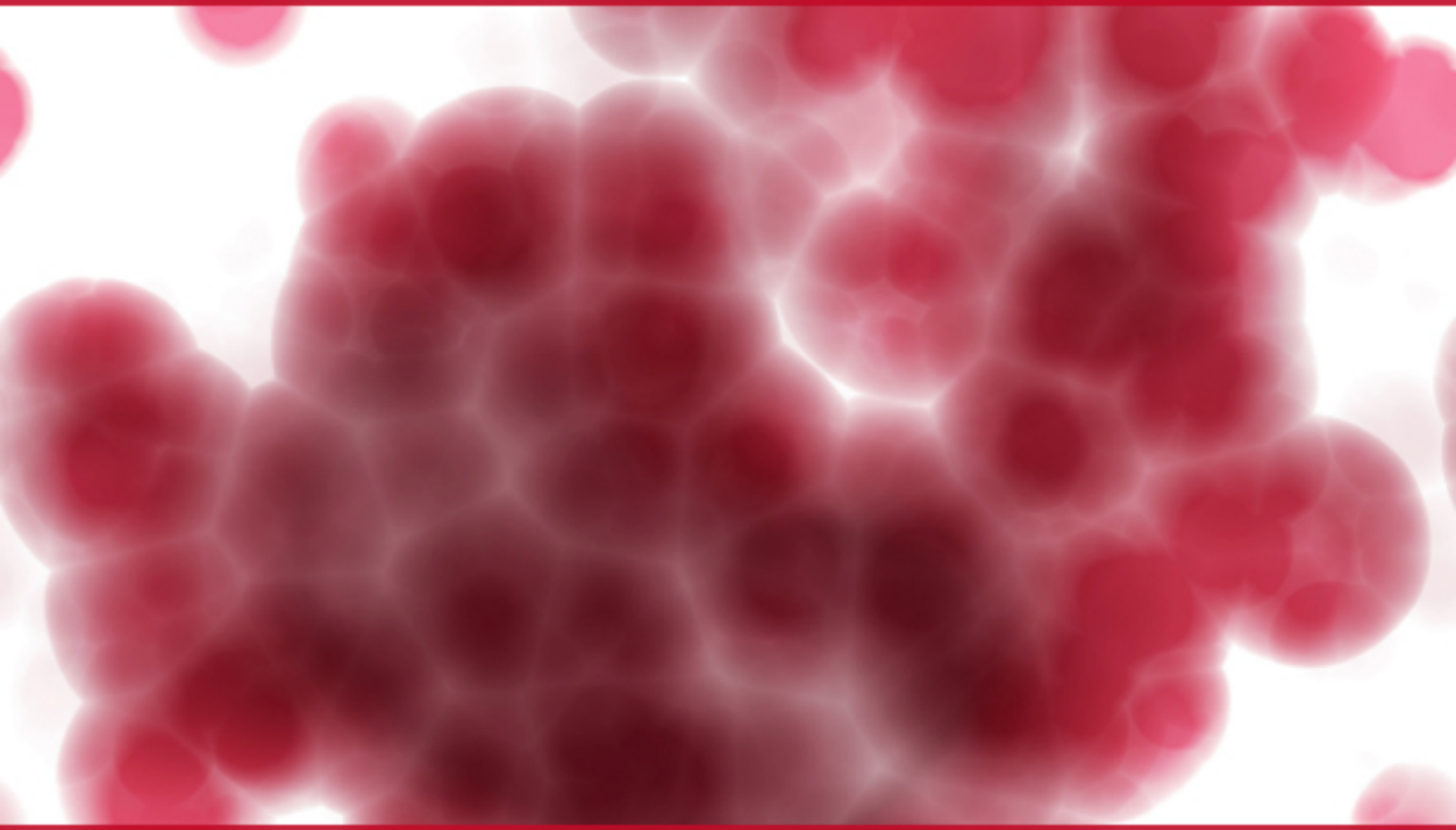
Hematology and Blood Transfusion Medicine



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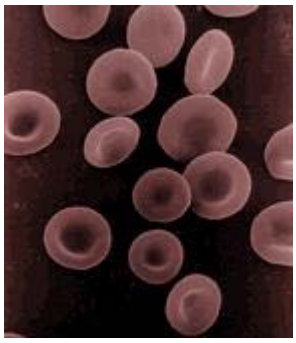
Red Blood Cell



