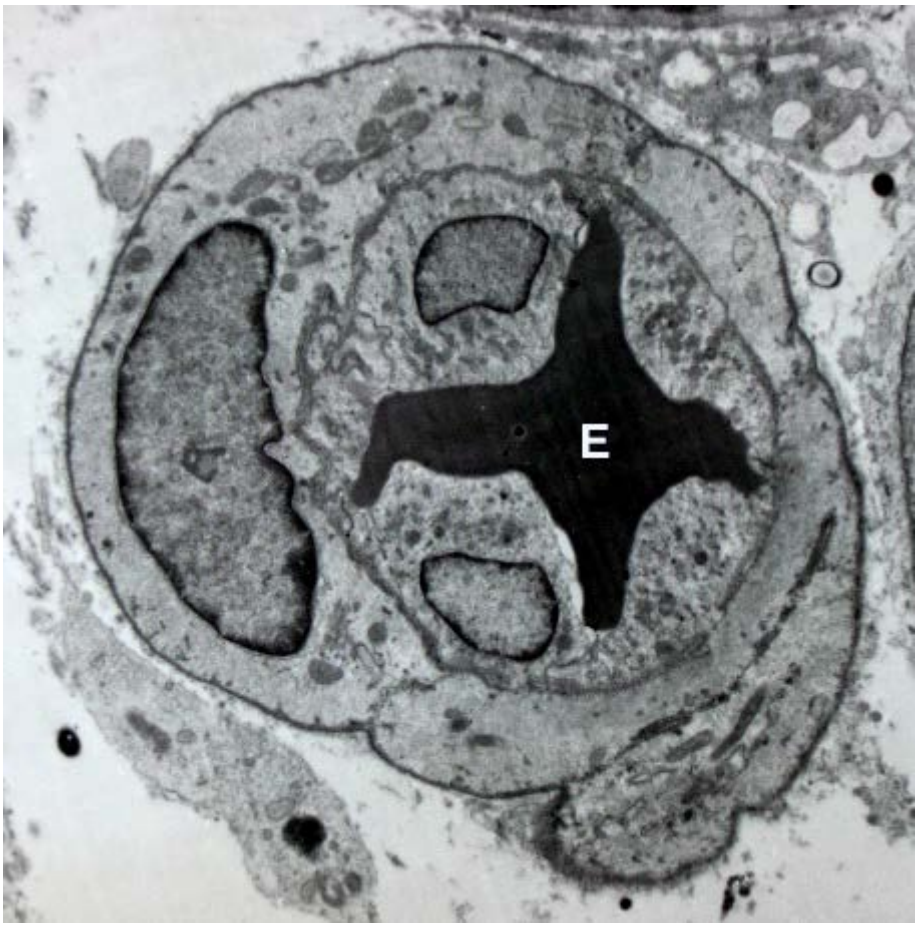
- *Tunica intima* (the thinnest layer): a single layer of simple squamous endothelial cells glued by a polysaccharide intercellular matrix, surrounded by a thin layer of subendothelial connective tissue interlaced with a number of circularly arranged elastic bands called the *internal elastic lamina*.
- *Tunica media* (the thickest layer): circularly arranged elastic fiber, connective tissue, polysaccharide substances, the second and third layer are separated by another thick elastic band called external elastic lamina. The tunica media may (especially in arteries) be rich in vascular smooth muscle, which controls the caliber of the vessel.
- *Tunica adventitia*: entirely made of connective tissue. It also contains nerves that supply the vessel

as well as nutrient capillaries (vasa vasorum) in the larger blood vessels.

Capillaries consist of little more than a layer of endothelium and occasional connective tissue.

When blood vessels connect to form a region of diffuse vascular supply it is called an anastomosis (pl. anastomoses). Anastomoses provide critical alternative routes for blood to flow in case of blockages.

Types



Blood vessel with an erythrocyte (red blood cell, E) within its lumen, endothelial cells forming its *tunica intima* (inner layer), and pericytes forming its *tunica adventitia* (outer layer).

There are various kinds of blood vessels:

- Arteries
  - Aorta (the largest artery, carries blood out of the heart)
  - Branches of the aorta, such as the carotid artery, the subclavian artery, the celiac trunk, the mesenteric arteries, the renal artery and the iliac artery.
- Arterioles
- Capillaries (the smallest blood vessels)
- Venules
- Veins
  - Large collecting vessels, such as the subclavian vein, the jugular vein, the renal vein and the iliac vein.
  - Venae cavae (the 2 largest veins, carry blood into the heart)

They are roughly grouped as *arterial* and *venous*, determined by whether the blood in it is flowing *away from* (arterial) or *toward* (venous) the heart. The term "arterial blood" is nevertheless used to indicate blood high in oxygen, although the pulmonary artery carries "venous blood" and blood flowing in the pulmonary vein is rich in oxygen. This is because they are carrying the blood to and from the lungs, respectively, to be oxygenated.

Physiology

Blood vessels do not actively engage in the transport of blood (they have no appreciable peristalsis), but arteries - and veins to a degree - can regulate their inner diameter by contraction of the muscular layer. This changes the blood flow to downstream organs, and is determined by the autonomic nervous system. Vasodilation and vasoconstriction are also used antagonistically as methods of thermoregulation.

Oxygen (bound to hemoglobin in red blood cells) is the most critical nutrient carried by the blood. In all arteries apart from the pulmonary artery, hemoglobin is highly saturated (95-100%) with oxygen. In all veins apart from the pulmonary vein, the hemoglobin is desaturated at about 75%. (The values are reversed in the pulmonary circulation.)

The blood pressure in blood vessels is traditionally expressed in millimetres of mercury (1 mmHg = 133 Pa). In the arterial system, this is usually around 120 mmHg systolic (high pressure wave due to contraction of the heart) and 80 mmHg diastolic (low pressure wave). In contrast, pressures in the venous system are constant and rarely exceed 10 mmHg.

Vasoconstriction is the constriction of blood vessels (narrowing, becoming smaller in cross-sectional area) by contracting the vascular smooth muscle in the vessel walls. It is regulated by vasoconstrictors (agents that cause vasoconstriction). These include paracrine factors (e.g. prostaglandins), a number of hormones (e.g. vasopressin and angiotensin) and neurotransmitters (e.g. epinephrine) from the nervous system.

Vasodilation is a similar process mediated by antagonistically acting mediators. The most prominent vasodilator is nitric oxide (termed endothelium-derived relaxing factor for this reason).

Permeability of the endothelium is pivotal in the release of nutrients to the tissue. It is also increased in inflammation in response to histamine, prostaglandins and interleukins, which leads to most of the symptoms of inflammation (swelling, redness and warmth).

Role in disease

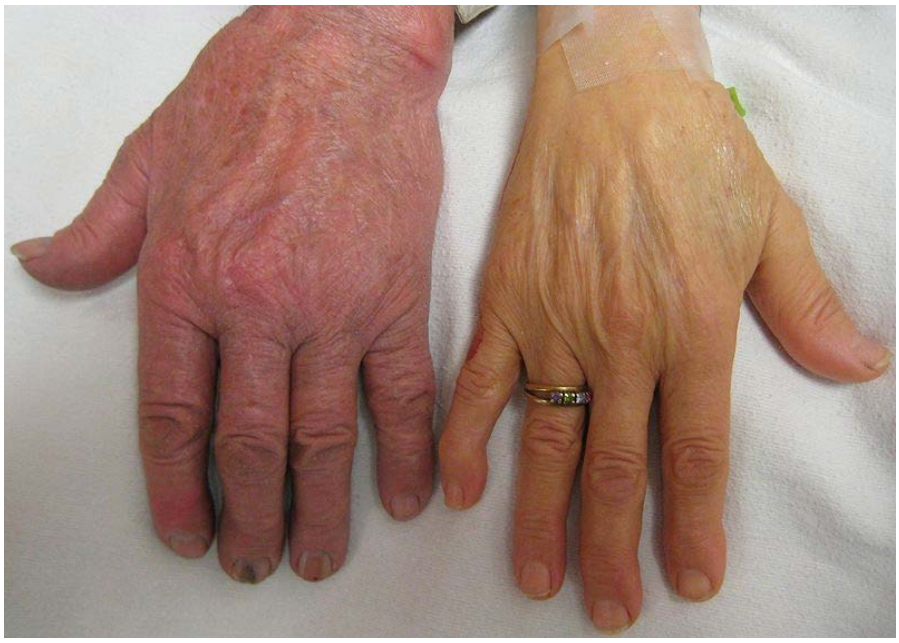
Blood vessels play a huge role in virtually every medical condition. Cancer, for example, cannot progress unless the tumor causes angiogenesis (formation of new blood vessels) to supply the malignant cells' metabolic demand. Atherosclerosis, the formation of lipid lumps (atheromas) in the blood vessel wall, is the most common cardiovascular disease, the main cause of death in the Western world.

Blood vessel permeability is increased in inflammation. Damage, due to trauma or spontaneously, may lead to haemorrhage due to mechanical damage to the vessel endothelium. In contrast, occlusion of the blood vessel by atherosclerotic plaque, by an embolised blood clot or a foreign body leads to downstream ischemia (insufficient blood supply) and possibly necrosis. Vessel occlusion tends to be a positive feedback system; an occluded vessel creates eddies in the normally laminar flow or plug flow blood currents. These eddies create abnormal fluid velocity gradients which push blood elements such as cholesterol or chylomicron bodies to the endothelium. These deposit onto the arterial walls which are already partially occluded and build upon the blockage.

Chapter 6

Anemia

Anemia



The pale hand of a woman with severe anemia (right) in comparison to the normal hand of her husband (left).

ICD-10	D50.-D64.
ICD-9	280-285
DiseasesDB	663
MedlinePlus	000560
eMedicine	med/132 emerg/808 emerg/734
MeSH	D000740

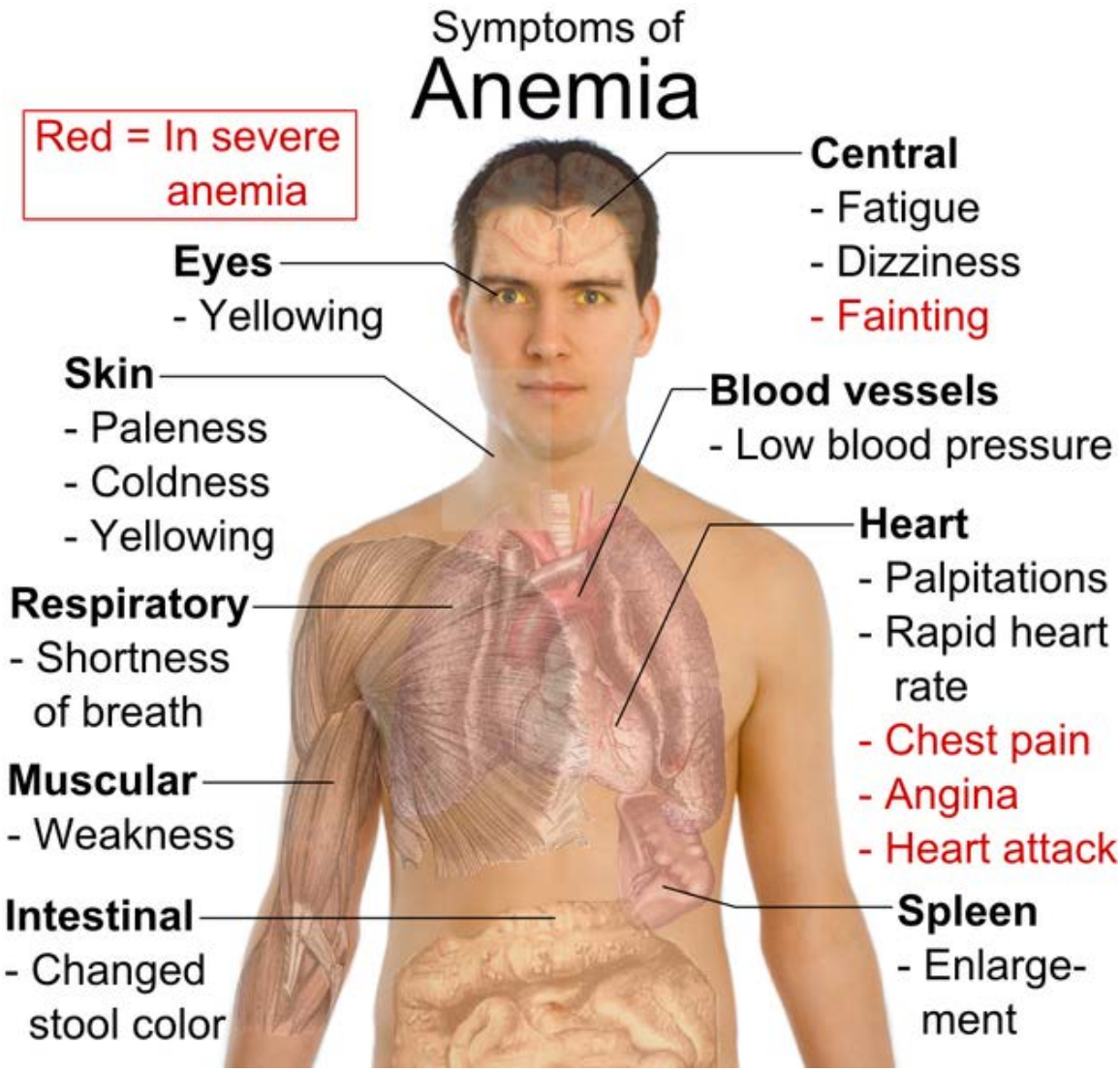
Anemia is a decrease in number of red blood cells (RBCs) or less than the normal quantity of hemoglobin in the blood. However, it can include decreased oxygen-binding ability of each hemoglobin molecule due to deformity or lack in numerical development as in some other types of hemoglobin deficiency.

Because hemoglobin (found inside RBCs) normally carries oxygen from the lungs to the tissues, anemia leads to hypoxia (lack of oxygen) in organs. Because all human cells depend on oxygen for survival, varying degrees of anemia can have a wide range of clinical consequences.

Anemia is the most common disorder of the blood. There are several kinds of anemia, produced by a variety of underlying causes. Anemia can be classified in a variety of ways, based on the morphology of RBCs, underlying etiologic mechanisms, and discernible clinical spectra, to mention a few. The three main classes of anemia include excessive blood loss (acutely such as a hemorrhage or chronically through low-volume loss), excessive blood cell destruction (hemolysis) or deficient red blood cell production (ineffective hematopoiesis).

There are two major approaches: the "kinetic" approach which involves evaluating production, destruction and loss, and the "morphologic" approach which groups anemia by red blood cell size. The morphologic approach uses a quickly available and low cost lab test as its starting point (the MCV). On the other hand, focusing early on the question of production may allow the clinician to more rapidly expose cases where multiple causes of anemia coexist.

Signs and symptoms



Main symptoms that may appear in anemia.

Anemia goes undetermined in many people, and symptoms can be minor or vague. The signs and symptoms can be related to the anemia itself, or the underlying cause.

Most commonly, people with anemia report non-specific symptoms of a feeling of weakness, or fatigue, general malaise and sometimes poor concentration. They may also report dyspnea (shortness of breath) on exertion. In very severe anemia, the body may compensate for the lack of oxygen carrying capability of the blood by increasing cardiac output. The patient may have symptoms related to this, such as palpitations, angina (if preexisting heart disease is present), intermittent claudication of the legs, and symptoms of heart failure.

On examination, the signs exhibited may include pallor (pale skin, mucosal linings and nail beds) but this is not a reliable sign. There may be signs of specific causes of anemia, e.g., koilonychia (in iron deficiency), jaundice (when anemia results from abnormal break down of red blood cells — in hemolytic anemia), bone deformities (found in thalassaemia major) or leg ulcers (seen in sickle cell disease).

In severe anemia, there may be signs of a hyperdynamic circulation: a fast heart rate (tachycardia), flow murmurs, and cardiac enlargement. There may be signs of heart failure.

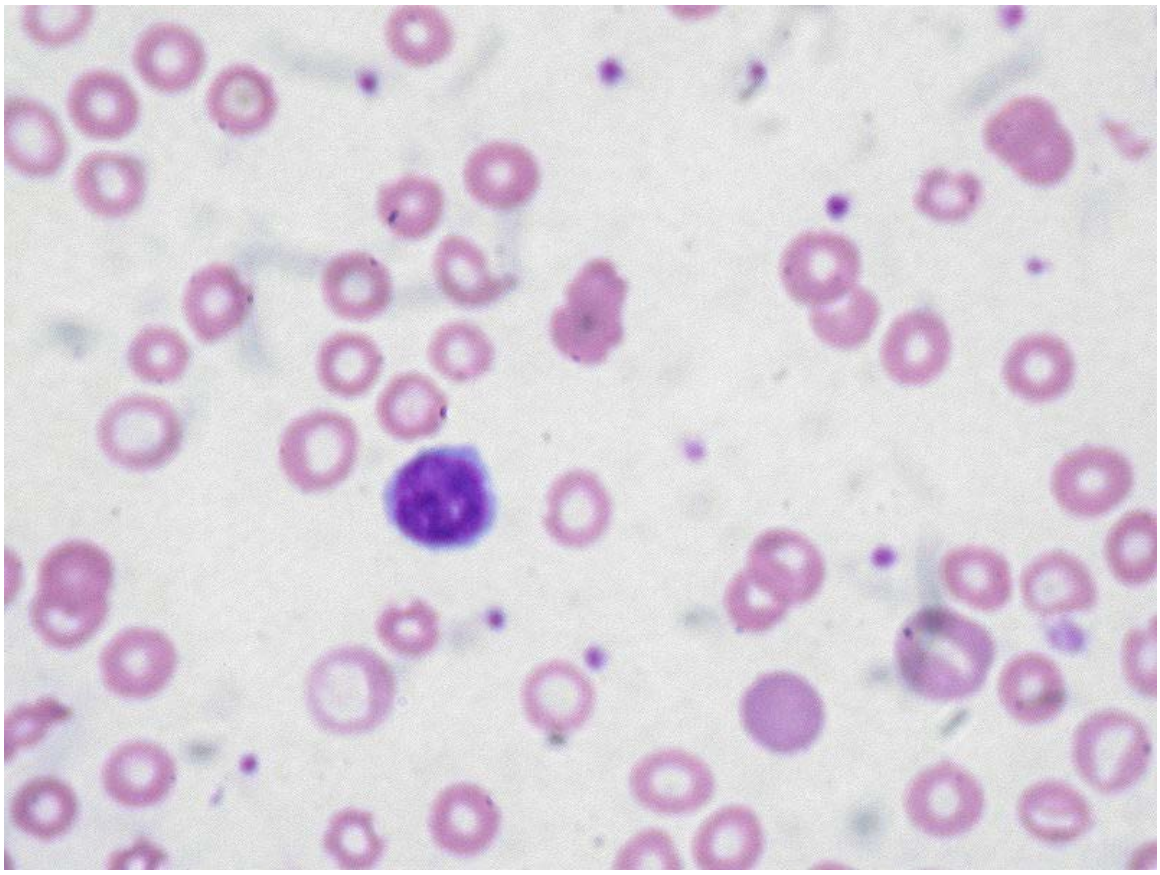
Pica, the consumption of non-food based items such as dirt, paper, wax, grass, ice, and hair, may be a symptom of iron deficiency, although it occurs often in those who have normal levels of hemoglobin.

Chronic anemia may result in behavioral disturbances in children as a direct result of impaired neurological development in infants, and reduced scholastic performance in children of school age.

Restless legs syndrome is more common in those with iron deficiency anemia.

Less common symptoms may include swelling of the legs or arms, chronic heartburn, vague bruises, vomiting, increased sweating, and blood in stool.

Diagnosis



Peripheral blood smear microscopy of a patient with iron-deficiency anemia.

Anemia is typically diagnosed on a complete blood count. Apart from reporting the number of red blood cells and the hemoglobin level, the automatic counters also measure the size of the red blood cells by flow cytometry, which is an important tool in distinguishing between the causes of anemia. Examination of a stained blood smear using a microscope can also be helpful, and is sometimes a necessity in regions of the world where automated analysis is less accessible.

In modern counters, four parameters (RBC count, hemoglobin concentration, MCV and RDW) are measured, allowing others (hematocrit, MCH and MCHC) to be calculated, and compared to values adjusted for age and sex. Some counters estimate hematocrit from direct measurements.

WHO's Hemoglobin thresholds used to define anemia (1 g/dL = 0.6206 mmol/L)

Age or gender group	Hb threshold (g/dl)	Hb threshold (mmol/l)
Children (0.5–5.0 yrs)	11.0	6.8
Children (5–12 yrs)	11.5	7.1
Teens (12–15 yrs)	12.0	7.4

Women, non-pregnant (>15yrs)	12.0	7.4
Women, pregnant	11.0	6.8
Men (>15yrs)	13.0	8.1

Reticulocyte counts, and the "kinetic" approach to anemia, have become more common than in the past in the large medical centers of the United States and some other wealthy nations, in part because some automatic counters now have the capacity to include reticulocyte counts. A reticulocyte count is a quantitative measure of the bone marrow's production of new red blood cells. The reticulocyte production index is a calculation of the ratio between the level of anemia and the extent to which the reticulocyte count has risen in response. If the degree of anemia is significant, even a "normal" reticulocyte count actually may reflect an inadequate response.