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

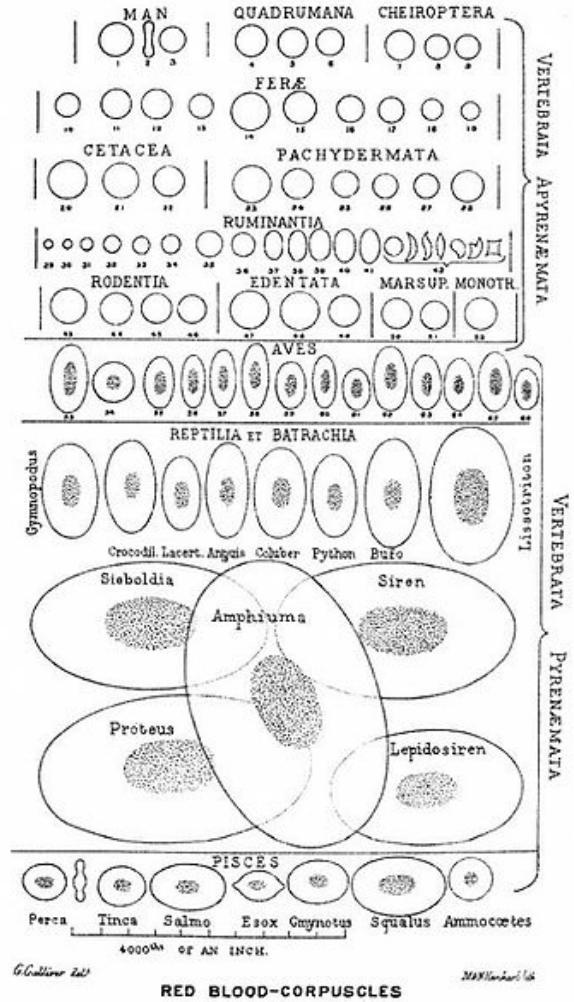
The first person to describe red blood cells was the young Dutch biologist Jan Swammerdam, who had used an early microscope in 1658 to study the blood of a frog.

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.

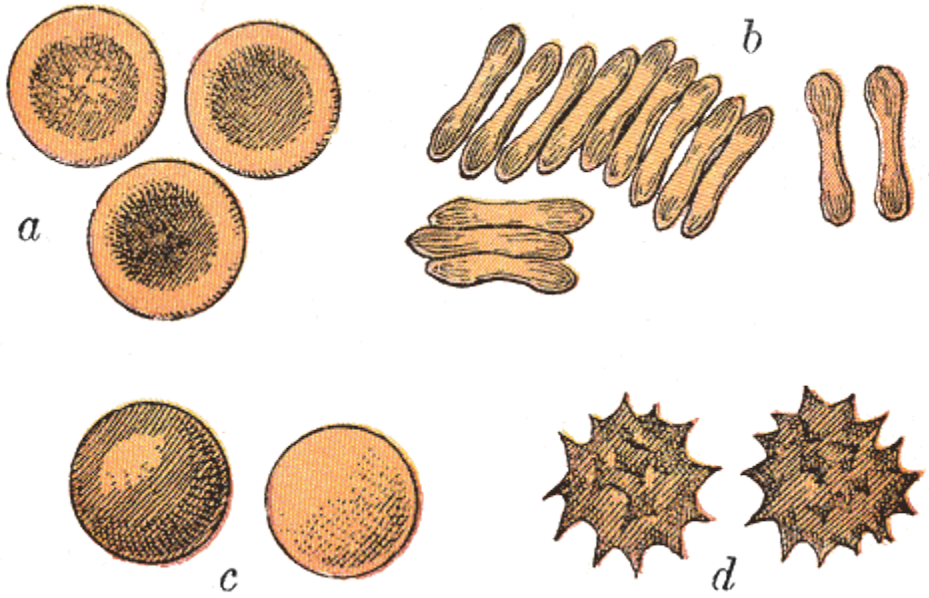
When their hemoglobin molecules are deoxygenated, erythrocytes release S-nitrosothiols which also acts to dilate vessels, thus directing more blood to areas of the body depleted of oxygen.