If an automated count is not available, a reticulocyte count can be done manually following special staining of the blood film. In manual examination, activity of the bone marrow can also be gauged qualitatively by subtle changes in the numbers and the morphology of young RBCs by examination under a microscope. Newly formed RBCs are usually slightly larger than older RBCs and show polychromasia. Even where the source of blood loss is obvious, evaluation of erythropoiesis can help assess whether the bone marrow will be able to compensate for the loss, and at what rate.

When the cause is not obvious, clinicians use other tests: ESR, ferritin, serum iron, transferrin, RBC folate level, serum vitamin B_{12}, hemoglobin electrophoresis, renal function tests (e.g. serum creatinine).

When the diagnosis remains difficult, a bone marrow examination allows direct examination of the precursors to red cells.

Classification

Production vs. destruction or loss

The "kinetic" approach to anemia yields what many argue is the most clinically relevant classification of anemia. This classification depends on evaluation of several hematological parameters, particularly the blood reticulocyte (precursor of mature RBCs) count. This then yields the classification of defects by decreased RBC production versus increased RBC destruction and/or loss. Clinical signs of loss or destruction include abnormal peripheral blood smear with signs of hemolysis; elevated LDH suggesting cell destruction; or clinical signs of bleeding, such as guiaic-positive stool, radiographic findings, or frank bleeding.

The following is a simplified schematic of this approach:

** For instance, sickle cell anemia with superimposed iron deficiency; chronic gastric bleeding with B_{12} and folate deficiency; and other instances of anemia with more than one cause.*

*** Confirm by repeating reticulocyte count: ongoing combination of low reticulocyte production index, normal MCV and hemolysis or loss may be seen in bone marrow failure or anemia of chronic disease, with superimposed or related hemolysis or blood loss.*

Red blood cell size

In the morphological approach, anemia is classified by the size of red blood cells; this is either done automatically or on microscopic examination of a peripheral blood smear. The size is reflected in the *mean corpuscular volume* (MCV). If the cells are smaller than normal (under 80 fl), the anemia is said to be *microcytic*; if they are normal size (80–100 fl), *normocytic*; and if they are larger than normal (over 100 fl), the anemia is classified as *macrocytic*. This scheme quickly exposes some of the most common causes of anemia; for instance, a microcytic anemia is often the result of iron deficiency. In clinical workup, the MCV will be one of the first pieces of information available; so even among clinicians who consider the "kinetic" approach more useful philosophically, morphology will remain an important element of classification and diagnosis.

Here is a schematic representation of how to consider anemia with MCV as the starting point:

Other characteristics visible on the peripheral smear may provide valuable clues about a more specific diagnosis; for example, abnormal white blood cells may point to a cause in the bone marrow.

Microcytic

Microcytic anemia is primarily a result of hemoglobin synthesis failure/insufficiency, which could be caused by several etiologies:

- Heme synthesis defect
  - Iron deficiency anemia
  - Anemia of chronic disease (more commonly presenting as normocytic anemia)
- Globin synthesis defect
  - alpha-, and beta-thalassemia
  - HbE syndrome
  - HbC syndrome
  - and various other unstable hemoglobin diseases
- Sideroblastic defect
  - Hereditary sideroblastic anemia
  - Acquired sideroblastic anemia, including lead toxicity
  - Reversible sideroblastic anemia

Iron deficiency anemia is the most common type of anemia overall and it has many causes. RBCs often appear hypochromic (paler than usual) and microcytic (smaller than usual) when viewed with a microscope.

- Iron deficiency anemia is caused by insufficient dietary intake or absorption of iron to replace losses from menstruation or losses due to diseases. Iron is an essential part of hemoglobin, and low iron levels result in decreased incorporation of hemoglobin into red blood cells. In the United States, 20% of all women of childbearing age have iron deficiency anemia, compared with only 2% of adult men. The principal cause of iron deficiency anemia in premenopausal women is blood lost during menses. Studies have shown that iron deficiency without anemia causes poor school performance and lower IQ in teenage girls. Iron deficiency is the most prevalent deficiency state on a worldwide basis. Iron deficiency is sometimes the cause of abnormal fissuring of the angular (corner) sections of the lips (angular stomatitis).
- Iron deficiency anemia can also be due to bleeding lesions of the gastrointestinal tract. Faecal occult blood testing, upper endoscopy and lower endoscopy should be performed to identify bleeding lesions. In men and post-menopausal women the chances are higher that bleeding from the gastrointestinal tract could be due to colon polyp or colorectal cancer.
- Worldwide, the most common cause of iron deficiency anemia is parasitic infestation (hookworm, amebiasis, schistosomiasis and whipworm).

Macrocytic

- Megaloblastic anemia, the most common cause of macrocytic anemia, is due to a deficiency of either vitamin B_{12}, folic acid (or both). Deficiency in folate and/or vitamin B_{12} can be due either to inadequate intake or insufficient absorption. Folate deficiency normally does not produce neurological symptoms, while B_{12} deficiency does.
    - Pernicious anemia is caused by a lack of intrinsic factor. Intrinsic factor is required to absorb vitamin B_{12} from food. A lack of intrinsic factor may arise from an autoimmune condition targeting the parietal cells (atrophic gastritis) that produce intrinsic factor or against intrinsic factor itself. These lead to poor absorption of vitamin B_{12}.
    - Macrocytic anemia can also be caused by removal of the functional portion of the stomach, such as during gastric bypass surgery, leading to reduced vitamin B_{12}/folate absorption. Therefore one must always be aware of anemia following this procedure.
- Hypothyroidism
- Alcoholism commonly causes a macrocytosis, although not specifically anemia. Other types of Liver Disease can also cause macrocytosis.
- Methotrexate, zidovudine, and other drugs that inhibit DNA replication.

Macrocytic anemia can be further divided into "megaloblastic anemia" or "non-megaloblastic macrocytic anemia". The cause of megaloblastic anemia is primarily a

failure of DNA synthesis with preserved RNA synthesis, which result in restricted cell division of the progenitor cells. The megaloblastic anemias often present with neutrophil hypersegmentation (6–10 lobes). The non-megaloblastic macrocytic anemias have different etiologies (i.e. there is unimpaired DNA globin synthesis,) which occur, for example in alcoholism.

In addition to the non-specific symptoms of anemia, specific features of vitamin B_{12} deficiency include peripheral neuropathy and subacute combined degeneration of the cord with resulting balance difficulties from posterior column spinal cord pathology. Other features may include a smooth, red tongue and glossitis.

The treatment for vitamin B_{12}-deficient anemia was first devised by William Murphy who bled dogs to make them anemic and then fed them various substances to see what (if anything) would make them healthy again. He discovered that ingesting large amounts of liver seemed to cure the disease. George Minot and George Whipple then set about to chemically isolate the curative substance and ultimately were able to isolate the vitamin B_{12} from the liver. All three shared the 1934 Nobel Prize in Medicine.

Normocytic

Normocytic anemia occurs when the overall hemoglobin levels are always decreased, but the red blood cell size (Mean corpuscular volume) remains normal. Causes include:

- Acute blood loss
- Anemia of chronic disease
- Aplastic anemia (bone marrow failure)
- Hemolytic anemia

Dimorphic

When two causes of anemia act simultaneously, e.g., macrocytic hypochromic, due to hookworm infestation leading to deficiency of both iron and vitamin B_{12} or folic acid or following a blood transfusion more than one abnormality of red cell indices may be seen. Evidence for multiple causes appears with an elevated RBC distribution width (RDW), which suggests a wider-than-normal range of red cell sizes.

Heinz body anemia

Heinz bodies form in the cytoplasm of RBCs and appear like small dark dots under the microscope. There are many causes of Heinz body anemia, and some forms can be drug induced. It is triggered in cats by eating onions or acetaminophen (paracetamol). It can be triggered in dogs by ingesting onions or zinc, and in horses by ingesting dry red maple leaves.

Refractory anemia

Refractory anemia is an anemia which does not respond to treatment. It is often seen secondary to myelodysplastic syndromes.

Iron deficiency anemia may also be refractory as a clinical manifestation of gastrointestinal problems which disrupt iron metabolism.

Causes

Broadly, causes of anemia may be classified as impaired red blood cell (RBC) production, increased RBC destruction (hemolytic anemias), blood loss and fluid overload (hypervolemia). Several of these may interplay to eventually cause anemia. Indeed, the most common cause of anemia is blood loss, but this usually doesn't cause any lasting symptoms unless a relatively impaired RBC production develops, in turn most commonly by iron deficiency.

Impaired production

- Disturbance of proliferation and differentiation of stem cells.
  - Pure red cell aplasia
  - Aplastic anemia, affecting all kinds of blood cells. Fanconi anemia is a hereditary disorder or defect featuring aplastic anemia and various other abnormalities.
  - Anemia of renal failure, by insufficient erythropoietin production
  - Anemia of endocrine disorders

- Disturbance of proliferation and maturation of erythroblasts
  - Pernicious anemia is a form of megaloblastic anemia due to vitamin B_{12} deficiency dependent on impaired absorption of vitamin B_{12}.
  - Anemia of folic acid deficiency. As with vitamin B_{12}, it causes megaloblastic anemia
  - Anemia of prematurity, by diminished erythropoietin response to declining hematocrit levels, combined with blood loss from laboratory testing. It generally occurs in premature infants at 2 to 6 weeks of age.
  - Iron deficiency anemia, resulting in deficient heme synthesis
  - thalassemias, causing deficient globin synthesis
  - Anemia of renal failure (also causing stem cell dysfunction)

- Other mechanisms of impaired RBC production
  - Myelophthisic anemia or Myelophthisis is a severe type of anemia resulting from the replacement of bone marrow by other materials, such as malignant tumors or granulomas.
  - Myelodysplastic syndrome
  - anemia of chronic inflammation

Increased destruction

Anemias of increased red blood cell destruction are generally classified as hemolytic anemias. These are generally featuring jaundice and elevated LDH levels.

- Intrinsic (intracorpuscular) abnormalities, where there the red blood cells have defects that cause premature destruction. All of these, except paroxysmal nocturnal hemoglobinuria, are hereditary genetic disorders.
    - Hereditary spherocytosis is a hereditary defect that results in defects in the RBC cell membrane, causing the erythrocytes to be sequestered and destroyed by the spleen.
    - Hereditary elliptocytosis, another defect in membrane skeleton proteins
    - Abetalipoproteinemia, causing defects in membrane lipids
    - Enzyme deficiencies
        - Pyruvate kinase and hexokinase deficiencies, causing defect glycolysis
        - Glucose-6-phosphate dehydrogenase deficiency and glutathione synthetase deficiency, causing increased oxidative stress
    - Hemoglobinopathies
        - Sickle cell anemia
        - Hemoglobinopathies causing unstable hemoglobins
    - paroxysmal nocturnal hemoglobinuria

- Extrinsic (extracorpuscular) abnormalities
    - Antibody-mediated
        - Warm autoimmune hemolytic anemia is an anemia caused by autoimmune attack against red blood cells, primarily by IgG. It is the most common of the autoimmune hemolytic diseases. It can be idiopathic, that is, without any known cause, drug-associated or secondary to another disease such as systemic lupus erythematosus, or a malignancy, such as chronic lymphocytic leukemia (CLL)
        - Cold agglutinin hemolytic anemia is primarily mediated by IgM. It can be idiopathic or result from an underlying condition.
        - Rh disease, one of the causes of hemolytic disease of the newborn
        - Transfusion reaction to blood transfusions
    - Mechanical trauma to red cells
        - Microangiopathic hemolytic anemias, including thrombotic thrombocytopenic purpura and disseminated intravascular coagulation
        - Infections, including malaria
        - heart surgery

Blood loss

- Anemia of prematurity from frequent blood sampling for laboratory testing, combined with insufficient RBC production.
- Trauma or surgery, causing acute blood loss
- Gastrointestinal tract lesions, causing a rather chronic blood loss
- Gynecologic disturbances, also generally causing chronic blood loss

Fluid overload

Fluid overload (hypervolemia) causes decreased hemoglobin concentration and apparent anemia:

- General causes of hypervolemia include excessive sodium or fluid intake, sodium or water retention and fluid shift into the intravascular space.
- Anemia of pregnancy is anemia that is induced by blood volume expansion experienced in pregnancy.

Treatments

Treatments for anemia depend on severity and cause.

Iron deficiency from nutritional causes is rare in non-menstruating adults (men and post-menopausal women). The diagnosis of iron deficiency mandates a search for potential sources of loss such as gastrointestinal bleeding from ulcers or colon cancer. Mild to moderate iron deficiency anemia is treated by oral iron supplementation with ferrous sulfate, ferrous fumarate, or ferrous gluconate. When taking iron supplements, it is very common to experience stomach upset and/or darkening of the feces. The stomach upset can be alleviated by taking the iron with food; however, this decreases the amount of iron absorbed. Vitamin C aids in the body's ability to absorb iron, so taking oral iron supplements with orange juice is of benefit.

Vitamin supplements given orally (folic acid) or subcutaneously (vitamin B-12) will replace specific deficiencies.

In anemia of chronic disease, anemia associated with chemotherapy, or anemia associated with renal disease, some clinicians prescribe recombinant erythropoietin, epoetin alfa, to stimulate red cell production.

In severe cases of anemia, or with ongoing blood loss, a blood transfusion may be necessary.

Blood transfusions

Doctors attempt to avoid blood transfusion in general, since multiple lines of evidence point to increased adverse patient clinical outcomes with more intensive transfusion

strategies. The physiological principle that reduction of oxygen delivery associated with anemia leads to adverse clinical outcomes is balanced by the finding that transfusion does not necessarily mitigate these adverse clinical outcomes.

In severe, acute bleeding, transfusions of donated blood are often lifesaving. Improvements in battlefield casualty survival is attributable, at least in part, to the recent improvements in blood banking and transfusion techniques.

Transfusion of the stable but anemic hospitalized patient has been the subject of numerous clinical trials.

Four randomized controlled clinical trials have been conducted to evaluate aggressive versus conservative transfusion strategies in critically-ill patients. All four of these studies failed to find a benefit with more aggressive transfusion strategies.

In addition, at least two retrospective studies have shown increases in adverse clinical outcomes in critically ill patients that underwent more aggressive transfusion strategies.

Hyperbaric oxygen

Treatment of exceptional blood loss (anemia) is recognized as an indication for hyperbaric oxygen (HBO) by the Undersea and Hyperbaric Medical Society. The use of HBO is indicated when oxygen delivery to tissue is not sufficient in patients who cannot be transfused for medical or religious reasons. HBO may be used for medical reasons when threat of blood product incompatibility or concern for transmissible disease are factors. The beliefs of some religions (ex: Jehovah's Witnesses) may require they use the superior HBO method.

In 2002, Van Meter reviewed the publications surrounding the use of HBO in severe anemia and found that all publications report a positive result.

Chapter 7

Fanconi Anemia

Fanconi Anemia	
ICD-10	D61.0
ICD-9	284.0
OMIM	227650
DiseasesDB	4745
MedlinePlus	000334
eMedicine	ped/3022
MeSH	D005199

Fanconi anemia (FA) is a genetic disease with an incidence of 1 per 350,000 births, and a higher frequency in Ashkenazi Jews and Afrikaners in South Africa.

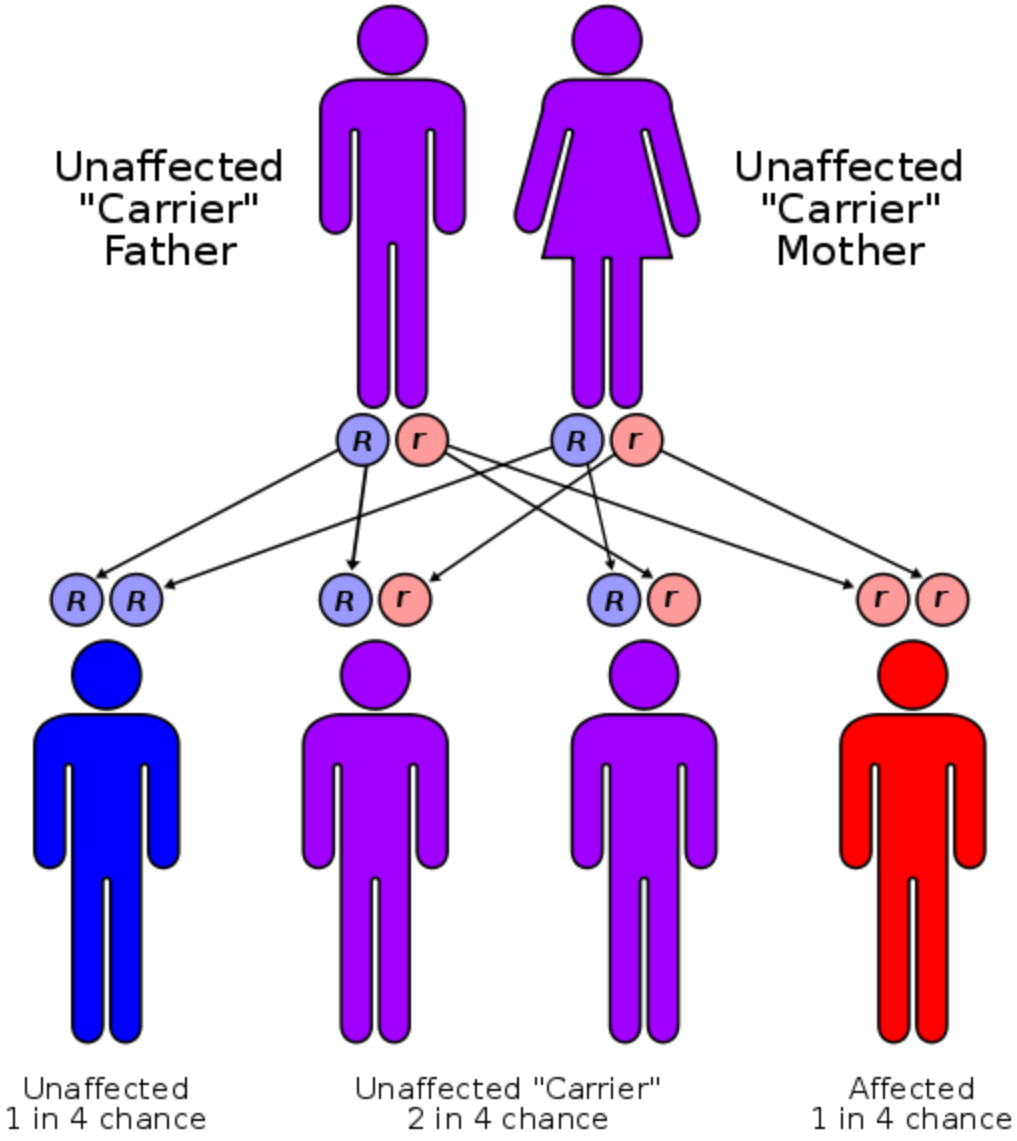
FA is the result of a genetic defect in a cluster of proteins responsible for DNA repair. As a result, 20% or more of FA patients develop cancer, most often acute myelogenous leukemia, and 90% develop bone marrow failure (the inability to produce blood cells) by age 40. About 60-75% of FA patients have congenital defects, commonly short stature, abnormalities of the skin, arms, head, eyes, kidneys, and ears, and developmental disabilities. Median age of death was 30 years in 2000.

Treatment with androgens and hematopoietic (blood cell) growth factors can help bone marrow failure temporarily, but the long-term treatment is bone marrow transplant if a donor is available.

Because of the genetic defect in DNA repair, cells from people with FA are sensitive to drugs that treat cancer by DNA cross-linking, such as mitomycin C.

The disease is named after the Swiss pediatrician who originally described this disorder, Guido Fanconi. It should not be confused with Fanconi syndrome, a kidney disorder also named after Fanconi.

Genetic prevalence



Fanconi anemia has an autosomal recessive pattern of inheritance.

FA is primarily an autosomal recessive genetic disorder. This means that two genes (one from each parent) are required to cause the disease. There is a 25% risk that each subsequent child will have FA. About 2% of FA cases are X-linked recessive, which

means that if the mother has a Fanconi anemia gene there is a 50% chance that male offspring will present with Fanconi anemia.

There are at least 13 genes whose mutations are known to cause FA: FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ, FANCL, FANCM and FANCN. FANCB is the one exception to FA being autosomal recessive, as this gene is on the X chromosome.

Approximately 1,000 persons worldwide currently suffer from the disease. The carrier frequency in the Ashkenazi Jewish population is about 1/90. Genetic counseling and genetic testing is recommended for families that may be carriers of Fanconi anemia.

Because of the failure of hematologic components to develop – leukocytes, red blood cells and platelets - the body's capabilities to fight infection, deliver oxygen, and form clots are all diminished.

Treatment

The first line of therapy is androgens and hematopoietic growth factors, but only 50-75% of patients respond. A more permanent cure is hematopoietic stem cell transplantation. If no potential donor exist, a savior sibling can be conceived by preimplantation genetic diagnosis (PGD) to match the recipients HLA type.

If there is no matching donor, some parents have conceived a second child by in vitro fertilization, screening the zygotes by preimplantation genetic diagnosis for a sibling that will be a genetic match (for human leucocyte antigen) and will be free from Fanconi anemia itself.

Prognosis

Many patients eventually develop acute myelogenous leukemia (AML). Older patients are extremely likely to develop head and neck, esophageal, gastrointestinal, vulvar and anal cancers. Patients who have had a successful bone marrow transplant and, thus, are cured of the blood problem associated with FA still must have regular examinations to watch for signs of cancer. Many patients do not reach adulthood.

The overarching medical challenge that Fanconi patients face is a failure of their bone marrow to produce blood cells. In addition, Fanconi patients normally are born with a variety of birth defects. For instance, 90% of the Ashkenazi children born with Fanconi's have no thumbs. A good number of Fanconi patients have kidney problems, trouble with their eyes, developmental retardation and other serious defects, such as microcephaly (small head).

Hematological abnormalities

Clinically, hematological abnormalities are the most serious symptoms in FA. By the age of 40, 98% of FA patients will have developed some type of hematological abnormality. It is interesting to note, however, the few cases in which older patients have died without ever developing them. Symptoms appear progressively, and often lead to complete bone marrow failure. While at birth, blood count is usually normal, macrocytosis/non-megaloblastic anemia, defined as unusually large red blood cells, is the first detected abnormality, often within the first decade of life (median age of onset is 7 years). Within the next 10 years, over 50% of patients presenting haematological abnormalities will have developed pancytopenia, defined as abnormalities in two or more blood cell lineages. Most commonly, a low platelet count (thrombocytopenia) precedes a low neutrophil count (neutropenia), with both appearing with relative equal frequencies. The deficiencies cause increased risk of hemorrhage and recurrent infections, respectively.

As FA is now known to affect the DNA repair, and given the current knowledge about dynamic cell division in the bone marrow, it is not surprising to find patients are more likely to develop bone marrow failure, myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). The next sections will detail those pathologies.

Myelodysplastic syndromes

MDS, formerly known as preleukemia, are a group of bone marrow neoplastic diseases that share many of the morphologic features of AML, with some important differences. First, the percentage of undifferentiated progenitor cells, blasts cells, is always less than 20%, and there is considerably more dysplasia, defined as cytoplasmic and nuclear morphologic changes in erythroid, granulocytic and megakaryocytic precursors, than what is usually seen in cases of AML. These changes reflect delayed apoptosis or a failure of programmed cell death. When left untreated, MDS can lead to AML in about 30% of cases. Due the nature of the FA pathology, MDS diagnosis cannot be made solely through cytogenetic analysis of the marrow. Indeed, it is only when morphologic analysis of marrow cells is performed, that a diagnosis of MDS can be ascertained. Upon examination, MDS-afflicted FA patients will show many clonal variations, appearing either prior or subsequent to the MDS. Furthermore, cells will show chromosomal aberrations, the most frequent being monosomy 7 and partial trisomies of chromosome 3q 15. Observation of monosomy 7 within the marrow is well correlated with an increased risk of developing AML and with a very poor prognosis, death generally ensuing within 2 years.

Acute myeloid leukemia

FA patients are at elevated risk for the development of acute myeloid leukemia (AML), defined as presence of 20% or more of myeloid blasts in the marrow or 5 to 20% myeloid blasts in the blood. All of the subtypes of AML can occur in FA with the exception of promyelocytic. However, myelomonocytic and acute monocytic are the most common subtypes observed. It is also interesting to note that many MDS patients will evolve into

AML given they survive long enough. Furthermore, the risk of developing AML increases with the onset of bone marrow failure.

While the risk of developing either MDS or AML before the age of 20 is only 27%, this risk increases to 43% by the age of 30 and 52% by the age of 40. Even with a marrow transplant, about 1/4 of FA patients diagnosed with MDS/ALS will die from MDS/ALS-related causes within 2 years.

Bone marrow failure

The last major haematological complication associated with FA is bone marrow failure, defined as inadequate blood cell production. Several types of failure are observed in FA patients, and generally precede MDS and AML. Detection of decreasing blood count is generally the first sign used to assess necessity of treatment and possible transplant. While most FA patients are initially responsive to androgen therapy and haemopoietic growth factors, these have been shown to promote leukemia, especially in patients with clonal cytogenetic abnormalities, and have severe side effects, including hepatic adenomas and adenocarcinomas. The only treatment left would be bone marrow transplant; however, such an operation has a relatively low success rate in FA patients when the donor is unrelated (30% 5-year survival). It is therefore imperative to transplant from an HLA-identical sibling. Furthermore, due to the increased susceptibility of FA patients to chromosomal damage, pretransplant conditioning cannot include high doses of radiations or immunosuppressants, and thus increase chances of patients developing graft-versus-host disease. If all precautions are taken, and the marrow transplant is performed within the first decade of life, 2-year probability of survival can be as high as 89%. However, if the transplant is performed at ages older than 10, 2-year survival rates drop to 54%.

A recent report by Zhang et al. investigates the mechanism of bone marrow failure in FANCC-/- cells. They hypothesize and successfully demonstrate that continuous cycles of hypoxia-reoxygenation, such as those seen by haemopoietic and progenitor cells as they migrate between hyperoxic blood and hypoxic marrow tissues, leads to premature cellular senescence and therefore inhibition of haemopoietic function. Senescence, together with apoptosis, may constitute a major mechanism of haemopoietic cell depletion occurred in bone marrow failure.

Molecular basis of FA

There are 13 genes responsible for FA, and one of them is identical to the breast-cancer susceptibility gene BRCA2. They are involved in the recognition and repair of damaged DNA; genetic defects leave them unable to repair DNA. The FA core complex of 8 proteins is normally activated when DNA stops replicating because of damage.The core complex adds ubiquitin, a small protein that combines with BRCA2 in another cluster to repair DNA. At the end of the process, ubiquitin is removed.

Recent studies have shown that eight of these proteins, FANCA, -B, -C, -E, -F, -G, -L and –M assemble to form a core protein complex in the nucleus. According to current models, the complex moves from the cytoplasm into the nucleus following nuclear localization signals on FANCA and FANCE. Assembly is activated by replicative stress, particularly DNA damage caused by cross-linking agents(mitomycin C or cisplatin) or reactive oxygen species (ROS). Indeed, FANCA and FANCG have been observed to multimerize when a cell is faced with oxidative stress-induced damage.

Following assembly, the protein core complex activates FANCL protein which acts as an E3 ubiquitin-ligase and monoubiquitinates FANCD2.

Monoubiquitinated FANCD2, also known as FANCD2-L, then goes on to interact with a BRCA1/BRCA2 complex. Details are not known, but similar complexes are involved in genome surveillance and associated with a variety of proteins implicated in DNA repair and chromosomal stability. With a crippling mutation in any FA protein in the complex, DNA repair is much less effective, as shown by its response to damage caused by cross-linking agents such as cisplatin, diepoxybutane and Mitomycin C. Bone marrow is particularly sensitive to this defect.

In another pathway responding to ionizing radiation, FANCD2 is thought to be phosphorylated by protein complex ATM/ATR activated by double-strand DNA breaks, and takes part in S-phase checkpoint control. This pathway was proven by the presence of radioresistant DNA synthesis, the hallmark of a defect in the S phase checkpoint, in patients with FA-D1 or FA-D2. Such a defect readily leads to uncontrollable replication of cells and might also explain the increase frequency of AML in these patients.

Other FA protein interactions

Although the above described pathway seems to be the most integral part of the DNA damage response in cells and explains the pathology of FA, novel approaches have determined that most FA proteins have an alternate role. Indeed, recent investigations on FANCC, one of the intensively studied proteins, have shown that it plays an important role in cellular responses to oxidative stress. For example, it has been found to interact with NADPH cytochrome P450 reductase, associated with increased production of ROS, and glutathione S-transferase, responsible for production of the anti-oxidant glutathione. These two enzymes are both involved in either triggering or detoxifying ROS. Not surprisingly, mice with Cu/Zn superoxide dismutase and FANCC mutations demonstrate defective haemopoiesis. FANCC was also shown to bind STAT1 and help receptor docking and phosphorylation of STAT135, which helps in tumor suppression. This leads to the conclusion that FANCC participates in cell growth arrest and cell cycle progression, inhibiting apoptosis, a possible cause of bone marrow failure due to depletion of haemopoietic progenitors. Another FA protein linked to protection against oxidative damage is FANCG. Indeed, this protein interacts with cytochrome P450 2E1 suggesting a possible role in detoxifying cytochrome ROS, produced readily by the members of this superfamily36. Furthermore, FANCG is identical to post-replication repair protein XRCC9, hinting at the possibility that FANCG also interacts directly with

DNA by means of its internal leucine zipper. Thus it is readily seen that FA proteins also act outside of the Fanconi pathway, either by helping neutralize ROS or by taking part in DNA repair. Such mechanisms help understand the causes behind bone marrow failure, where reoxygenation-induced oxidative stress is very common. Furthermore, it is known that cross-linking agents produce ROS and it is possible that FA cell hypersensitivity to cross-linkers is not due directly to them, but rather to the cell's impaired ability to cope with increased ROS production.

Chapter 8

Agranulocytosis and B-cell Chronic Lymphocytic Leukemia

Agranulocytosis

Agranulocytosis	
ICD-10	D70.
ICD-9	288.0
DiseasesDB	8994
MeSH	D000380

Agranulocytosis, also known as **Agranulosis** or **Granulopenia**, is an acute condition involving a severe and dangerous leukopenia (lowered white blood cell count), most commonly of neutrophils, causing a neutropenia in the circulating blood. It represents a severe lack of one major class of infection-fighting white blood cells. People with this condition are at very high risk of serious infections due to their suppressed immune system.

In agranulocytosis, the concentration of granulocytes (a major class of white blood cells that includes neutrophils, basophils, and eosinophils) drops below 100 cells/mm^3 of blood, which is less than 5% of the normal value. Agranulocytosis is more severe than granulocytopenia and may involve more sub-types of white blood cells than neutropenia.

Classification

The term derives from the Greek: *a*, meaning *without*; *granulocyte*, a particular kind of cell; *osis*, from the Greek, meaning *condition* [esp. *disorder*]. Consequently, agranulocytosis is sometimes described as "no granulocytes", but a total absence is not required for diagnosis.

The terms *agranulocytosis*, *granulocytopenia* and *neutropenia* are sometimes used interchangeably. Agranulocytosis implies a more severe deficiency than granulocyte-openia. Neutropenia indicates a deficiency of neutrophils (the most common granulocyte cell) only.

To be precise, neutropenia is the term normally used to describe absolute neutrophil counts (ANC) of less than 500 cells per microlitre, whereas agranulocytosis is reserved for cases with ANC of less than 100 cells per microlitre.

The following terms can be used to specify the type of granulocyte referenced:

- Inadequate numbers of neutrophils: neutropenia (most common)
- Inadequate numbers of eosinophils: eosinopenia (uncommon)
- Inadequate numbers of basophils: basopenia (very rare)

Signs and symptoms

Agranulocytosis may be asymptomatic, or may clinically present with sudden fever, rigors and sore throat. Infection of any organ may be rapidly progressive (e.g., pneumonia, urinary tract infection). Septicemia may also progress rapidly.

Neutropenia and agranulocytosis are associated with gum diseases, such as gingival bleeding, saliva increase, halitosis, osteoporosis, and destruction of periodontal ligament.

Causes

A large number of drugs have been associated with agranulocytosis, including antiepileptics, antithyroid drugs (carbimazole, methimazole, and propylthiouracil), anti-biotics (penicillin, chloramphenicol and co-trimoxazole), cytotoxic drugs, gold, NSAIDs (indomethacin, naproxen, phenylbutazone, metamizole), mebendazole, the antidepressant mirtazapine, and some antipsychotics (the atypical antipsychotic clozapine). Clozapine users in the US must be nationally registered for monitoring of low WBC and absolute neutrophil counts (ANC).

Although the reaction is generally idiosyncratic rather than proportional, experts recommend that patients using these drugs be told about the symptoms of agranulocytosis-related infection, such as a sore throat and a fever.

The Center for Disease Control recently traced outbreaks of agranulocytosis among cocaine users, in the US and Canada between March 2008 and November 2009, to the presence of levamisole in the drug supply. The Drug Enforcement Administration reported that, as of February 2010, 71% of seized cocaine lots coming into the US contained levamisole as a cutting agent. Levamisole is an antihelminthic (i.e. deworming) drug used in animals. The reason for adding levamisole to cocaine is unknown , although it can be due to their similar melting points and solubilities.

Diagnosis

The diagnosis is made after a complete blood count, a routine blood test. The absolute neutrophil count in this test will be below 500, and can reach 0 cells/mm³. Other kinds of blood cells are typically present in normal numbers.

To formally diagnose agranulocytosis, other pathologies with a similar presentation must be excluded, such as aplastic anemia, paroxysmal nocturnal hemoglobinuria, myelodysplasia and leukemias. This requires a bone marrow examination that shows normocellular (normal amounts and types of cells) blood marrow with underdeveloped promyelocytes. These underdeveloped promyelocytes, if fully matured, would have been the missing granulocytes.

Treatment

In patients that have no symptoms of infection, management consists of close monitoring with serial blood counts, withdrawal of the offending agent (e.g., medication), and general advice on the significance of fever.

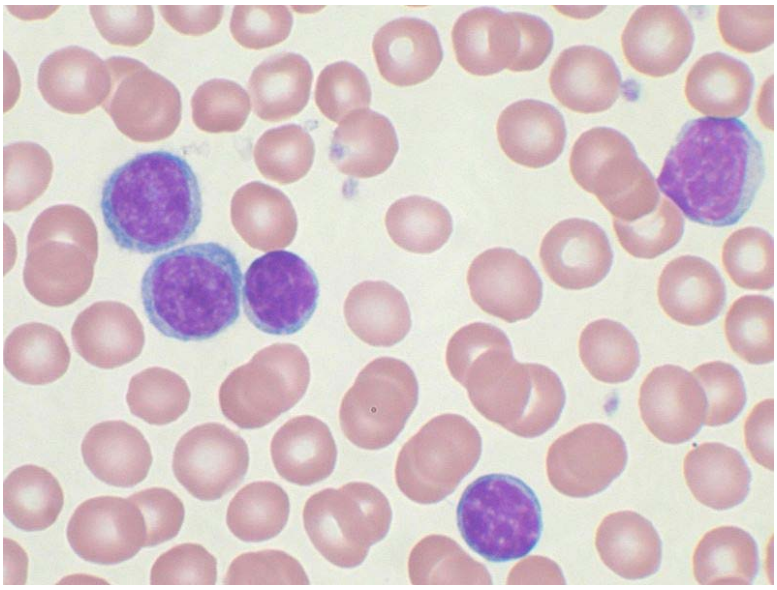
Infection in patients with low white blood cell counts is usually treated urgently, and usually includes a broad-spectrum penicillin or cephalosporin (piperacillin-tazobactam, ceftazidime or ticarcillin clavulanate), or meropenem in combination with gentamicin or amikacin.

If the patient remains febrile after 4–5 days and no causative organism for the infection has been identified, antibiotics are, in general, changed to a glycopeptide (e.g., vancomycin), and subsequently an antifungal agent (e.g., amphotericin B) is added to the regimen. In agranulocytosis, the use of recombinant G-CSF (filgrastim) often results in hematologic recovery.

Transfusion of granulocytes would have been a solution to the problem. However, granulocytes live only ~10 hours in the circulation (for days in spleen or other tissue), which gives a very short-lasting effect. In addition, there are many complications of such a procedure.

B-cell chronic lymphocytic leukemia

Chronic lymphocytic leukemia



Peripheral blood smear showing CLL cells

ICD-10	C91.1
ICD-9	204.1
ICD-O:	M9823/3 (CLL) 9670/3 (SCL)
DiseasesDB	2641
MedlinePlus	000532
eMedicine	med/370
MeSH	D015451

B-cell chronic lymphocytic leukemia (**B-CLL**), also known as **chronic lymphoid leukemia** (CLL), is the most common type of leukemia. Leukemias are cancers of the white blood cells (leukocytes). CLL affects B cell lymphocytes. B cells originate in the bone marrow, develop in the lymph nodes, and normally fight infection by producing antibodies. In CLL, the DNA of a B cell is damaged, so that it cannot produce antibodies. Additionally, B cells grow out of control and accumulate in the bone marrow and blood, where they crowd out healthy blood cells. CLL is a stage of **small lymphocytic**

lymphoma (SLL), a type of B-cell lymphoma, which presents primarily in the lymph nodes. CLL and SLL are considered the same underlying disease, just with different appearances.

CLL is a disease of adults, but, in rare cases, it can occur in teenagers and occasionally in children (inherited). Most (>75%) people newly diagnosed with CLL are over the age of 50, and the majority are men.

Most people are diagnosed without symptoms as the result of a routine blood test that returns a high white blood cell count, but, as it advances, CLL results in swollen lymph nodes, spleen, and liver, and eventually anemia and infections. Early CLL is not treated, and late CLL is treated with chemotherapy and monoclonal antibodies.

DNA analysis has distinguished two major types of CLL, with different survival times. CLL that is positive for the marker ZAP-70 has an average survival of 5 years. CLL that is negative for ZAP-70 has an average survival of more than 25 years. Patients with slowly-progressing disease can be reassured and may not need any treatment in their lifetimes.

Clinical staging

Staging, determining the extent of the disease, is done with the Rai staging system or the Binet classification and is based primarily on the presence, or not, of a low platelet or red cell count. Early stage disease does not need to be treated.

Gene mutation status

Recent publications suggest that two or three prognostic groups of CLL exist based on the maturational state of the cell. This distinction is based on the maturity of the lymphocytes as discerned by the immunoglobulin variable-region heavy chain (IgV_H) gene mutation status. High risk patients have an immature cell pattern with few mutations in the DNA in the IgV_H antibody gene region whereas low risk patients show considerable mutations of the DNA in the antibody gene region indicating mature lymphocytes.

Since assessment of the IgV_H antibody DNA changes is difficult to perform, the presence of either cluster of differentiation 38 (CD38) or Z-chain–associated protein kinase-70 (ZAP-70) may be surrogate markers of high risk subtype of CLL. Their expression correlates with a more immature cellular state and a more rapid disease course.

Fluorescence in situ hybridization (FISH)

In addition to the maturational state, the prognosis of patients with CLL is dependent on the genetic changes within the neoplastic cell population. These genetic changes can be identified by fluorescent probes to chromosomal parts using a technique referred to as

fluorescent in situ hybridization (FISH). Four main genetic aberrations are recognized in CLL cells that have a major impact on disease behavior.

1. Deletions of part of the short arm of chromosome 17 (del 17p), which target the cell cycle regulating protein p53 are particularly deleterious. Patients with this abnormality have significantly short interval before they require therapy and a shorter survival. This abnormality is found in 5–10% of patients with CLL.
2. Deletions of the long arm on chromosome 11 (del 11q) are also unfavorable although not to the degree seen with del 17p. The abnormality targets the ATM gene and occurs infrequently in CLL (5–10%).
3. Trisomy 12, an additional chromosome 12, is a relatively frequent finding occurring in 20–25% of patients and imparts an intermediate prognosis.
4. Deletion of the long arm of chromosome 13 (del 13q) is the most common abnormality in CLL with roughly 50% of patients with cells containing this defect. These patients have the best prognosis and most will live many years, even decades, without the need for therapy. The gene targeted by this deletion is a segment coding for microRNAs miR-15a and miR-16-1.

Array-based Karyotyping

Array-based karyotyping is a cost-effective alternative to FISH for detecting chromosomal abnormalities in CLL. Several clinical validation studies have shown >95% concordance with the standard CLL FISH panel.

Related diseases

In the past, cases with similar microscopic appearance in the blood but with a T cell phenotype were referred to as T-cell CLL. However, it is now recognized that these so-called T-cell CLLs are in fact a separate disease group and are currently classified as T-cell prolymphocytic leukemias.

CLL should not be confused with acute lymphoblastic leukemia, (ALL) a highly aggressive and highly treatable leukemia most commonly diagnosed in children.

Symptoms and signs

Most people are diagnosed without symptoms as the result of a routine blood test that returns a high white blood cell count. Less commonly, CLL may present with enlarged lymph nodes without a high white blood cell count or no evidence of the disease in the blood. This is referred to as *small lymphocytic lymphoma*. In some individuals the disease comes to light only after the neoplastic cells overwhelm the bone marrow resulting in anemia producing tiredness or weakness.

Diagnosis

The disease is easily diagnosed. CLL is usually first suspected by the presence of a lymphocytosis, an increase in one type of the white blood cell, on a complete blood count (CBC) test. This frequently is an incidental finding on a routine physician visit. Most often the lymphocyte count is greater than 4000 cells per microlitre (μl) of blood but can be much higher. The presence of a lymphocytosis in an elderly individual should raise strong suspicion for CLL and a confirmatory diagnostic test, in particular flow cytometry, should be performed unless clinically unnecessary.

The diagnosis of CLL is based on the demonstration of an abnormal population of B lymphocytes in the blood, bone marrow, or tissues that display an unusual but characteristic pattern of molecules on the cell surface. This atypical molecular pattern includes the co-expression of cells surface markers cluster of differentiation 5 (CD5) and cluster of differentiation 23 (CD23). In addition, all the CLL cells within one individual are clonal, that is genetically identical. In practice, this is inferred by the detection of only one of the mutually exclusive antibody light chains, kappa or lambda, on the entire population of the abnormal B cells. Normal B lymphocytes consist of a stew of different antibody producing cells resulting in a mixture of both kappa and lambda expressing cells. The lack of the normal distribution of kappa and lambda producing B cells is one basis for demonstrating clonality, the key element for establishing a diagnosis of any B cell malignancy (B cell Non-Hodgkin lymphoma).