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



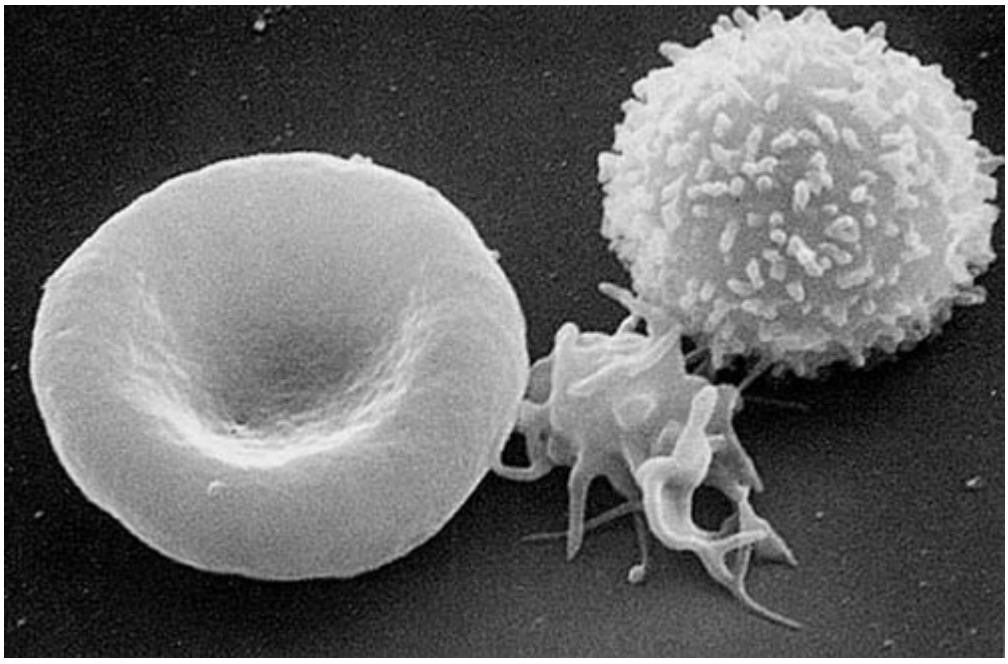
Typical mammalian erythrocytes: (a) seen from surface; (b) in profile, forming rouleaux; (c) rendered spherical by water; (d) rendered crenate by salt. (c) and (d) do not normally occur in the body.

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.

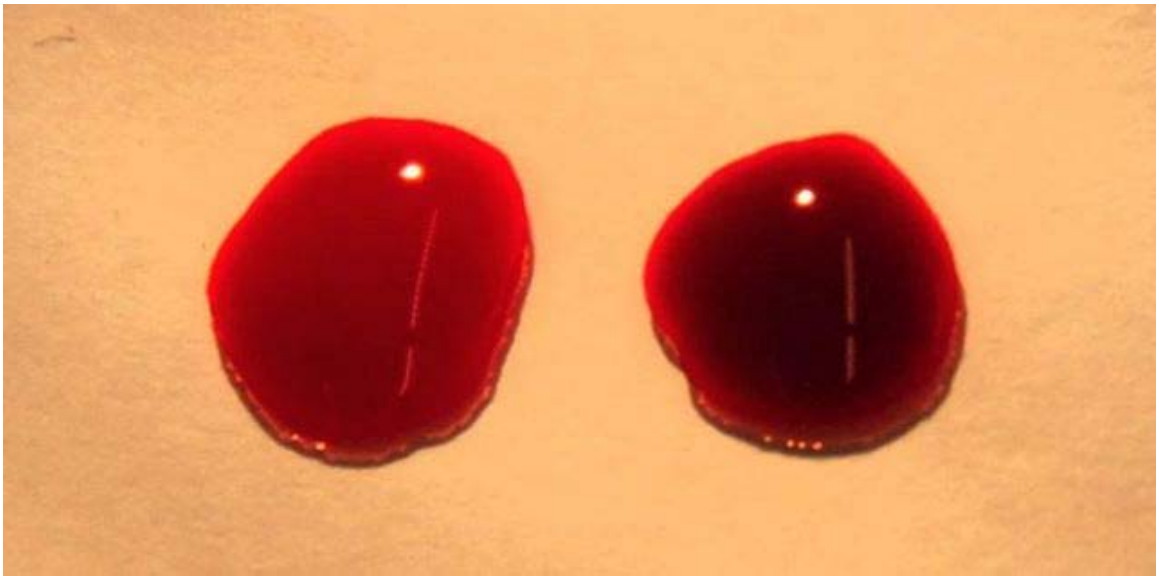
In large blood vessels, red blood cells sometimes occur as a stack, flat side next to flat side. This is known as *rouleaux formation*, and it occurs more often if the levels of certain serum proteins are elevated, as for instance during inflammation.

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.