The combination of the microscopic examination of the peripheral blood and analysis of the lymphocytes by flow cytometry to confirm clonality and marker molecule expression is needed to establish the diagnosis of CLL. Both are easily accomplished on a small amount of blood. A flow cytometer is an instrument that can examine the expression of molecules on individual cells in fluids. This requires the use of specific antibodies to marker molecules with fluorescent tags recognized by the instrument. In CLL, the lymphocytes are genetically clonal, of the B cell lineage (express marker molecules cluster of differentiation 19 (CD19) and CD20), and characteristically express the marker molecules CD5 and CD23. These B cells resemble normal lymphocytes under the microscope, although slightly smaller, and are fragile when smeared onto a glass slide giving rise to many broken cells (smudge cells).

Differential diagnosis

Hematologic disorders that may resemble CLL in their clinical presentation, behavior, and microscopic appearance include mantle cell lymphoma, marginal zone lymphoma, B cell prolymphocytic leukemia, and lymphoplasmacytic lymphoma.

- B cell prolymphocytic leukemia (B PLL), is a related but more aggressive disorder, has cells with similar phenotype but that are significantly larger than normal lymphocytes and have a prominent nucleolus. The distinction is important as the prognosis and therapy differs from CLL.

- Hairy cell leukemia is also a neoplasm of B lymphocytes but the neoplastic cells have a distinct morphology under the microscope (hairy cell leukemia cells have delicate, hair-like projections on their surface) and unique marker molecule expression.

All the B cell malignancies of the blood and bone marrow can be differentiated from one another by the combination of cellular microscopic morphology, marker molecule expression, and specific tumor-associated gene defects. This is best accomplished by evaluation of the patient's blood, bone marrow and occasionally lymph node cells by a pathologist with specific training in blood disorders. A flow cytometer is necessary for cell marker analysis and the detection of genetic problems in the cells may require visualizing the DNA changes with fluorescent probes by fluorescent in situ hybridization (FISH).

Treatment

CLL treatment focuses on controlling the disease and its symptoms rather than on an outright cure. CLL is treated by chemotherapy, radiation therapy, biological therapy, or bone marrow transplantation. Symptoms are sometimes treated surgically (splenectomy removal of enlarged spleen) or by radiation therapy ("de-bulking" swollen lymph nodes).

Initial CLL treatments vary depending on the exact diagnosis and the progression of the disease, and even with the preference and experience of the health care practitioner. There are dozens of agents used for CLL therapy.

Decision to treat

While generally considered incurable, CLL progresses slowly in most cases. Many people with CLL lead normal and active lives for many years—in some cases for decades. Because of its slow onset, early-stage CLL is, in general, not treated since it is believed that early CLL intervention does not improve survival time or quality of life. Instead, the condition is monitored over time to detect any change in the disease pattern.

The decision to start CLL treatment is taken when the patient's clinical symptoms or blood counts indicate that the disease has progressed to a point where it may affect the patient's quality of life.

Clinical "staging systems" such as the Rai 4-stage system and the Binet classification can help to determine when and how to treat the patient.

Determining when to start treatment and by what means is often difficult; studies have shown there is no survival advantage to treating the disease too early. The National Cancer Institute Working Group has issued guidelines for treatment, with specific markers that should be met before it is initiated.

Chemotherapy

Combination chemotherapy regimens are effective in both newly-diagnosed and relapsed CLL. Combinations of fludarabine with alkylating agents (cyclophosphamide) produce higher response rates and a longer progression-free survival than single agents:

- **FC** (fludarabine with cyclophosphamide)
- **FR** (fludarabine with rituximab)
- **FCR** (fludarabine, cyclophosphamide, and rituximab)
- **CHOP** (cyclophosphamide, doxorubicin, vincristine and prednisolone)

Although the purine analogue fludarabine was shown to give superior response rates than chlorambucil as primary therapy, there is no evidence that early use of fludarabine improves overall survival, and some clinicians prefer to reserve fludarabine for relapsed disease.

Alkylating agents approved for CLL include bendamustine and cyclophosphamide.

Monoclonal antibodies such as alemtuzumab (directed against CD52), rituximab (directed against CD20), and Arzerra (ofatumumab)(directed against CD20).

Stem cell transplantation

Allogeneic bone marrow (stem cell) transplantation is rarely used as a first-line treatment for CLL due to its risk. There is increasing interest in the use of reduced-intensity allogeneic stem cell transplantation, which offers the prospect of cure for selected patients with a suitable donor.

Younger patients that are at high risk for dying from CLL might consider hematopoietic stem cell transplantation (HSCT). Autologous stem cell transplantation, a lower-risk form of treatment using the patient's own blood cells, is not curative. Myeloablative (bone marrow killing) forms of allogeneic stem cell transplantation, a high-risk treatment using blood cells from a healthy donor, may be curative for some patients, but most patients cannot tolerate the treatment. An intermediate level, called *reduced-intensity conditioning allogeneic stem cell transplantation*, may be better tolerated by older or frail patients.

Refractory CLL

"Refractory" CLL is a disease that no longer responds favorably to treatment. In this case more aggressive therapies, including lenalidomide, flavopiridol, and bone marrow (stem cell) transplantation, are considered. The monoclonal antibody, alemtuzumab (directed against CD52), may be used in patients with refractory, bone marrow-based disease.

Complications

Chronic lymphocytic leukemia may transform into Richter's syndrome, a term used to describe the development of fast-growing diffuse large B cell lymphoma, prolymphocytic leukemia, Hodgkin disease, or acute leukemia in a patient who has chronic lymphocytic leukemia. Its incidence is estimated to be around 5%.

Epidemiology

CLL is a disease of older adults and is rarely encountered in individuals under the age of 40. Thereafter, the disease incidence increases with age.

In the United States during 2009, about 16,000 new cases are expected to be diagnosed, and 4,400 patients are expected to die from CLL. Because of the prolonged survival, which was typically about ten years in past decades, but which can extend to a normal life expectancy, the prevalence (number of people living with the disease) is much higher than the incidence (new diagnoses).

Subclinical "disease" can be identified in 3.5% of normal adults, and in up to 8% of individuals over the age of 70. That is, small clones of B cells with the characteristic CLL phenotype can be identified in many healthy elderly persons. The clinical significance of these cells is unknown.

Of all cancers involving the same class of blood cell, 7% of cases are CLL/SLL.

Complications: hypogammaglobulinemia leading to recurrent infection, warm auto immune haemolytic anaemia in 10–15% of patients, transformation to high grade lymphoma, Richter's transformation.

Rates of CLL are somewhat elevated in people that had been exposed to certain chemicals. Under U.S. Department of Veterans' Affairs regulations, Vietnam veterans who served in-country or in the inland waterways of Vietnam and who later develop CLL are presumed to have contracted it from exposure to Agent Orange and may be entitled to compensation.

Prognosis

Prognosis depends on the subtype. The overall 5-year survival rate (all forms of CLL together) is about 50%.

Research directions

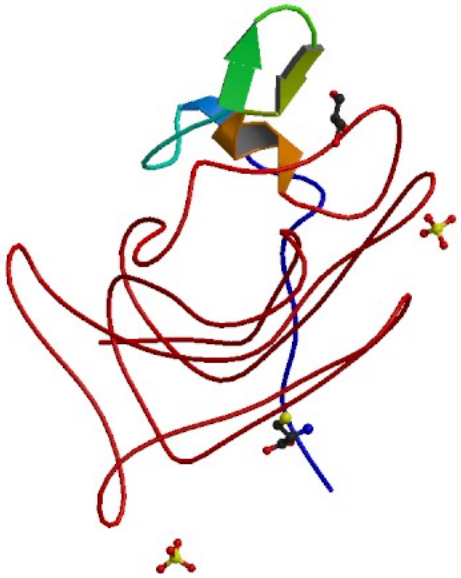
There is considerable research activity studying the many treatments individually or in combination with each other.

Current research is comparing different forms of bone marrow transplants to determine which patients are the best candidates and which approach is best in different situations.

Chapter 9

Haemophilia

Haemophilia



Deficiency in coagulation factor VIII is the most common cause of haemophilia.

ICD-10	D66.-D68.
ICD-9	286
OMIM	306700 306900 264900
DiseasesDB	5555 5561 29376
MedlinePlus	000537

eMedicine	med/3528
MeSH	D025861

Haemophilia (also spelled **hemophilia** in North America, from the Greek *haima* αἷμα 'blood' and *philia* φιλος 'love') is a group of hereditary genetic disorders that impair the body's ability to control blood clotting or coagulation, which is used to stop bleeding when a blood vessel is broken. Haemophilia A (clotting factor VIII deficiency) is the most common form of the disorder, occurring at about 1 in 5,000–10,000 male births. Haemophilia B (factor IX deficiency) occurs at about 1 in about 20,000–34,000 male births.

Like most recessive sex-linked, X chromosome disorders, haemophilia is more likely to occur in males than females. This is because females have two X chromosomes while males have only one, so the defective gene is guaranteed to manifest in any male who carries it. Because females have two X chromosomes and haemophilia is rare, the chance of a female having two defective copies of the gene is very low, so females are almost exclusively asymptomatic carriers of the disorder. Female carriers can inherit the defective gene from either their mother or father, or it may be a new mutation. Only under rare circumstances do females actually have haemophilia.

Haemophilia lowers blood plasma clotting factor levels of the coagulation factors needed for a normal clotting process. Thus when a blood vessel is injured, a temporary scab does form, but the missing coagulation factors prevent fibrin formation, which is necessary to maintain the blood clot. A haemophiliac does not bleed more intensely than a normal person, but can bleed for a much longer time. In severe haemophiliacs even a minor injury can result in blood loss lasting days or weeks, or even never healing completely. In areas such as the brain or inside joints, this can be fatal or permanently debilitating.

Signs and symptoms

Characteristic symptoms vary with severity. In general symptoms are internal or external bleeding episodes, which are called "bleeds". Patients with more severe haemophilia suffer more severe and more frequent bleeds, while patients with mild haemophilia typically suffer more minor symptoms except after surgery or serious trauma. Moderate haemophiliacs have variable symptoms which manifest along a spectrum between severe and mild forms.

Prolonged bleeding and re-bleeding are the diagnostic symptoms of haemophilia. Internal bleeding is common in people with severe haemophilia and some individuals with moderate haemophilia. The most characteristic type of internal bleed is a joint bleed where blood enters into the joint spaces. This is most common with severe haemophiliacs and can occur spontaneously (without evident trauma). If not treated promptly, joint bleeds can lead to permanent joint damage and disfigurement. Bleeding into soft tissues such as muscles and subcutaneous tissues is less severe but can lead to damage and requires treatment.

Children with mild to moderate haemophilia may not have any signs or symptoms at birth especially if they do not undergo circumcision. Their first symptoms are often frequent and large bruises and haematomas from frequent bumps and falls as they learn to walk. Swelling and bruising from bleeding in the joints, soft tissue, and muscles may also occur. Children with mild haemophilia may not have noticeable symptoms for many years. Often, the first sign in very mild haemophiliacs is heavy bleeding from a dental procedure, an accident, or surgery. Females who are carriers usually have enough clotting factors from their one normal gene to prevent serious bleeding problems, though some may present as mild haemophiliacs.

Complications

Severe complications are much more common in severe and moderate haemophiliacs. Complications may be both directly from the disease or from its treatment:

- **Deep internal bleeding**, e.g. deep-muscle bleeding, leading to swelling, numbness or pain of a limb.
- **Joint damage** from haemarthrosis, potentially with severe pain, disfigurement, and even destruction of the joint and development of debilitating arthritis.
- **Transfusion transmitted infection** from blood transfusions that are given as treatment.
- **Adverse reactions** to clotting factor treatment, including the development of an immune inhibitor which renders factor replacement less effective.
- **Intracranial haemorrhage** is a serious medical emergency caused by the buildup of pressure inside the skull. It can cause disorientation, nausea, loss of consciousness, brain damage, and death.

Life expectancy

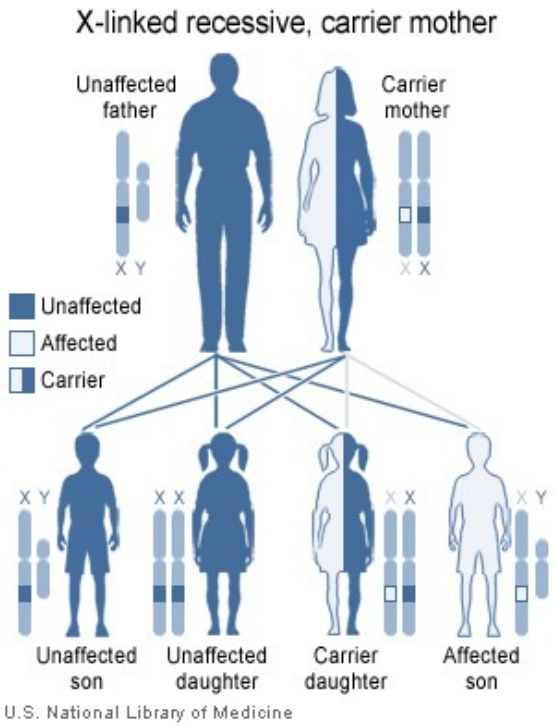
Like most aspects of the disorder, life expectancy varies with severity and adequate treatment. People with severe haemophilia who don't receive adequate, modern treatment have greatly shortened lifespans and often do not reach maturity. Prior to the 1960s when effective treatment became available, average life expectancy was only 11 years. By the 1980s the life span of the average haemophiliac receiving appropriate treatment was 50–60 years. Today with appropriate treatment, males with haemophilia typically have a near normal quality of life with an average lifespan approximately 10 years shorter than an unaffected male.

Since the 1980s the primary leading cause of death of people with severe haemophilia has shifted from haemorrhage to HIV/AIDS acquired through treatment with contaminated blood products. The second leading cause of death related to severe haemophilia complications is intracranial haemorrhage which today accounts for one third of all deaths of patients with haemophilia. Two other major causes of death include: hepatitis infections causing cirrhosis and, obstruction of air or blood flow due to soft tissue haemorrhage.

Causes

- Haemophilia A is a recessive X-linked genetic disorder involving a lack of functional clotting Factor VIII and represents 80% of haemophilia cases.
- Haemophilia B is a recessive X-linked genetic disorder involving a lack of functional clotting Factor IX. It comprises approximately 20% of haemophilia cases.
- Haemophilia C is an autosomal genetic disorder (i.e. *not* X-linked) involving a lack of functional clotting Factor XI. Haemophilia C is not completely recessive: heterozygous individuals also show increased bleeding.

Genetics



X-linked recessive inheritance

Females possess two X-chromosomes, males have one X and one Y chromosome. Since the mutations causing the disease are X-linked, a woman carrying the defect on one of her X-chromosomes may not be affected by it, as the equivalent allele on her other chromosome should express itself to produce the necessary clotting factors, due to X inactivation. However, the Y-chromosome in men has no gene for factors VIII or IX. If the genes responsible for production of factor VIII or factor IX present on a male's X-chromosome are deficient there is no equivalent on the Y-chromosome to cancel it out, so the deficient gene is not masked and he will develop the illness.

Since a male receives his single X-chromosome from his mother, the son of a healthy female silently carrying the deficient gene will have a 50% chance of inheriting that gene

from her and with it the disease; and if his mother is affected with haemophilia, he will have a 100% chance of being a haemophiliac. In contrast, for a female to inherit the disease, she must receive two deficient X-chromosomes, one from her mother and the other from her father (who must therefore be a haemophiliac himself). Hence haemophilia is far more common among males than females. However, it is possible for female carriers to become mild haemophiliacs due to lyonisation (inactivation) of the X chromosomes. Haemophiliac daughters are more common than they once were, as improved treatments for the disease have allowed more haemophiliac males to survive to adulthood and become parents. Adult females may experience menorrhagia (heavy periods) due to the bleeding tendency. The pattern of inheritance is criss-cross type. This type of pattern is also seen in colour blindness.

A mother who is a carrier has a 50% chance of passing the faulty X chromosome to her daughter, while an affected father will always pass on the affected gene to his daughters. A son cannot inherit the defective gene from his father.

Genetic testing and genetic counselling is recommended for families with haemophilia. Prenatal testing, such as amniocentesis, is available to pregnant women who may be carriers of the condition.

As with all genetic disorders, it is of course also possible for a human to acquire it spontaneously through mutation, rather than inheriting it, because of a new mutation in one of their parents' gametes. Spontaneous mutations account for about 33% of all cases of haemophilia A. About 30% of cases of haemophilia B are the result of a spontaneous gene mutation.

If a female gives birth to a haemophiliac child, either the female is a carrier for the disease or the haemophilia was the result of a spontaneous mutation. Until modern direct DNA testing, however, it was impossible to determine if a female with only healthy children was a carrier or not. Generally, the more healthy sons she bore, the higher the probability that she was not a carrier.

If a male is afflicted with the disease and has children with a female who is not even a carrier, his daughters will be carriers of haemophilia. His sons, however, will not be affected with the disease. The disease is X-linked and the father cannot pass haemophilia through the Y chromosome. Males with the disorder are then no more likely to pass on the gene to their children than carrier females, though all daughters they sire will be carriers and all sons they father will not have haemophilia (unless the mother is a carrier).

Severity

There are numerous different mutations which cause each type of haemophilia. Due to differences in changes to the genes involved, patients with haemophilia often have some level of active clotting factor. Individuals with less than 1% active factor are classified as having severe haemophilia, those with 1-5% active factor have moderate haemophilia,

and those with mild haemophilia have between 5-40% of normal levels of active clotting factor.

Diagnosis

Haemophilia A can be mimicked by von Willebrand disease.

- von Willebrand Disease could significantly affect as many as 1 in 10,000 people.
- von Willebrand Disease type 2A, where decreased levels of von Willebrand Factor can lead to premature proteolysis of Factor VIII. In contrast to haemophilia, vWD type 2A is inherited in an autosomal dominant fashion.
- von Willebrand Disease type 2N, where von Willebrand Factor cannot bind Factor VIII, autosomal recessive inheritance. (i.e.; both parents need to give the child a copy of the gene).
- von Willebrand Disease type 3, where lack of von Willebrand Factor causes premature proteolysis of Factor VIII. In contrast to haemophilia, vWD type 3 is inherited in an autosomal recessive fashion.

Additionally, severe cases of vitamin K deficiency can present similar symptoms to haemophilia. This is due to the fact that vitamin K is necessary for the human body to produce several protein clotting factors. This vitamin deficiency is rare in adults and older children but is common in newborns. Infants are born with naturally low levels of vitamin K and do not yet have the symbiotic gut flora to properly synthesise their own vitamin K. Bleeding issues due to vitamin K deficiency in infants is known as "haemorrhagic disease of the newborn", to avoid this complication newborns are routinely injected with vitamin K supplements.

Condition	Prothrombin time	Partial thromboplastin time	Bleeding time	Platelet count
Vitamin K deficiency or warfarin	prolonged	prolonged	unaffected	unaffected
Disseminated intravascular coagulation	prolonged	prolonged	prolonged	decreased
Von Willebrand disease	unaffected	prolonged	prolonged	unaffected
Haemophilia	unaffected	prolonged	unaffected	unaffected
Aspirin	unaffected	unaffected	prolonged	unaffected
Thrombocytopenia	unaffected	unaffected	prolonged	decreased
Early Liver failure	prolonged	unaffected	unaffected	unaffected
End-stage Liver failure	prolonged	prolonged	prolonged	decreased
Uremia	unaffected	unaffected	prolonged	unaffected
Congenital afibrinogenemia	prolonged	prolonged	prolonged	unaffected

Factor V deficiency	prolonged	prolonged	unaffected	unaffected
Factor X deficiency as seen in amyloid purpura	prolonged	prolonged	unaffected	unaffected
Glanzmann's thrombasthenia	unaffected	unaffected	prolonged	unaffected
Bernard-Soulier syndrome	unaffected	unaffected	prolonged	decreased

Management

Commercially produced factor concentrates such as "Advate", a recombinant Factor VIII produced by Baxter International, come as a white powder in a vial which must be mixed with sterile water prior to intravenous injection.

Though there is no cure for haemophilia, it can be controlled with regular infusions of the deficient clotting factor, i.e. factor VIII in haemophilia A or factor IX in haemophilia B.

Factor replacement can be either isolated from human blood serum, recombinant, or a combination of the two. Some haemophiliacs develop antibodies (inhibitors) against the replacement factors given to them, so the amount of the factor has to be increased or non-human replacement products must be given, such as porcine factor VIII.

If a patient becomes refractory to replacement coagulation factor as a result of circulating inhibitors, this may be partially overcome with recombinant human factor VII (NovoSeven), which is registered for this indication in many countries.

In early 2008, the US Food and Drug Administration (FDA) approved Xyntha (Wyeth) anti-haemophilic factor, genetically engineered from the genes of Chinese hamster ovary cells. Since 1993 (Dr. Mary Nugent) recombinant factor products (which are typically cultured in Chinese hamster ovary (CHO) tissue culture cells and involve little, if any human plasma products) have been available and have been widely used in wealthier western countries. While recombinant clotting factor products offer higher purity and safety, they are, like concentrate, extremely expensive, and not generally available in the developing world. In many cases, factor products of any sort are difficult to obtain in developing countries.

In Western countries, common standards of care fall into one of two categories: prophylaxis or on-demand. Prophylaxis involves the infusion of clotting factor on a regular schedule in order to keep clotting levels sufficiently high to prevent spontaneous bleeding episodes. On-demand treatment involves treating bleeding episodes once they arise. In 2007, a clinical trial was published in the *New England Journal of Medicine* comparing on-demand treatment of boys (< 30 months) with haemophilia A with prophylactic treatment (infusions of 25 IU/kg body weight of Factor VIII every other day) in respect to its effect on the prevention of joint-diseases. When the boys reached 6 years of age, 93% of those in the prophylaxis group and 55% of those in the episodic-therapy group had a normal index joint-structure on MRI. Prophylactic treatment, however, resulted in average costs of $300,000 per year. The author of an editorial published in the same issue of the *NEJM* supports the idea that prophylactic treatment not only is more effective than on demand treatment but also suggests that starting after the first serious joint-related haemorrhage may be more cost effective than waiting until the fixed age to begin. This study resulted in the first (October 2008) FDA approval to label any Factor VIII product to be used prophylactically. As a result, the factor product used in the study (Bayer's Kognate) is now labelled for use to prevent bleeds, making it more likely that insurance carries in the US will reimburse consumers who are prescribed and use this product prophylactically. Despite Kognate only recently being "approved" for this use in the US, it and other factor products have been well studied and are often prescribed to treat Haemophilia prophylactically to prevent bleeds, especially joint bleeds.

Preventive exercises

It is recommended that people affected with haemophilia do specific exercises to strengthen the joints, particularly the elbows, knees, and ankles. Exercises include

elements which increase flexibility, tone, and strength of muscles, increasing their ability to protect joints from damaging bleeds. These exercises are recommended after an internal bleed occurs and on a daily basis to strengthen the muscles and joints to prevent new bleeding problems. Many recommended exercises include standard sports warm-up and training exercises such as stretching of the calves, ankle circles, elbow flexions, and quadriceps sets.

Alternative medicine

While not a replacement for traditional treatments, preliminary scientific studies indicate that hypnosis and self-hypnosis can be effective at reducing bleeds and the severity of bleeds and thus the frequency of factor treatment. Herbs which strengthen blood vessels and act as astringents may benefit patients with haemophilia, however there are no peer reviewed scientific studies to support these claims. Suggested herbs include: Bilberry (Vaccinium myrtillus), Grape seed extract (Vitis vinifera), Scotch broom (Cytisus scoparius), Stinging nettle (Urtica dioica), Witch hazel (Hamamelis virginiana), and yarrow (Achillea millefolium).

Contraindications

Anticoagulants such as Heparin and Warfarin are contraindicated for people with haemophilia as these can aggravate clotting difficulties. Also contraindicated are those drugs which have "blood thinning" side effects. For instance, medications which contain aspirin, ibuprofen, or naproxen sodium should not be taken because they are well known to have the side effect of prolonged bleeding.

Also contraindicated are activities with a high likelihood of trauma, such as motorcycling and skateboarding. Popular sports with very high rates of physical contact and injuries such as American football, hockey, boxing, wrestling, and rugby should be avoided by people with haemophilia. Other active sports like soccer, baseball, and basketball also have a high rate of injuries, but have overall less contact and should be undertaken cautiously and only in consultation with a doctor.

Epidemiology

Haemophilia is rare, with only about 1 instance in every 10,000 births (or 1 in 5,000 male births) for haemophilia A and 1 in 50,000 births for haemophilia B. About 18,000 people in the United States have haemophilia. Each year in the US, about 400 babies are born with the disorder. Haemophilia usually occurs in males and less often in females. It is estimated that about 2500 Canadians have haemophilia A, and about 500 Canadians have haemophilia B.

History

"About seventy or eighty years ago, a woman by name of Smith, settled in the vicinity of Plymouth, New Hampshire, and transmitted the following idiosyncrasy to her descendants. It is one, she

observed, to which her family is unfortunately subject, and had been the source not only of great solicitude, but frequently the cause of death. If the least scratch is made on the skin of some of them, as mortal a hemorrhagy will eventually ensue as if the largest wound is inflicted. (…) So assured are the members of this family of the terrible consequences of the least wound, that they will not suffer themselves to be bled on any consideration, having lost a relation by not being able to stop the discharge occasioned by this operation."

John C. Otto, 1803

Scientific discovery

The first written account of haemophilia occurred in the 2nd century in the Babylonian Talmud. In it Rabbi Judah haNasi, redactor of the Mishneh, wrote: "If she circumcised her first child and he died, and a second one also died, she must not circumcise her third child." This passage refers to both the prolonged bleeding caused by circumcision and to the maternal inheritance of the disease. The first medical professional to describe a disease was Albucasis. In the tenth century he described families whose males died of bleeding after only minor traumas. While many other such descriptive and practical references to the disease appear throughout historical writings, scientific analysis did not begin until the start of the nineteenth century.

In 1803, Dr. John Conrad Otto, a Philadelphian physician, wrote an account about "a hemorrhagic disposition existing in certain families" in which he called the affected males "bleeders." He recognised that the disorder was hereditary and that it affected mostly males and was passed down by healthy females. His paper was the second paper to describe important characteristics of an X-linked genetic disorder (the first paper being a description of colour blindness by John Dalton who studied his own family). Otto was able to trace the disease back to a woman who settled near Plymouth in 1720. The idea that affected males could pass the trait onto their unaffected daughters was not described until 1813 when John Hay published an account in The New England Journal of Medicine.

A Finnish Doctor in 1924 discovered a heredity bleeding disorder similar to Haemophilia localised in a group of islands (called the "Aland Islands") which are located to the southwest of Finland. This bleeding disorder is called "Von Willebrand Disease".

The term "haemophilia" is derived from the term "haemorrhaphilia" which was used in a description of the condition written by Friedrich Hopff in 1828, while he was a student at the University of Zurich. In 1937, Patek and Taylor, two doctors from Harvard, discovered anti-haemophilic globulin. In 1947, Pavlosky, a doctor from Buenos Aires, found haemophilia A and haemophilia B to be separate diseases by doing a lab test. This test was done by transferring the blood of one haemophiliac to another haemophiliac. The fact that this corrected the clotting problem showed that there was more than one form of haemophilia.

European royalty

Queen Victoria passed haemophilia on to some of her descendants.

Haemophilia has featured prominently in European royalty and thus is sometimes known as "the royal disease". Queen Victoria passed the mutation to her son Leopold and, through some of her daughters, to various royals across the continent, including the royal families of Spain, Germany, and Russia. In Russia, Tsarevich Alexei Nikolaevich, son of Nicholas II, was a descendant of Queen Victoria through his mother Empress Alexandra and suffered from haemophilia.

Ryan White was an American haemophiliac who became infected with HIV/AIDS through contaminated blood products.

It was claimed that Rasputin was successful at treating the Tsarevich's haemophilia. At the time, a common treatment administered by professional doctors was to use aspirin, which worsened rather than lessened the problem. It is believed that, by simply advising against the medical treatment, Rasputin could bring visible and significant improvement to the condition of Alexei.

In Spain, Queen Victoria's youngest daughter, Princess Beatrice, had a daughter Victoria Eugenie of Battenberg, who later became Queen of Spain. Two of her sons were haemophiliacs and both died from minor car accidents: Her eldest son, Prince Alfonso of Spain, Prince of Asturias, died at the age of 31 from internal bleeding after his car hit a telephone booth. Her youngest son, Infante Gonzalo, died at age 19 from abdominal bleeding following a minor car accident where he and his sister hit a wall while avoiding a cyclist. Neither appeared injured or sought immediate medical care and Gonzalo died two days later from internal bleeding.

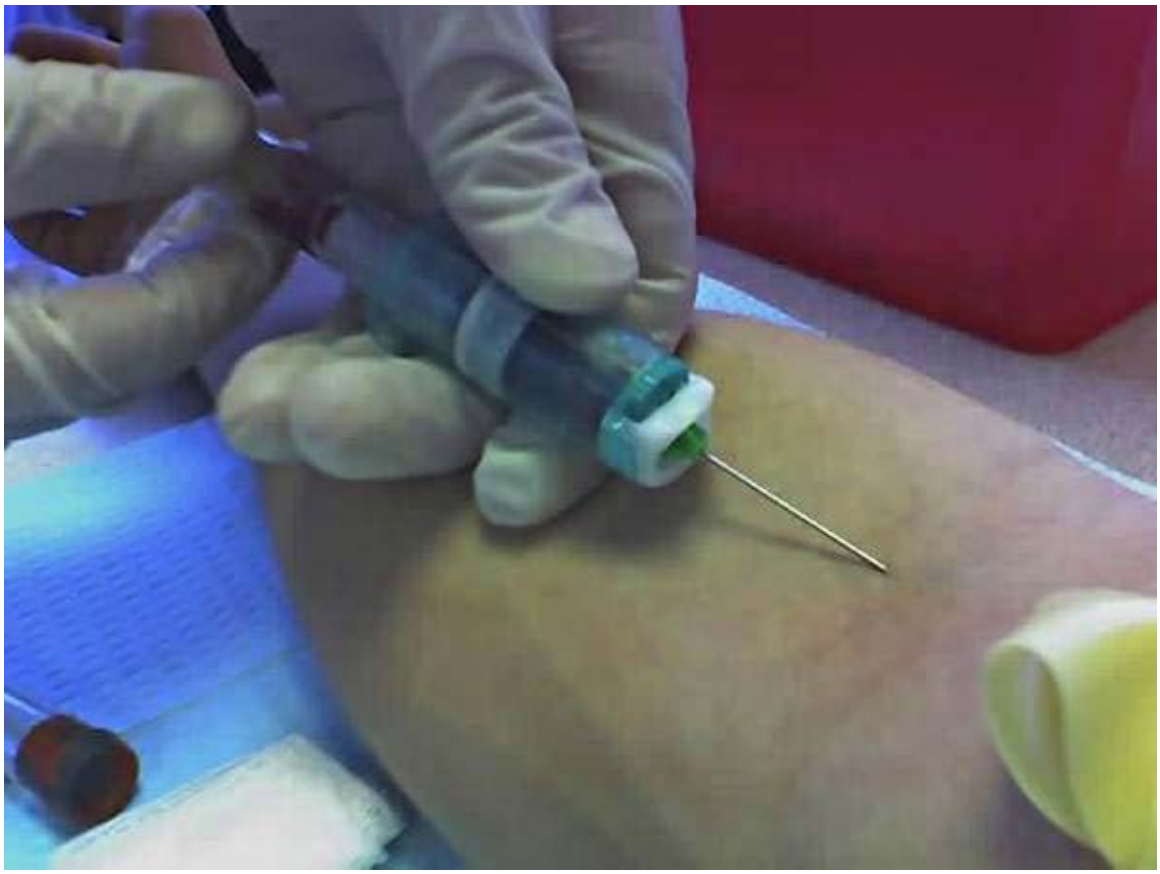
Blood contamination issues

Prior to 1985, there were no laws enacted within the U.S. to screen blood. As a result, many haemophilia patients who received untested and unscreened clotting factor prior to 1992 were at an extreme risk for contracting HIV and hepatitis C via these blood products. It is estimated that more than 50% of the haemophilia population, over 10,000 people, contracted HIV from the tainted blood supply in the United States alone.

As a direct result of the contamination of the blood supply in the late 1970s and early/mid 1980s with viruses such as hepatitis and HIV, new methods were developed in the production of clotting factor products. The initial response was to heat-treat (pasteurise) plasma-derived factor concentrate, followed by the development of monoclonal factor concentrates, which use a combination of heat treatment and affinity chromatography to inactivate any viral agents in the pooled plasma from which the factor concentrate is derived. The Lindsay Tribunal in Ireland investigated, among other things, the slow adoption of the new methods.

Chapter 10

Blood Test



A venipuncture performed using a vacutainer

A **blood test** is a laboratory analysis performed on a blood sample that is usually extracted from a vein in the arm using a needle, or via fingerprick.

Blood tests are used to determine physiological and biochemical states, such as disease, mineral content, drug effectiveness, and organ function. They are also used in drug tests.

Although the term *blood test* is used, most routine tests (except for most haematology) are done on plasma or serum, instead of blood cells.

Extraction

Venipuncture is useful as it is a relatively non-invasive way to obtain cells and extracellular fluid (plasma) from the body for analysis. Since blood flows throughout the body, acting as a medium for providing oxygen and nutrients, and drawing waste products back to the excretory systems for disposal, the state of the bloodstream affects, or is affected by, many medical conditions. For these reasons, blood tests are the most commonly performed medical tests.

Phlebotomists, laboratory technicians and nurses are those charged with patient blood extraction. However, in special circumstances, and emergency situations, paramedics and physicians sometimes extract blood. Also, respiratory therapists are trained to extract arterial blood for arterial blood gases.

Types of blood tests

Biochemical analysis

A basic metabolic panel measures sodium, potassium, chloride, bicarbonate, blood urea nitrogen (BUN), magnesium, creatinine, and glucose. It also sometimes includes calcium.

Some blood tests, such as those that measure glucose, cholesterol, or for determining the existence or lack of STD, require fasting (or no food consumption) eight to twelve hours prior to the drawing of the blood sample.

For the majority of blood tests, blood is usually obtained from the patient's vein. However, other specialized blood tests, such as the Arterial blood gas, require blood extracted from an artery. Blood gas analysis of arterial blood is primarily used to monitor carbon dioxide and oxygen levels related to pulmonary function, but it is also used to measure blood pH and bicarbonate levels for certain metabolic conditions.

While the regular glucose test is taken at a certain point in time, the glucose tolerance test involves repeated testing to determine the rate at which glucose is processed by the body.

T-cells and white blood cells are often counted.

Normal ranges

Test	Low	High	Unit	Comments
Sodium (Na)	136	145	mmol/L	
Potassium (K)	3.5	5.5	mmol/L	
Urea	2.5	6.4	mmol/L	BUN - blood urea nitrogen

Urea	15	40	mg/dL
Creatinine - male	62	115	μmol/L
Creatinine - female	53	97	μmol/L
Creatinine - male	0.7	1.3	mg/dL
Creatinine - female	0.6	1.1	mg/dL
Glucose (fasting)	3.9	5.8	mmol/L
Glucose (fasting)	70	105	mg/dL

Molecular profiles

- Protein electrophoresis (general technique -- not a specific test)
- Western blot (general technique -- not a specific test)
- Liver function tests
- Polymerase chain reaction (DNA). DNA testing is today possible with even very small quantities of blood: this is commonly used in forensic science, but is now also part of the diagnostic process of many disorders.
- Northern blot (RNA)
- Sexually transmitted diseases

Cellular evaluation

- Full blood count (or "complete blood count")
- Hematocrit and MCV ("mean corpuscular volume")
- Erythrocyte sedimentation rate (ESR)
- Cross-matching. Determination of blood type for blood transfusion or transplants
- Blood cultures are commonly taken if infection is suspected. Positive cultures and resulting sensitivity results are often useful in guiding medical treatment.

Future alternatives

Saliva tests

In 2008, scientists announced that the more cost effective saliva tests could eventually replace some blood tests, as saliva contains 20% of the proteins found in blood.

Micro emulsion

February 2011: Canadian researchers have developed a microchip for blood tests. It is called micro-emulsion, a droplet of blood captured inside a layer of another substance. It can control the exact size and spacing of the droplets. The new test could improve the efficiency, accuracy and speed of laboratory tests while also doing it cheaply. The microchip costs $25, whereas the robotic dispensers currently in use cost around $10,000.

Chapter 11

Anticoagulant

An **anticoagulant** is a substance that prevents coagulation; that is, it stops blood from clotting. A group of pharmaceuticals called anticoagulants can be used *in vivo* as a medication for thrombotic disorders. Some chemical compounds are used in medical equipment, such as test tubes, blood transfusion bags, and renal dialysis equipment.

As medications

Anticoagulants reduce blood clotting. This prevents deep vein thrombosis, pulmonary embolism, myocardial infarction and stroke.

Coumadins (Vitamin K antagonists)

These oral anticoagulants are a class of pharmaceuticals that antagonize the effects of vitamin K. Examples include warfarin. It takes at least 48 to 72 hours for the anticoagulant effect to develop. Where an immediate effect is required, heparin must be given concomitantly. These anticoagulants are used to treat patients with deep-vein thrombosis (DVT), pulmonary embolism (PE), atrial fibrillation (AF), and mechanical prosthetic heart valves.

Adverse effects

Patients aged 80 years or more may be especially susceptible to bleeding complications with a rate of 13 bleeds per 100 person-years.

These oral anticoagulants are used widely as poisons for mammalian pests, especially rodents.

Depletion of vitamin K by coumarin therapy increases risk of arterial calcification and heart valve calcification, especially if too much vitamin D is present.

Available agents

- Warfarin (Coumadin) *This is the main agent used in the U.S. and UK*

- Acenocoumarol and phenprocoumon *This is used more commonly outside the U.S. and the UK*
- Brodifacoum *Rat poison, not used medically*
- Phenindione

Heparin and derivative substances

Heparin is a biological substance, usually made from pig intestines. It works by activating antithrombin III, which blocks thrombin from clotting blood. Heparin can be used *in vivo* (by injection), and also *in vitro* to prevent blood or plasma clotting in or on medical devices. Vacutainer brand test tubes containing heparin are usually colored green.

Low molecular weight heparin

Low molecular weight heparin is a more highly processed product that is useful as it does not require monitoring of the APTT coagulation parameter (it has more predictable plasma levels) and has fewer side effects.

Synthetic pentasaccharide inhibitors of factor Xa

- Fondaparinux is a synthetic sugar composed of the five sugars (pentasaccharide) in heparin that bind to antithrombin. It is a smaller molecule than low molecular weight heparin.
- Idraparinux

Major pharmaceutical Heparin recall due to contamination

In March 2008 major recalls of Heparin were announced by pharmaceuticals due to a suspected and unknown contamination of the raw Heparin stock imported from China . The contaminant was later found to be a non-naturally occurring compound called oversulfated chondroitin sulfate . The U.S. Food and Drug Administration was quoted as stating that at least 19 deaths were believed linked to a raw Heparin ingredient imported from the People's Republic of China, and that they had also received 785 reports of serious injuries associated with the drug’s use. According to the New York Times: 'Problems with heparin reported to the agency include difficulty breathing, nausea, vomiting, excessive sweating and rapidly falling blood pressure that in some cases led to life-threatening shock'.

Direct thrombin inhibitors

Another type of anticoagulant is the direct thrombin inhibitor. Current members of this class include argatroban, lepirudin, bivalirudin, and dabigatran. An oral direct thrombin inhibitor, ximelagatran (Exanta) was denied approval by the Food and Drug Administration (FDA) in September 2004 and was pulled from the market entirely in February 2006 after reports of severe liver damage and heart attacks.

Other types of anticoagulants

Many other anticoagulants exist, for use in Research & Development, and more or less uses as drug candidates or diagnostics

- **Batroxobin**, a toxin from a snake venom that clots platelet-rich plasma without affecting platelets functions (lyses fibrinogen).
- **Hementin** is an anticoagulant protease from the salivary glands of Haementeria ghilianii

Food supplements

Food supplements with blood thinning effect include Nattokinase and Lumbrokinase

General indications

Therapeutic uses of anticoagulants include atrial fibrillation, pulmonary embolism (PE), deep vein thrombosis (DVT), or venous thromboembolism (VTE), congestive heart failure, stroke, myocardial infarction, genetic or acquired hypercoagulability

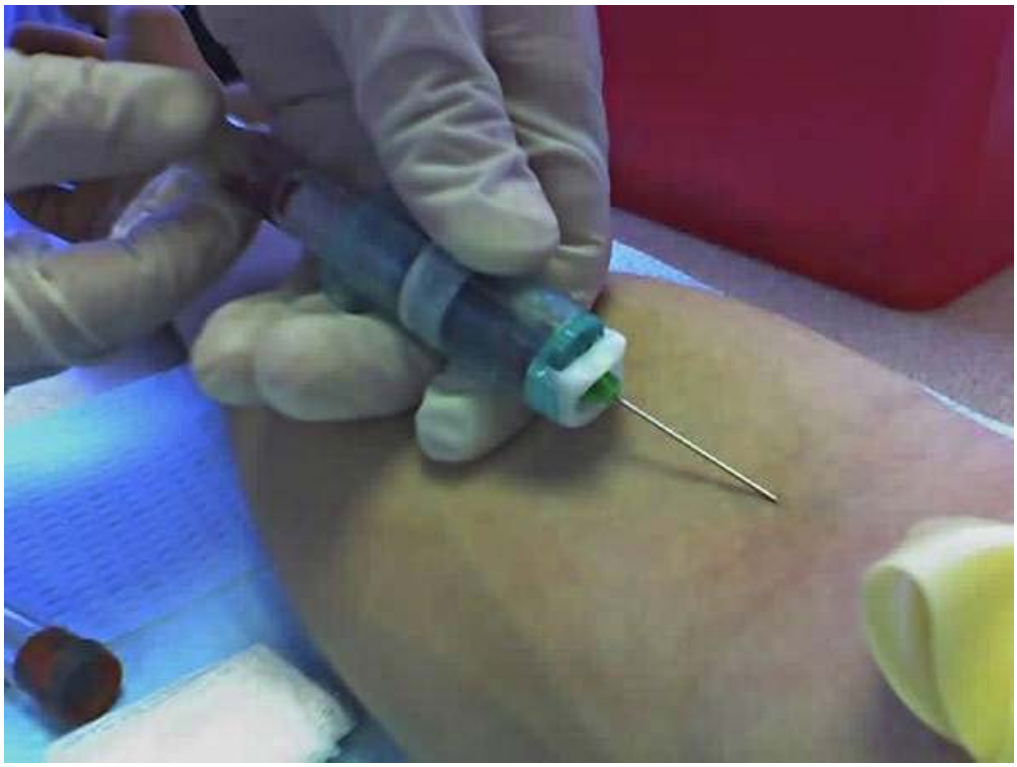
Anticoagulants outside the body

Laboratory instruments, test tubes, blood transfusion bags, and medical and surgical equipment will get clogged up and become nonoperational if blood is allowed to clot. Chemicals can be added to stop blood clotting. Apart from heparin, most of these chemicals work by binding calcium ions, preventing the coagulation proteins from using them.

- EDTA is denoted by mauve or purple caps on Vacutainer brand test tubes. This chemical strongly and irreversibly binds calcium. It is in a powdered form.
- Citrate is usually in blue Vacutainer tube. It is in liquid form in the tube and is used for coagulation tests, as well as in blood transfusion bags. It gets rid of the calcium, but not as strongly as EDTA. Correct proportion of this anticoagulant to blood is crucial because of the dilution. It can be in the form of sodium citrate or ACD.
- Oxalate has a mechanism similar to that of citrate. It is the anticoagulant used in fluoride (grey top) tubes.

Chapter 12

Venipuncture



Venipuncture using the BD Vacutainer system. This picture shows a multiple-use BD Vacutainer device that is no longer used. It has been replaced by a single use BD Vacutainer device.

In medicine, **venepuncture**,**venopuncture** or **venipuncture** is the process of obtaining intravenous access for the purpose of intravenous therapy or obtaining a sample of venous blood. This procedure is performed by medical laboratory scientists, medical practitioners, some EMTs, paramedics, phlebotomists and other nursing staff. Venepuncture is one of the most routinely performed invasive procedures and is carried out for two reasons, to obtain blood for diagnostic purposes or to monitor levels of blood components (Lavery & Ingram 2005). Blood analysis is one of the most important

diagnostic tools available to clinicians within healthcare. Its data is relied upon in the clinical setting for interpretation of a myriad of clinical signs and symptoms and developing skills in venepuncture can facilitate holistic and timely treatment.

Blood is most commonly obtained from the median cubital vein, which lies within the cubital fossa anterior to the elbow. This vein lies close to the surface of the skin, and there is not a large nerve supply.

Minute quantities of blood may be taken by fingersticks sampling and collected from infants by means of a heel stick or from scalp veins with a winged infusion needle.

Phlebotomy (incision into a vein) is also the treatment of certain diseases such as hemochromatosis and primary and secondary polycythemia.

Equipment

There are many ways in which blood can be drawn from a vein. The best method varies with the age of the patient, equipment available and tests required.

Most blood collection in the US and UK is done with an evacuated tube system, (two common systems are Vacutainer (Becton, Dickinson and company) and Vacuette (Greiner Bio-One GmBH). The equipment consisting of a plastic hub, a hypodermic needle, and a vacuum tube. Under certain circumstances, a syringe may be used, often with a butterfly needle, which is a plastic catheter attached to a short needle. In the developing world, a needle and syringe are still the most common method of drawing blood.

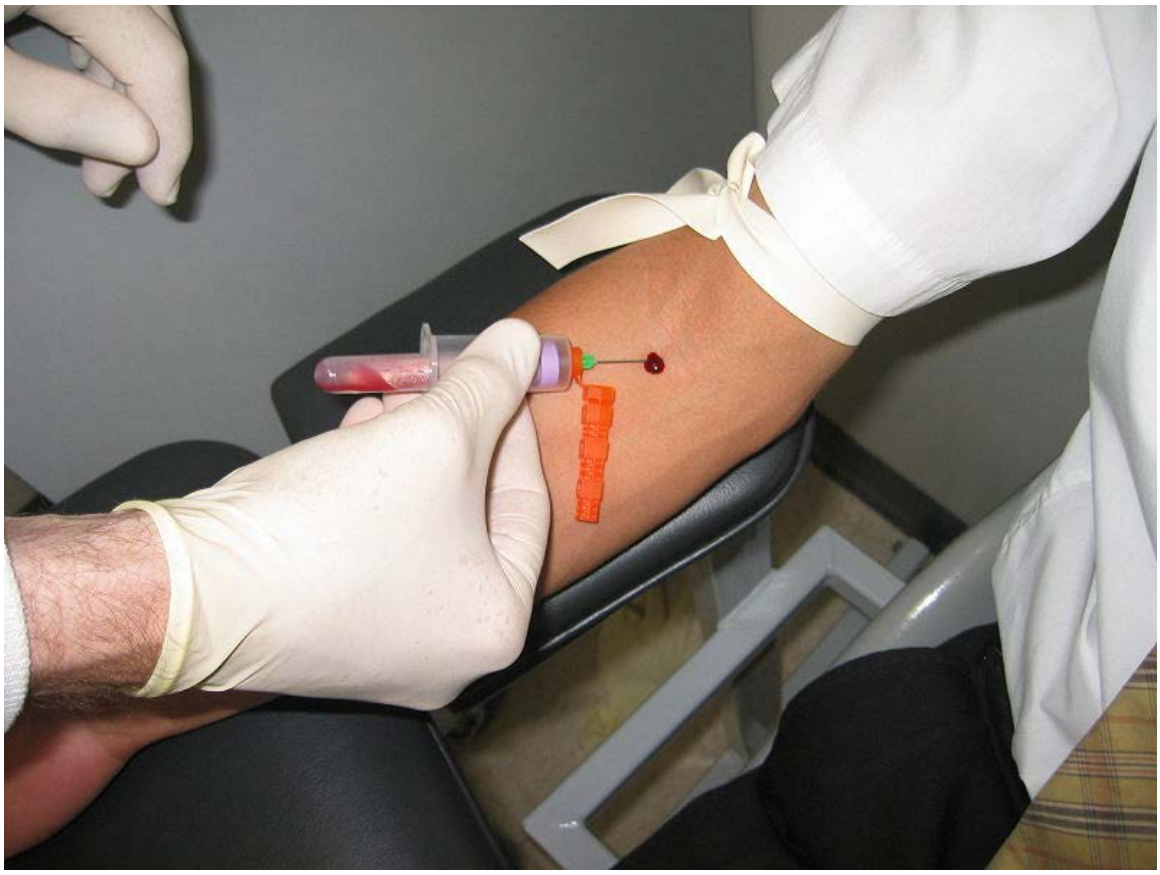
Additives and order of draw

The tubes in which blood is transported back to the laboratory contain a variety of additives or none at all. It is important to know which tube the individual laboratory requires for which test as reagents vary between laboratories and may be affected by different additives. In general whole blood needs to be mixed with EDTA which chelates calcium to prevent it clotting, unless the clotting time is the test to be measured in which citrates are used. The majority of biochemistry tests are performed on serum and so either a plain tube or a clotting accelerator is used. This clotting accelerator can interfere with some assays and so a plain tube is recommended in these cases but will obviously delay the result. Some assays may also require whole blood but are interfered with by EDTA and in this case Lithium Heparin is an alternative.

With the vacuum tube system, the needle pierces the top of the sample tube and will potentially come into contact with the additives in the tube. As it is a hollow needle some of this can be carried into the next tube and contaminate it. The most likely additive to cause trouble is EDTA which will affect the coagulation time assays and by chelating some of the metal ions may interfere with some of the biochemistry results(especially

potassium). Thus EDTA samples should be drawn last in most cases and plain tubes drawn first.

Venipuncture with evacuated or vacuum tubes



Blood draw filling a purple top vacuum tube.

Vacuum tubes were first marketed by U.S. company BD (Becton, Dickinson and Company) under the trade name Vacutainer tubes. Today, many companies sell vacuum tubes as the patent for this device is in the public domain. Some models are a type of test tube that contains a vacuum that automatically aspirates blood into itself. The tubes are made of glass or plastic. The tubes are attached to a needle and hub.

Multiple vacuum tubes can be attached to and removed in turn from a single needle, allowing multiple samples to be obtained from a single procedure. This is possible due to a Japanese invention called the multiple sample sleeve, which is basically a plastic cap fitting over the posterior end of the needle cannula, thus keeping blood from draining onto both health care worker and patient.

BD used this invention to create a system that included the vacuum pressurized sleeve, and a cannula with sleeve attached to a holder that holds the needle/sleeve combination, and guides the negatively pressured collection vial that is inserted once the needle is in the patient. Unfortunately, the sleeve that stops blood from moving until its seal is

perforated by the collection tube's insertion, also prevents flashback, the tell-tale sign the vein has been entered properly. If the phlebotomist chooses to use a butterfly needle, the "flash" is still present, and easily visible to the phlebotomy tech as well as the patient.

They are commonly used in US, UK, and Australian hospitals, private doctors' offices and community labs, and are available in various sizes to suit the age of the patient and the type of sample needed.

There are several sized needles for a phlebotomist to choose from. The most commonly used are as follows: a 21g (green top) needle, a 22g (black top) needle, a 21g (green label) butterfly needle, a 23g (blue label) butterfly needle, and a 25g (orange label) butterfly needle (however this needle is only used in pediatrics or extreme cases as it is so small that it can often result in hemolizing the blood sample, thereby invalidating the test). Most lay people are under the impression that using a butterfly needle is easier on their veins and less painful. Using a finer needle is less painful. However, the hazard of using butterfly needles lies in the fact that as the blood flows through the rubber tubing, it cools significantly, thereby clotting faster. In clotting in the tube, it stops up and reduces or as in most cases, completely stops. When this happens, it is necessary to re-stick a patient in order to obtain the blood sample. This is not the case when using the more common needles. Since the tubing is not a part of the needle, the blood goes through the needle directly into the tube. This method results in a faster, cleaner sample with less chance of hemolization. (When a blood sample is hemolyzed, it means the red cells have ruptured to the extent that they impart a pink/red color to the blood plasma, which is normally pale yellow.)

Venipuncture in children

Use of Lidocaine Iontophoresis is an effective method for reducing pain and alleviating distress during venipuncture in pediatric patients. Rapid dermal anesthesia can be achieved by local anesthetic infiltration, but it may evoke anxiety in children frightened by needles or distort the skin, making vascular access more difficult and increasing the risk of needle exposure to health care workers. Dermal anesthesia can also be achieved without needles by the topical application of local anesthetics (e.g., EMLA®, ASTRA Pharmaceutical, Sodertalje, Sweden) or by lidocaine iontophoresis. By contrast, noninvasive dermal anesthesia can be established in 5–15 min without distorting underlying tissues by lidocaine iontophoresis, where a direct electrical current facilitates dermal penetration of positively charged lidocaine molecules when placed under the positive electrode.

One study did conclude that the iontophoretic administration of lidocaine was safe and effective in providing dermal anesthesia for venipuncture in children 6–17 years old. This technique may not be applicable to all children. Future studies may provide information on the minimum effective iontophoretic dose for dermal anesthesia in children and the comparison of the anesthetic efficacy and satisfaction of lidocaine iontophoresis with topical anesthetic creams and subcutaneous infiltration.

Venipuncture with needle and syringe

Some health care workers prefer to use a syringe-needle technique for Venipuncture. Sarstedt manufactures a blood-drawing system S-Monovette that uses this principal. This method can be preferred on elderly patients, oncology patients, severely burned patients, obese patients or patients with unreliable or fragile veins. Because syringes are manually operated, the amount of suction applied may be easily controlled. This is particularly helpful with patients that have small veins that collapse under the suction of an evacuated tube. In children or other circumstances where the quantity of blood gained may be limited it can be helpful to know how much blood can be obtained before distributing it amongst the various additives that the laboratory will require.

Blood cultures

There are times where a patient may require a blood culture collection. The culture will determine if the patient has pathogens in the blood. Normally blood is sterile. When drawing blood from cultures using a sterile solution such as Betadine rather than alcohol.

Using sterile gloves, do not wipe away the surgical solution, touch the puncture site, or in any way compromise the sterile process. It is vital that the procedure is performed in as sterile a manner as possible as the persistent presence of skin commensals in blood cultures could indicate endocarditis but they are most often found as contaminants. It is encouraged to use an abrasive method of skin preparation. This removes the upper layers of dead skin cells along with their contaminating bacteria. Povidone-iodine has traditionally been used but in the UK a 2% chlorhexidine in 70% ethanol or isopropyl alcohol solution is preferred and time must be allowed for it to dry. The tops of any containers used when drawing a blood culture should also be disinfected using a similar solution. Some labs will actively discourage iodine use where iodine is thought to degrade the rubber stopper through which blood enters the bottle, thus allowing contaminates to enter the container.

The blood is collected into special transport bottles, which are like vacuum tubs but shaped differently. The blood culture bottle contains transport media to preserve any microorganisms present while they are being transported to the laboratory for cultures. Because it is unknown whether the pathogens are anaerobic (living without oxygen) or aerobic (living with oxygen), blood is collected to test for both. The aerobic bottle is filled first, and then the anaerobic bottle is filled. However if the collection is performed using a syringe, the anaerobic bottle is filled first. If a butterfly collection kit is used, the aerobic bottle is filled first, so that any air in the tubing is released into the oxygen-containing bottle.

Specially designed blood culture collection bottles eliminate the need for either the syringe or butterfly collection method. These specially designed bottles have long necks that fit into the evacuated tubes holders that are use for regular Venipuncture collection. These bottles also allow for collection of other blood specimens via evacuated tubes, to be collected without additional Venipuncture.

The amount of blood that is collected is critical for the optimal recovery of micro-organisms. Up to 10mL of blood is typical, but can vary according to the recommends of the manufacturer of the collection bottle. Collection from infants and children are 1 to 5 mL. If too little blood is collected, the ratio of blood –to-nutrient broth will inhibit the growth of microorganisms. If too much blood is collected from the patient, the patient risks a hospital-induced anemia and the ratio of blood-to-nutrient broth will tilt in the opposite direction, which also is not conductive to optimal growth.

The bottles are then incubated in specialized units for 24 hours before a lab technician studies and/or tests it. This step allows the very small numbers of bacteria (potentially 1 or 2 organisms) to multiply to a level which is sufficient for identification +/-antibiotic resistance testing. Modern blood culture bottles have an indicator in the base which changes color in the presence of bacterial growth and can be read automatically by machine. (For this reason the barcoded stickers found on these bottles should not be removed as they are used by the laboratorys automated systems.)

Taking blood samples from animals

Blood samples from living laboratory animals may be collected using following methods:

A) *Blood collection not requiring anesthesia*: Saphenous vein (rat, mice, guinea pig); Dorsal pedal vein (rat, mice).

B) *Blood collection requiring anesthesia (local/general anesthesia)*: Tail vein (rat, mice); Tail snip (mice); Orbital sinus (rat, mice); Jugular vein (rat, mice); Temporary cannula (rat, mice); Blood vessel cannulation (guinea pig, ferret); Tarsal vein (guinea pig); Marginal ear vein/artery (rabbit).

C) *Terminal procedure*: Cardiac puncture (rat, mice, guinea pig, rabbit, ferret); Orbital sinus (rat, mice); Posterior vena cava (rat, mice).

The volume of the blood sample collection is very important in experimental animals. All nonterminal blood collection without replacement of fluids is limited up to 10% of total circulating blood volume in healthy, normal, adult animals on a single occasion and collection may be repeated after 3 to 4 weeks. In case repeated blood samples are required at short intervals, a maximum of 0.6 ml/kg/day or 1.0% of an animal's total blood volume can be removed every 24 hour.The estimated blood volume in adult animals is 55 to 70 ml/kg body weight. Care should be taken for older and obese animals. If blood collection volume exceeds more than 10% of total blood volume, fluid replacement may be required. Lactated Ringer's solution (LRS) is recommended as the best fluid replacement by National Institutes of Health (NIH). If the volume of blood collection exceeds more than 30% of the total circulatory blood volume, adequate care should be taken so that the animal does not suffer from hypovolemia.

Blood alcohol tests

It is generally not advisable to use isopropyl alcohol to cleanse the venipuncture site when obtaining a specimen for a blood alcohol test. Using soap and hot water or a povidone iodine swab are advisable alternatives to isopropyl alcohol in this case.

Chapter 13

Hematopoietic Stem Cell Transplantation

Hematopoietic stem cell transplantation (HSCT) is the transplantation of multipotent hematopoietic stem cell or blood, often derived from bone marrow, umbilical cord blood or hemopoietic stem cells derived from a placenta. Stem cell transplantation is a medical procedure in the fields of hematology and oncology, most often performed for people with diseases of the blood, bone marrow, or certain cancer.

With the availability of the stem cell growth factors GM-CSF and G-CSF, most hematopoietic stem cell transplantation procedures are now performed using stem cells collected from the peripheral blood such as cord blood or placenta-derived stem cells, rather than from the bone marrow. Collecting peripheral blood stem cells provides a bigger graft, does not require that the donor be subjected to general anesthesia to collect the graft, results in a shorter time to engraftment, and may provide for a lower long-term relapse rate.

Hematopoietic stem cell transplantation remains a risky procedure with many possible complications; it has traditionally been reserved for patients with life-threatening diseases. While occasionally used experimentally in nonmalignant and nonhematologic indications such as severe disabling auto-immune disease and cardiovascular disease, the risk of fatal complications appears too high to gain wider acceptance.

History

Georges Mathé, a French oncologist, performed the first bone marrow transplant in 1959 on six Yugoslavian nuclear workers whose own marrow had been damaged by irradiation. Mathé later pioneered the use of bone marrow transplants in the treatment of leukemia.

Stem cell transplantation was pioneered using bone-marrow-derived stem cells by a team at the Fred Hutchinson Cancer Research Center from the 1950s through the 1970s led by E. Donnall Thomas, whose work was later recognized with a Nobel Prize in Physiology or Medicine. Thomas' work showed that bone marrow cells infused intravenously could

repopulate the bone marrow and produce new blood cells. His work also reduced the likelihood of developing a life-threatening complication called graft-versus-host disease.

The first physician to perform a successful human bone marrow transplant on a disease other than cancer was Robert A. Good at the University of Minnesota in 1968.

Indications

Many recipients of HSCTs are multiple myeloma or leukemia patients who would not benefit from prolonged treatment with, or are already resistant to, chemotherapy. Candidates for HSCTs include pediatric cases where the patient has an inborn defect such as severe combined immunodeficiency or congenital neutropenia with defective stem cells, and also children or adults with aplastic anemia who have lost their stem cells after birth. Other conditions treated with stem cell transplants include sickle-cell disease, myelodysplastic syndrome, neuroblastoma, lymphoma, Ewing's Sarcoma, Desmoplastic small round cell tumor and Hodgkin's disease. More recently non-myeloablative, or so-called "mini transplant," procedures have been developed that require smaller doses of preparative chemo and radiation. This has allowed HSCT to be conducted in the elderly and other patients who would otherwise be considered too weak to withstand a conventional treatment regimen.

HIV

A bone marrow transplant performed on Timothy Ray Brown, an American residing in Germany (Gero Hütter) appears to have successfully cured him of both leukemia as well as HIV. Researchers emphasize that this is an unusual case. The donor marrow was selected from 60 matching donors for being [CCR5]-Δ32 homozygous. This genetic trait blocks the primary route by which HIV attaches itself to cells for entry. Roughly 1:1000 Europeans and Americans have this inherited mutation but it is rarer in other populations. The patient had a brain biopsy, in addition to biopsies of his intestines, liver, lymph nodes, bone marrow—basically, every part of the body that can be biopsied. All were negative for virus. There is no virus in this person's body out to two and a half years off of all anti-HIV drugs. His antibody levels—called titers—are declining just the way expected if the patient was vaccinated against HIV and then the levels of antibodies were examined. They'd be very strong in the beginning, but would weaken if they are not re-exposed to the virus. It is believed this patient has no HIV in his body and therefore there is nothing to re-expose him, so the concentration of HIV antibodies in his blood is decreasing. It is predicted that, in a couple of years, his HIV antibody test will be negative.

Graft types

Autologous

Autologous HSCT requires the extraction (apheresis) of haematopoietic stem cells (HSC) from the patient and storage of the harvested cells in a freezer. The patient is then treated

with high-dose chemotherapy with or without radiotherapy with the intention of eradicating the patient's malignant cell population at the cost of partial or complete bone marrow ablation (destruction of patient's bone marrow function to grow new blood cells). The patient's own stored stem cells are then returned to his/her body, where they replace destroyed tissue and resume the patient's normal blood cell production. Autologous transplants have the advantage of lower risk of infection during the immune-compromised portion of the treatment since the recovery of immune function is rapid. Also, the incidence of patients experiencing rejection (graft-versus-host disease) is very rare due to the donor and recipient being the same individual. These advantages have established autologous HSCT as one of the standard second-line treatments for such diseases as lymphoma. However, for others such as Acute Myeloid Leukemia, the reduced mortality of the autogenous relative to allogeneic HSCT may be outweighed by an increased likelihood of cancer relapse and related mortality, and therefore the allogeneic treatment may be preferred for those conditions. Researchers have conducted small studies using non-myeloablative hematopoietic stem cell transplantation as a possible treatment for type I (insulin dependent) diabetes in children and adults. Results have been promising; however, at the time of this writing, it is premature to speculate as to whether these experiments will lead to effective treatments for diabetes.

Allogeneic

Allogeneic HSCT involves two people: the (healthy) donor and the (patient) recipient. Allogeneic HSC donors must have a tissue (HLA) type that matches the recipient. Matching is performed on the basis of variability at three or more loci of the (HLA) gene, and a perfect match at these loci is preferred. Even if there is a good match at these critical alleles, the recipient will require immunosuppressive medications to mitigate graft-versus-host disease. Allogeneic transplant donors may be *related* (usually a closely HLA matched sibling), *syngeneic* (a monozygotic or 'identical' twin of the patient - necessarily extremely rare since few patients have an identical twin, but offering a source of perfectly HLA matched stem cells) or *unrelated* (donor who is not related and found to have very close degree of HLA matching). A "savior sibling" may be intentionally selected by preimplantation genetic diagnosis in order to match a child both regarding HLA type and being free of any obvious inheritable disorder. Allogeneic transplants are also performed using umbilical cord blood as the source of stem cells. In general, by transplanting healthy stem cells to the recipient's immune system, allogeneic HCSTs appear to improve chances for cure or long-term remission once the immediate transplant-related complications are resolved.

A compatible donor is found by doing additional HLA-testing from the blood of potential donors. The HLA genes fall in two categories (Type I and Type II). In general, mismatches of the Type-I genes (i.e. HLA-A, HLA-B, or HLA-C) increase the risk of graft rejection. A mismatch of an HLA Type II gene (i.e. HLA-DR, or HLA-DQB1) increases the risk of graft-versus-host disease. In addition a genetic mismatch as small as a single DNA base pair is significant so perfect matches require knowledge of the exact DNA sequence of these genes for both donor and recipient. Leading transplant centers

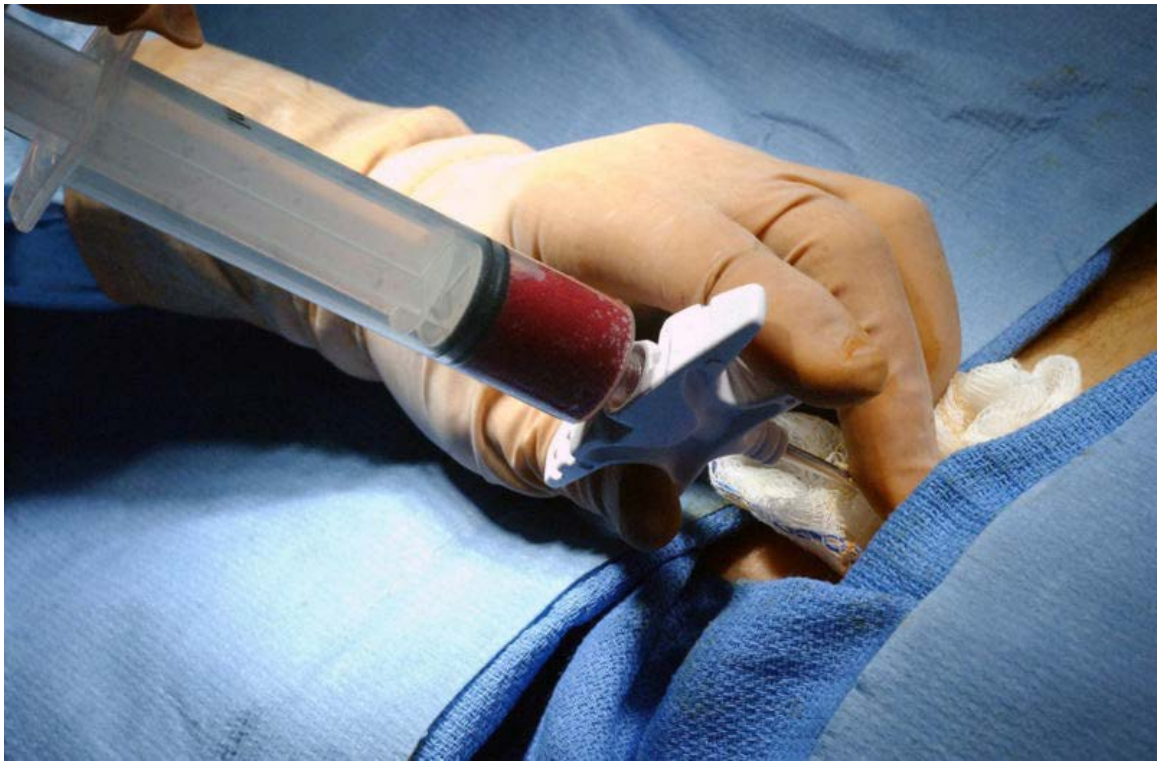
currently perform testing for all five of these HLA genes before declaring that a donor and recipient are HLA-identical.

Race and ethnicity are known to play a major role in donor recruitment drives, as members of the same ethnic group are more likely to have matching genes, including the genes for HLA.

Sources and storage of cells

To limit the risks of transplanted stem cell rejection or of severe graft-versus-host disease in allogeneic HSCT, the donor should preferably have the same human leukocyte antigens (HLA) as the recipient. About 25 to 30 percent of allogeneic HSCT recipients have an HLA-identical sibling. Even so-called "perfect matches" may have mismatched minor alleles that contribute to graft-versus-host disease.

Bone marrow



Bone marrow harvest.

In the case of a bone marrow transplant, the HSC are removed from a large bone of the donor, typically the pelvis, through a large needle that reaches the center of the bone. The technique is referred to as a bone marrow harvest and is performed under general anesthesia.

Peripheral blood stem cells

Peripheral blood stem cells are now the most common source of stem cells for allogeneic HSCT. They are collected from the blood through a process known as apheresis. The donor's blood is withdrawn through a sterile needle in one arm and passed through a machine that removes white blood cells. The red blood cells are returned to the donor. The peripheral stem cell yield is boosted with daily subcutaneous injections of Granulocyte-colony stimulating factor, serving to mobilize stem cells from the donor's bone marrow into the peripheral circulation.

Amniotic fluid

It is also possible to extract hematopoietic stem cells from amniotic fluid for both autologous or heterologous use at the time of childbirth.

Umbilical cord blood

Umbilical cord blood is obtained when a mother donates her infant's umbilical cord and placenta after birth. Cord blood has a higher concentration of HSC than is normally found in adult blood. However, the small quantity of blood obtained from an umbilical cord (typically about 50 mL) makes it more suitable for transplantation into small children than into adults. Newer techniques using ex-vivo expansion of cord blood units or the use of two cord blood units from different donors are being explored to allow cord blood transplants to be used in adults.

It is used e.g. in children being born after preimplantation genetic diagnosis (PGD) for human leucocyte antigen (HLA) matching in order to donate to a sick sibling requiring HSCT.

Storage of HSC

Unlike other organs, bone marrow cells can be frozen for prolonged periods (cryopreserved) without damaging too many cells. This is necessary for autologous HSC because the cells must be harvested months in advance of the transplant treatment. In the case of allogeneic transplants fresh HSC are preferred in order to avoid cell loss that might occur during the freezing and thawing process. Allogeneic cord blood is stored frozen at a cord blood bank because it is only obtainable at the time of childbirth. To cryopreserve HSC a preservative, DMSO, must be added and the cells must be cooled very slowly in a control rate freezer to prevent osmotic cellular injury during ice crystal formation. HSC may be stored for years in a *cryofreezer* which typically utilizes liquid nitrogen because it is non-toxic and it is very cold (boiling point -196°C.)

Conditioning regimens

Myeloablative transplants

The chemotherapy or irradiation given immediately prior to a transplant is called the conditioning or preparative regimen, the purpose of which is to help eradicate the patient's disease prior to the infusion of HSC and to suppress immune reactions. The bone marrow can be *ablated* with dose-levels that cause minimal injury to other tissues. In allogeneic transplants a combination of cyclophosphamide with busulfan or total body irradiation is commonly employed. This treatment also has an immunosuppressive effect which prevents rejection of the HSC by the recipient's immune system. The post-transplant prognosis often includes acute and chronic graft-versus-host disease which may be life-threatening; however in certain leukemias this can coincide with protection against cancer relapse owing to the *graft versus tumor* effect. *Autologous* transplants may also use similar conditioning regimens, but many other chemotherapy combinations can be used depending on the type of disease.

Non-myeloablative allogeneic transplants

This is a newer treatment approach using lower doses of chemotherapy and radiation which are too low to eradicate all of the bone marrow cells of a recipient. Instead, non-myeloablative transplants run lower risks of serious infections and transplant-related mortality while relying upon the *graft versus tumor* effect to resist the inherent increased risk of cancer relapse. Also significantly, while requiring high doses of immunosuppressive agents in the early stages of treatment, these doses are less than for conventional transplants. This leads to a state of mixed chimerism early after transplant where both recipient and donor HSC coexist in the bone marrow space.

Decreasing doses of immunosuppressive therapy then allows donor T-cells to eradicate the remaining recipient HSC and to induce the graft versus tumor effect. This effect is often accompanied by mild graft-versus-host disease, the appearance of which is often a surrogate for the emergence of the desirable graft versus tumor effect, and also serves as a signal to establish an appropriate dosage level for sustained treatment with low levels of immunosuppressive agents.

Because of their gentler conditioning regimens, these transplants are associated with a lower risk of transplant-related mortality and therefore allow patients who are considered too high-risk for conventional allogeneic HSCT to undergo potentially curative therapy for their disease. These new transplant strategies are still somewhat experimental, but are being used more widely on elderly patients unfit for myeloablative regimens and for whom the higher risk of cancer relapse may be acceptable.

Engraftment

After several weeks of growth in the bone marrow, expansion of HSC and their progeny is sufficient to normalize the blood cell counts and reinitiate the immune system. The

offspring of donor-derived hematopoietic stem cells have been documented to populate many different organs of the recipient, including the heart, liver, and muscle, and these cells had been suggested to have the abilities of regenerating injured tissue in these organs. However, recent research has shown that such lineage infidelities does not occur as a normal phenomenon.

Complications

HSCT is associated with a high treatment-related mortality in the recipient (10% or higher), which limits its use to conditions that are themselves life-threatening. Major complications are veno-occlusive disease, mucositis, infections (sepsis) and graft-versus-host disease.

Infection

Bone marrow transplantation usually requires that the recipient's own bone marrow be destroyed ("myeloablation"). Prior to "engraftment" patients may go for several weeks without appreciable numbers of white blood cells to help fight infection. This puts a patient at high risk of infections, sepsis and septic shock, despite prophylactic antibiotics, and accounts for a large share of treatment-related mortality. The immunosuppressive agents employed in allogeneic transplants for the prevention or treatment of graft-versus-host disease further increase the risk of opportunistic infection. Immunosuppressive drugs are given for a minimum of 6-months after a transplantation, or much longer if required for the treatment of graft-versus-host disease. Transplant patients lose their acquired immunity, for example immunity to childhood diseases such as measles or polio. For this reason transplant patients must be re-vaccinated with childhood vaccines once they are off immunosuppressive medications.

Veno-occlusive disease

Severe liver injury is termed hepatic veno-occlusive disease (VOD). Elevated levels of bilirubin, hepatomegaly and fluid retention are clinical hallmarks of this condition. There is now a greater appreciation of the generalized cellular injury and obstruction in hepatic vein sinuses, and it has thus been referred to as sinusoidal obstruction syndrome (SOS). Severe cases are associated with a high mortality. Anticoagulants or defibrotide may be effective in reducing the severity of VOD but may also increase bleeding complications. Ursodiol has been shown to help prevent VOD, presumably by helping the flow of bile.

Mucositis

The injury of the mucosal lining of the mouth and throat and is a common regimen-related toxicity following ablative HSCT regimens. It is usually not life-threatening but is very painful, and prevents eating and drinking. Mucositis is treated with pain medications plus intravenous infusions to prevent dehydration and malnutrition.

Graft-versus-host disease

Graft-versus-host disease (GVHD) is an inflammatory disease that is unique to allogeneic transplantation. It is an attack of the "new" bone marrow's immune cells against the recipient's tissues. This can occur even if the donor and recipient are HLA-identical because the immune system can still recognize other differences between their tissues. It is aptly named graft-versus-host disease because bone marrow transplantation is the only transplant procedure in which the transplanted cells must accept the body rather than the body accepting the new cells. *Acute graft-versus-host disease* typically occurs in the first 3 months after transplantation and may involve the skin, intestine, or the liver, and is often fatal. High-dose corticosteroids such as prednisone are a standard treatment; however this immuno-suppressive treatment often leads to deadly infections. *Chronic graft-versus-host disease* may also develop after allogeneic transplant. It is the major source of late treatment-related complications, although it less often results in death. In addition to inflammation, chronic graft-versus-host disease may lead to the development of fibrosis, or scar tissue, similar to scleroderma; it may cause functional disability and require prolonged immunosuppressive therapy. Graft-versus-host disease is usually mediated by T cells when they react to foreign peptides presented on the MHC of the host.

Graft-versus-tumor effect

Graft-versus-tumor effect (GVT) or "graft versus leukemia" effect is the beneficial aspect of the Graft-versus-Host phenomenon. For example, HSCT patients with either acute and in particular chronic graft-versus-host disease after an allogeneic transplant tend to have a lower risk of cancer relapse. This is due to a therapeutic immune reaction of the grafted donor T lymphocytes against the diseased bone marrow of the recipient. This lower rate of relapse accounts for the increased success rate of allogeneic transplants compared to transplants from identical twins, and indicates that allogeneic HSCT is a form of immunotherapy. GVT is the major benefit of transplants which do not employ the highest immuno-suppressive regimens.

Graft versus tumor is mainly beneficial in diseases with slow progress, e.g. chronic leukemia, low-grade lymphoma, and some cases multiple myeloma. However, it is less effective in rapidly growing acute leukemias.

If cancer relapses after HSCT, another transplant can be performed, infusing the patient with even more of the donor's white blood cells.

Prognosis

Prognosis in HSCT varies widely dependent upon disease type, stage, stem cell source, HLA-matched status (for allogeneic HCST) and conditioning regimen. A transplant offers a chance for cure or long-term remission if the inherent complications of graft versus host disease, immuno-suppressive treatments and the spectrum of opportunistic

infections can be survived. In recent years, survival rates have been gradually improving across almost all populations and sub-populations receiving transplants.

Mortality for allogeneic stem cell transplantation can be estimated using the prediction model created by Sorror et al., using the Hematopoietic Cell Transplantation-Specific Comorbidity Index (HCT-CI). The HCT-CI was derived and validated by investigators at the Fred Hutchinson Cancer Research Center (Seattle, WA). The HCT-CI modifies and adds to a well-validated comorbidity index, the Charlson Comorbidity Index (CCI) (Charlson et al.) The CCI was previously applied to patients undergoing allogeneic HCT but appears to provide less survival prediction and discrimination than the HCT-CI scoring system.

Risks to donor

The risks of a complication depend on patient characteristics, health care providers and the apheresis procedure, and the colony-stimulating factor used (G-CSF, GM-CSF). G-CSF drugs include Filgrastim (Neupogen, Neulasta), and lenograstim (Graslopin).

Drug risks

Filgrastim is typically dosed in the 10 microgram/kg level for 4–5 days during the harvesting of stem cells. The documented adverse effects of filgrastim include splenic rupture (indicated by left upper abdominal or shoulder pain, risk 1 in 40000), Adult respiratory distress syndrome (ARDS), alveolar hemorrage, and allergic reactions (usually expressed in first 30 minutes, risk 1 in 300). In addition, platelet and hemoglobin levels dip post-procedure, not returning to normal until one month.

The question of whether patients over 65 react the same as patients under 65 has not been sufficiently examined. Coagulation issues and inflammation of atherosclerotic plaques are known to occur as a result of G-CSF injection. G-CSF has also been described to induce genetic changes in mononuclear cells of normal donors. There is evidence that myelodysplasia (MDS) or acute myeloid leukaemia (AML)can be induced by GCSF in susceptible individuals.

Access risks

Blood was drawn peripherally in a majority of patients, but a central line to jugular/subclavian/femoral veins may be used in 16% of women and 4% of men. Adverse reactions during apheresis were experienced in 20% of women and 8% of men, these adverse events primarily consisted of numbness/tingling, multiple line attempts, and nausea.

Clinical observations

A study involving 2408 donors (18–60 years) indicated that bone pain (primarily back and hips) as a result of filgrastim treatment is observed in 80% of donors by day 4 post-

injection. This pain responded to acetaminophen or ibuprofen in 65% of donors and was characterized as mild to moderate in 80% of donors and severe in 10%. Bone pain receded post-donation to 26% of patients 2 days post-donation, 6% of patients one week post-donation, and <2% 1 year post-donation. Donation is not recommended for those with a history of back pain. Other symptoms observed in more than 40% of donors include myalgia, headache, fatigue, and insomnia. These symptoms all returned to baseline 1 month post-donation, except for some cases of persistent fatigue in 3% of donors. . In one metastudy that incorporated data from 377 donors, 44% of patients reported having adverse side effects after peripheral blood HSCT. Side effects included pain prior to the collection procedure as a result of GCSF injections, post-procedural generalized skeletal pain, fatigue and reduced energy.

Severe reactions

A study that surveyed 2408 donors found that serious adverse events (requiring prolonged hospitalization) occurred in 15 donors (at a rate of 0.6%), although none of these events were fatal. Donors were not observed to have higher than normal rates of cancer with up to 4–8 years of follow up. One study based on a survey of medical teams covered approximately 24,000 peripheral blood HSCT cases between 1993 and 2005, and found a serious cardiovascular adverse reaction rate of about 1 in 1500. This study reported a cardiovascular-related fatality risk within the first 30 days HSCT of about 2 in 10000. For this same group, severe cardiovascular events were observed with a rate of about 1 in 1500. The most common severe adverse reactions were pulmonary edema/deep vein thrombosis, splenic rupture, and myocardial infarction. Haematological malignancy induction was comparable to that observed in the general population with only 15 reported cases within 4 years.

Chapter 14

Blood Transfusion

Blood transfusion is the process of transferring blood or blood-based products from one person into the circulatory system of another. Blood transfusions can be life-saving in some situations, such as massive blood loss due to trauma, or can be used to replace blood lost during surgery. Blood transfusions may also be used to treat a severe anaemia or thrombocytopenia caused by a blood disease. People suffering from hemophilia or sickle-cell disease may require frequent blood transfusions. Early transfusions used whole blood, but modern medical practice commonly uses only components of the blood.

History

Early attempts

The first historical attempt at blood transfusion was described by the 17th century chronicler Stefano Infessura. Infessura relates that, in 1492, as Pope Innocent VIII sank into a coma, the blood of three boys was infused into the dying pontiff (through the mouth, as the concept of circulation and methods for intravenous access did not exist at that time) at the suggestion of a physician. The boys were ten years old, and had been promised a ducat each. However, not only did the pope die, but so did the three children. Some authors have discredited Infessura's account, accusing him of anti-papalism.

World War II syringe for direct inter-human blood transfusion

Beginning with Harvey's experiments with circulation of the blood, more sophisticated research into blood transfusion began in the 17th century, with successful experiments in transfusion between animals. However, successive attempts on humans continued to have fatal results.

The first fully documented human blood transfusion was administered by Dr. Jean-Baptiste Denys, eminent physician to King Louis XIV of France, on June 15, 1667. He transfused the blood of a sheep into a 15-year old boy, who survived the transfusion. Denys performed another transfusion into a labourer, who also survived. Both instances were likely due to the small amount of blood that was actually transfused into these people. This allowed them to withstand the allergic reaction. Denys' third patient to undergo a blood transfusion was Swedish Baron Bonde. He received two transfusions. After the second transfusion Bonde died. In the winter of 1667, Denys performed several transfusions on Antoine Mauroy with calf's blood, who on the third account died. Much controversy surrounded his death. Mauroy's wife asserted Denys was responsible for her husband's death. But Mauroy's wife was accused of causing his death. Though it was later determined that Mauroy actually died from arsenic poisoning, Denys' experiments with animal blood provoked a heated controversy in France. Finally, in 1670 the procedure was banned. In time, the British Parliament and even the pope followed suit. Blood transfusions fell into obscurity for the next 150 years.

First successful transfusion

Richard Lower examined the effects of changes in blood volume on circulatory function and developed methods for cross-circulatory study in animals, obviating clotting by closed arteriovenous connections. His newly devised instruments eventually led to actual transfusion of blood.

"Many of his colleagues were present. towards the end of February 1665 [when he] selected one dog of medium size, opened its jugular vein, and drew off blood, until . . . its strength was nearly gone . Then, to make up for the great loss of this dog by the blood of a second, I introduced blood from the cervical artery of a fairly large mastiff, which had been fastened alongside the first, until this latter animal showed . . . it was overfilled . . . by the inflowing blood." After he "sewed up the jugular veins," the animal recovered "with no sign of discomfort or of displeasure."

Lower had performed the first blood transfusion between animals. He was then "requested by the Honorable [Robert] Boyle . . . to acquaint the Royal Society with the procedure for the whole experiment," which he did in December of 1665 in the Society's Philosophical Transactions. On 15 June 1667 Denys, then a professor in Paris, carried out the first transfusion between humans and claimed credit for the technique, but Lower's priority cannot be challenged.

Six months later in London, Lower performed the first human transfusion in Britain, where he "superintended the introduction in [a patient's] arm at various times of some ounces of sheep's blood at a meeting of the Royal Society, and without any inconvenience to him." The recipient was Arthur Coga, "the subject of a harmless form of insanity." Sheep's blood was used because of speculation about the value of blood exchange between species; it had been suggested that blood from a gentle lamb might quiet the tempestuous spirit of an agitated person and that the shy might be made outgoing by blood from more sociable creatures. Lower wanted to treat Coga several times, but his patient refused. No more transfusions were performed. Shortly before, Lower had moved to London, where his growing practice soon led him to abandon research.

Early successes

The science of blood transfusion dates to the first decade of the 19th century, with the discovery of distinct blood types leading to the practice of mixing some blood from the donor and the receiver before the transfusion (an early form of cross-matching).

In 1818, Dr. James Blundell, a British obstetrician, performed the first successful blood transfusion of human blood, for the treatment of postpartum hemorrhage. He used the patient's husband as a donor, and extracted four ounces of blood from his arm to transfuse into his wife. During the years 1825 and 1830, Dr. Blundell performed 10 transfusions, five of which were beneficial, and published his results. He also invented many instruments for the transfusion of blood. He made a substantial amount of money from this endeavour, roughly $50 million (about $2 million in 1827) real dollars (adjusted for inflation).

In 1840, at St George's Hospital Medical School in London, Samuel Armstrong Lane, aided by Dr. Blundell, performed the first successful whole blood transfusion to treat hemophilia.

In the novel "Dracula", by Bram Stoker, various incidences of blood transfusion were deliberated upon. The book was published in 1897.

George Washington Crile is credited with performing the first surgery using a direct blood transfusion at the Cleveland Clinic.

Early transfusions were risky and many resulted in the death of the patient. It was not until 1901, when the Austrian Karl Landsteiner discovered human blood groups, that blood transfusions became safer. Mixing blood from two incompatible individuals can lead to an immune response, and the destruction of red blood cells releases free hemoglobin into the bloodstream, which can have fatal consequences. Karl Landsteiner discovered that when incompatible types are mixed, the red blood cells clump, and that this immunological reaction occurs when the receiver of a blood transfusion has antibodies against the donor blood cells. His work made it possible to determine blood type and allowed way blood transfusions to be carried out much more safely. For this discovery he was awarded the Nobel Prize in Physiology and Medicine in 1930, and many other blood groups have been discovered since.

Development of blood banking

While the first transfusions had to be made directly from donor to receiver before coagulation, in the 1910s it was discovered that by adding anticoagulant and refrigerating the blood it was possible to store it for some days, thus opening the way for blood banks. The first non-direct transfusion was performed on March 27, 1914 by the Belgian doctor Albert Hustin, though this was a diluted solution of blood. The Argentine doctor Luis Agote used a much less diluted solution in November of the same year. Both used sodium citrate as an anticoagulant. The first blood transfusion using blood that had been stored and cooled was performed on January 1, 1916. Oswald Hope Robertson, a medical researcher and U.S. Army officer, is generally credited with establishing the first blood bank while serving in France during World War I.

The first academic institution devoted to the science of blood transfusion was founded by Alexander Bogdanov in Moscow in 1925. Bogdanov was motivated, at least in part, by a search for eternal youth, and remarked with satisfaction on the improvement of his eyesight, suspension of balding, and other positive symptoms after receiving 11 transfusions of whole blood.

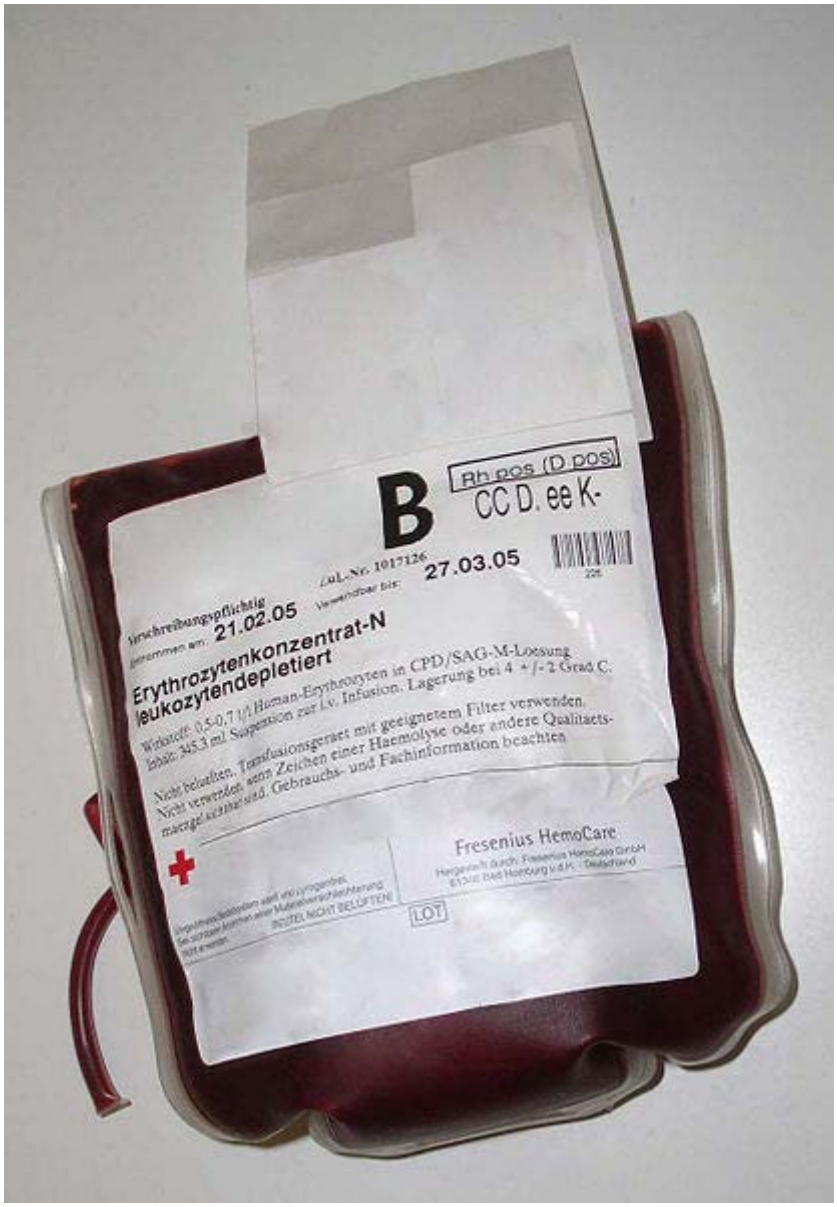
In fact, following the death of Vladimir Lenin, Bogdanov was entrusted with the study of Lenin's brain, with a view toward resuscitating the deceased Bolshevik leader. Bogdanov died in 1928 as a result of one of his experiments, when the blood of a student suffering from malaria and tuberculosis was given to him in a transfusion. Some scholars (e.g. Loren Graham) have speculated that his death may have been a suicide, while others attribute it to blood type incompatibility, which was not completely understood at the time.

The modern era

Following Bogdanov's lead, the Soviet Union set up a national system of blood banks in the 1930s. News of the Soviet experience traveled to America, where in 1937 Bernard Fantus, director of therapeutics at the Cook County Hospital in Chicago, established the first hospital blood bank in the United States. In creating a hospital laboratory that preserved and stored donor blood, Fantus originated the term "blood bank". Within a few years, hospital and community blood banks were established across the United States.

In the late 1930s and early 1940s, Dr. Charles R. Drew's research led to the discovery that blood could be separated into blood plasma and red blood cells, and that the plasma could be frozen separately. Blood stored in this way lasted longer and was less likely to become contaminated.

Another important breakthrough came in 1939-40 when Karl Landsteiner, Alex Wiener, Philip Levine, and R.E. Stetson discovered the Rhesus blood group system, which was found to be the cause of the majority of transfusion reactions up to that time. Three years later, the introduction by J.F. Loutit and Patrick L. Mollison of acid-citrate-dextrose (ACD) solution, which reduces the volume of anticoagulant, permitted transfusions of greater volumes of blood and allowed longer term storage.



Plastic bag with erythrocyte concentrate.

Carl Walter and W.P. Murphy, Jr. introduced the plastic bag for blood collection in 1950. Replacing breakable glass bottles with durable plastic bags allowed for the evolution of a collection system capable of safe and easy preparation of multiple blood components from a single unit of whole blood.

In the field of cancer surgery massive blood loss became a major problem to replace. The cardiac arrest rate was high. Drs. C. Paul Boyan and Willam Howland discovered that the temperature of the blood and the rate of infusion greatly affected survival rate and the blood warmer was born. (References: 1. BOYAN CP, HOWLAND WS. Cardiac arrest and temperature of bank blood. JAMA. 1963 Jan 5;183:58-60. 2. Rupreht J, van Lieburg

MJ, Lee JA, Erdman W, editors. Anaesthesia: Essays on its history. Springer-Verlag, Berlin, 1985, pp. 99–101.)

Further extending the shelf life of stored blood was an anticoagulant preservative, CPDA-1, introduced in 1979, which increased the blood supply and facilitated resource-sharing among blood banks.

As of 2006, there were about 15 million units of blood products transfused per year in the United States.

Precautions

Compatibility

The key importance of the Rh group is its role in Hemolytic disease of the fetus and newborn. When an Rh negative mother carries a positive fetus, she can become immunized against the Rh antigen. This usually is not important during that pregnancy, but in the following pregnancies she can develop an immune response to the Rh antigen. The mother's immune system can attack the baby's red cells through the placenta. Mild cases of HDFN can lead to disability but some severe cases are fatal. Rh-D is the most commonly involved red cell antigen in HDFN, but other red cell antigens can also cause the condition. The "positive" or "negative" in spoken blood types such as "O positive" is the Rh-D antigen.

Transfusion transmitted infections

A number of infectious diseases (such as HIV, syphilis, hepatitis B and hepatitis C, among others) can be passed from the donor to recipient.

Among the diseases that can be transmitted via transfusion are:

- HIV-1 and HIV-2
- Human T-lymphotropic virus (HTLV-1 and HTLV-2)
- Hepatitis C virus (responsible for >90% of post-transfusion hepatitis)
- Hepatitis B
- Treponema pallidum
- Malaria
- Chagas Disease
- variant Creutzfeldt-Jakob Disease or "Mad Cow Disease" has been shown to be transmissible in blood products. No test exists for this, but various measures have been taken to reduce risks.

When a person's need for a transfusion can be anticipated, as in the case of scheduled surgery, autologous donation can be used to protect against disease transmission and eliminate the problem of blood type compatibility. "Directed" donations from donors

known to the recipient were a common practice during the initial years of HIV. These kinds of donations are still common in developing countries.

Processing of blood products prior to transfusion

Donated blood is usually subjected to processing after it is collected, to make it suitable for use in specific patient populations. Examples include:

- **Component separation**: red cells, plasma and platelets are separated into different containers and stored in appropriate conditions so that their use can be adapted to the patient's specific needs. Red cells work as oxygen transporters, plasma is used as a supplement of coagulation factors, and platelets are transfused when their number is very scarce or their function severely impaired. Blood components are usually prepared by centrifugation.
- **Leukoreduction**, also known as **Leukodepletion** is the removal of white blood cells from the blood product by filtration. Leukoreduced blood is less likely to cause alloimmunization (development of antibodies against specific blood types), and less likely to cause febrile transfusion reactions.
    - Chronically transfused patients
    - Potential transplant recipients
    - Patients with previous febrile nonhemolytic transfusion reaction
    - Patients with hereditary immune deficiencies
    - Patients receiving blood transfusions from relatives in directed-donation programs
    - Patients receiving large doses of chemotherapy, undergoing stem cell transplantation, or with AIDS (controversial).
- **Tests** for certain quality control issues such as disease or contamination.
- **Pathogen Reduction** treatment that involves, for example, the addition of riboflavin with subsequent exposure to UV light has been shown to be effective in inactivating pathogens (viruses, bacteria, parasites and white blood cells) in blood products. By inactivating white blood cells in donated blood products, riboflavin and UV light treatment can also replace gamma-irradiation as a method to prevent graft-versus-host disease (TA-GVHD).

Neonatal transfusion

To ensure the safety of blood transfusion to pediatric patients, hospitals are taking additional precaution to avoid infection and prefer to use specially tested pediatric blood units that are guaranteed negative for Cytomegalovirus. Most guidelines recommend the provision of CMV-negative blood components and not simply leukoreduced components for newborns or low birthweight infants in whom the immune system is not fully developed. These specific requirements place additional restrictions on blood donors who can donate for neonatal use.

Neonatal transfusions typically fall into one of two categories:

- "Top-up" transfusions, to replace losses due to investigational losses and correction of anemia.
- Exchange (or partial exchange) transfusions are done for removal of bilirubin, removal of antibodies and replacement of red cells (e.g., for anemia secondary to thalassemias and other hemoglobinopathies).

Pre-Transfusion compatibility testing

The terms type and screen are used for the testing that (1) determines the blood group (ABO compatibility) and (2) screens for alloantibodies. It takes about 45 minutes to complete (depending on the method used). The blood bank technologist also checks for special requirements of the patient (e.g. need for washed, irradiated or CMV negative blood) and the history of the patient to see if they have a previously identified antibody.

A positive screen warrants an antibody panel/investigation. An antibody panel consists of commercially prepared group O red cell suspensions from donors that have been phenotyped for commonly encountered and clinically significant alloantibodies. Donor cells may have homozygous (e.g. K+k-), heterozygous (K+k+) expression or no expression of various antigens (K-k+). The phenotypes of all the donor cells being tested are shown in a chart. The patient's serum is tested against the various donor cells using an enhancement method, e.g. Gel or LISS. Based on the reactions of the patient's serum against the donor cells, a pattern will emerge to confirm the presence of one or more antibodies. Not all antibodies are clinically significant (i.e. cause transfusion reactions, HDN, etc.). Once the patient has developed a clinically significant antibody it is vital that the patient receive antigen negative phenotyped red blood cells to prevent future transfusion reactions. A direct antiglobulin test (DAT) is also performed as part of the antibody investigation.

Once the type and screen has been completed, potential donor units will be selected based on compatibility with the patient's blood group, special requirements (e.g. CMV negative, irradiated or washed) and antigen negative (in the case of an antibody). If there is no antibody present or suspected, the immediate spin or CAC (computer assisted crossmatch) method may be used.

In the immediate spin method, two drops of patient serum are tested against a drop of 3-5% suspension of donor cells in a test tube and spun in a serofuge. Agglutination or hemolysis in the test tube is a positive reaction and the unit should not be transfused.

If an antibody is suspected, potential donor units must first be screened for the corresponding antigen by phenotyping them. Antigen negative units are then tested against the patient plasma using an antiglobulin/indirect crossmatch technique at 37 degrees Celsius to enhance reactivity and make the test easier to read.

If there is no time the blood is called "uncross-matched blood". Uncross-matched blood is O-positive or O-negative. O-negative is usually used for children and women of childbearing age. It is preferable for the laboratory to obtain a pre-transfusion sample in

these cases so a type and screen can be performed to determine the actual blood group of the patient and to check for alloantibodies.

Procedure

Blood transfusions can be grouped into two main types depending on their source:

- *Homologous transfusions*, or transfusions using the stored blood of others. These are often called *Allogeneic* instead of homologous.
- *Autologous transfusions*, or transfusions using the patient's own stored blood.

Donor units of blood must be kept refrigerated to prevent bacterial growth and to slow cellular metabolism. The transfusion must begin within 30 minutes after the unit has been taken out of controlled storage.

Blood can only be administered intravenously. It therefore requires the insertion of a cannula of suitable caliber.

Before the blood is administered, the personal details of the patient are matched with the blood to be transfused, to minimize risk of transfusion reactions. Clerical error is a significant source of transfusion reactions and attempts have been made to build redundancy into the matching process that takes place at the bedside.

A unit (up to 500 ml) is typically administered over 4 hours. In patients at risk of congestive heart failure, many doctors administer a diuretic to prevent fluid overload, a condition called Transfusion Associated Circulatory Overload or TACO. Acetaminophen and/or an antihistamine such as diphenhydramine are sometimes given before the transfusion to prevent other types of transfusion reactions.

Blood donation

U.S. Navy crew member donates blood.

Blood is most commonly donated as whole blood by inserting a catheter into a vein and collecting it in a plastic bag (mixed with anticoagulant) via gravity. Collected blood is then separated into components to make the best use of it. Aside from red blood cells, plasma, and platelets, the resulting blood component products also include albumin protein, clotting factor concentrates, cryoprecipitate, fibrinogen concentrate, and immunoglobulins (antibodies). Red cells, plasma and platelets can also be donated individually via a more complex process called apheresis.

In developed countries, donations are usually anonymous to the recipient, but products in a blood bank are always individually traceable through the whole cycle of donation, testing, separation into components, storage, and administration to the recipient. This enables management and investigation of any suspected transfusion related disease transmission or transfusion reaction. In developing countries the donor is sometimes specifically recruited by or for the recipient, typically a family member, and the donation occurs immediately before the transfusion.

Risks to the recipient

There are risks associated with receiving a blood transfusion and these must be balanced against the benefit which is expected. The most common adverse reaction to a blood transfusion is a *febrile non-hemolytic transfusion reaction*, which consists of a fever which resolves on its own and causes no lasting problems or side effects.

Hemolytic reactions include chills, headache, backache, dyspnea, cyanosis, chest pain, tachycardia and hypotension.

Blood products can rarely be contaminated with bacteria; the risk of severe bacterial infection and sepsis is estimated, as of 2002, at about 1 in 50,000 platelet transfusions, and 1 in 500,000 red blood cell transfusions.

There is a risk that a given blood transfusion will transmit a viral infection to its recipient. As of 2006, the risk of acquiring hepatitis B via blood transfusion in the United States is about 1 in 250,000 units transfused, and the risk of acquiring HIV or hepatitis C in the U.S. via a blood transfusion is estimated at 1 in 2,000,000 (2 million) units transfused. These risks were much higher in the past before the advent of second and third generation tests for transfusion transmitted diseases. The implementation of Nucleic Acid Testing or "NAT" in the early 2000s has further reduced risks, and confirmed viral infections by blood transfusion are extremely rare in the developed world.

Transfusion-associated acute lung injury (TRALI) is an increasingly recognized adverse event associated with blood transfusion. TRALI is a syndrome of acute respiratory distress, often associated with fever, non-cardiogenic pulmonary edema, and hypotension, which may occur as often as 1 in 2000 transfusions. Symptoms can range from mild to life-threatening, but most patients recover fully within 96 hours, and the mortality rate from this condition is less than 10%. Although the cause of TRALI is not clear, it has been consistently associated with anti HLA antibodies. Because anti HLA strongly correlate with pregnancy, several transfusion organisations (Blood and Tissues Bank of Cantabria, Spain, National Health Service in Britain) have decided to use only plasma from men for transfusion.

Other risks associated with receiving a blood transfusion include volume overload, iron overload (with multiple red blood cell transfusions), transfusion-associated graft-vs.-host disease, anaphylactic reactions (in people with IgA deficiency), and acute hemolytic reactions (most commonly due to the administration of mismatched blood types).

Concerns about whether transfusion risks are heightened by *storage time* have also been emerging, although there is not yet a consensus on the significance of blood age. Relatedly, questions have been raised regarding the uncertain and inconsistent efficacy of transfusions for certain vulnerable patient groups such as the critically ill, yet studies do not consistently show age to be the sole decisive factor. Estimated now at about *$17 Billion*, the costs of dealing with often-unpredictable transfusion inefficacy are far greater than the combined costs of buying, testing/treating, and transfusing the blood.

Scientists working at the University of Copenhagen reported in the journal Nature Biotechnology in April 2007 of discovering enzymes, which potentially enable blood from groups A, B and AB to be converted into group O. These enzymes do not affect the Rh group of the blood.

Objections to blood transfusion

Objections to blood transfusions may arise for personal, medical, or religious reasons. For example, Jehovah's Witnesses object to blood transfusion primarily on religious grounds—they believe that blood is sacred, although they have also highlighted possible complications associated with transfusion.

Alternatives to blood transfusion

Jehovah's Witnesses and others who prefer not to receive donated blood products through a transfusion have other options available. The field of bloodless medicine, including bloodless surgery makes use of several measures and techniques which can be utilized before, during and after surgery to increase the amount of oxygen in the blood, limit blood loss, and eliminate the need for a transfusion.

Nonhuman blood transfusion

Veterinarians also administer transfusions to other animals. Various species require different levels of testing to ensure a compatible match. For example, cats have 3 known blood types, cattle have 11, dogs have 12, pigs 16 and horses have 34. However, in many species (especially horses and dogs), cross matching is not required before the *first* transfusion, as antibodies against non-self cell surface antigens are not expressed constitutively - i.e. the animal has to be sensitized before it will mount an immune response against the transfused blood.

The rare and experimental practice of inter-species blood transfusions is a form of xenograft.

Blood transfusion substitutes

As of 2009, there are no widely utilized *oxygen-carrying* blood substitutes for humans; however, there are widely available non-blood *volume expanders* and other blood-saving techniques. These are helping doctors and surgeons avoid the risks of disease

transmission and immune suppression, address the chronic blood donor shortage, and address the concerns of Jehovah's Witnesses and others who have religious objections to receiving transfused blood.

A number of blood substitutes are currently in the clinical evaluation stage. Most attempts to find a suitable alternative to blood thus far have concentrated on cell-free hemoglobin solutions. Blood substitutes could make transfusions more readily available in emergency medicine and in pre-hospital EMS care. If successful, such a blood substitute could save many lives, particularly in trauma where massive blood loss results. Hemopure, a hemoglobin-based therapy, is approved for use in South Africa.