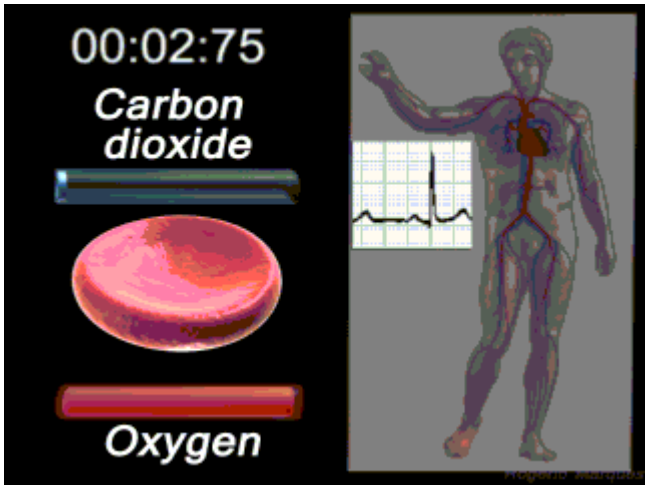


Scanning electron micrograph of blood cells. From left to right: human erythrocyte, thrombocyte (platelet), leukocyte.

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

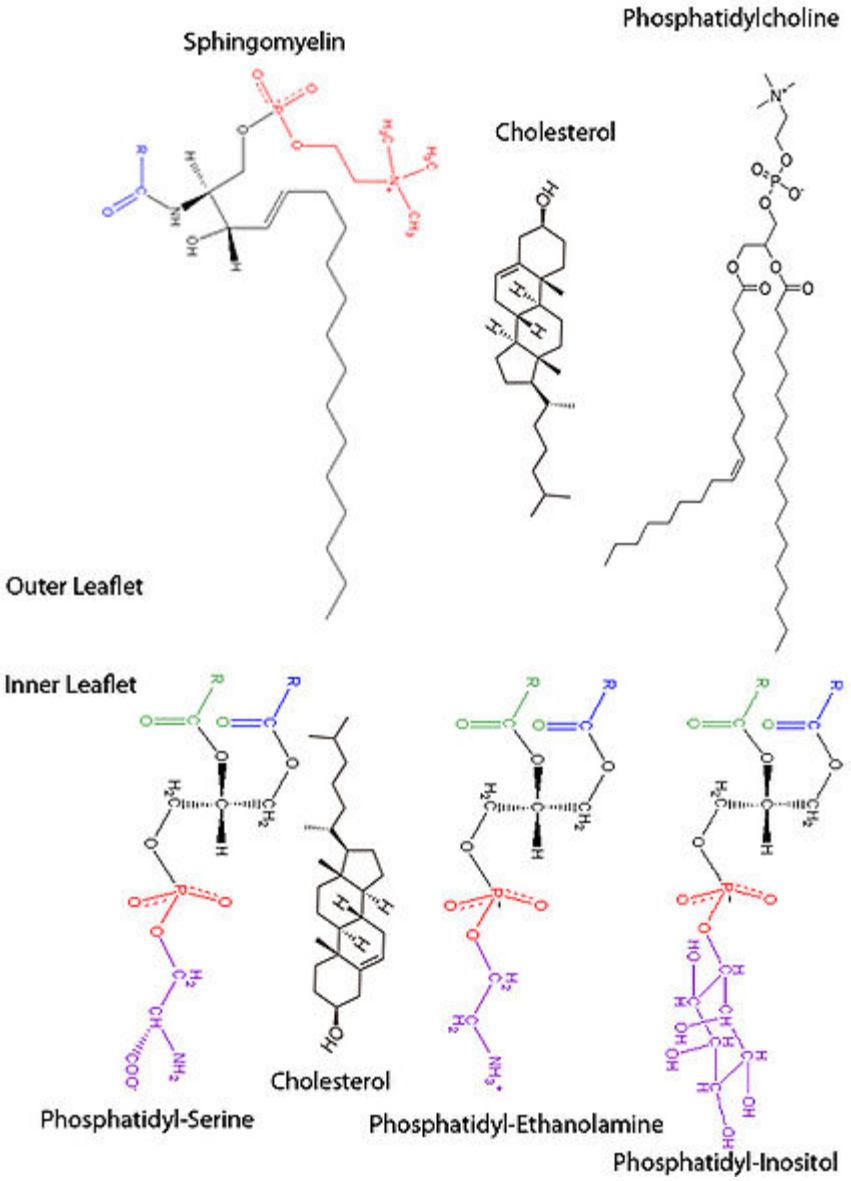
reticuloendothelial system (spleen, liver and bone marrow), thus removing old and defective cells and continually purging the blood. This process is termed eryptosis, or erythrocyte programmed cell death. This process normally occurs at the same rate of production by erythropoiesis, balancing the total 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.

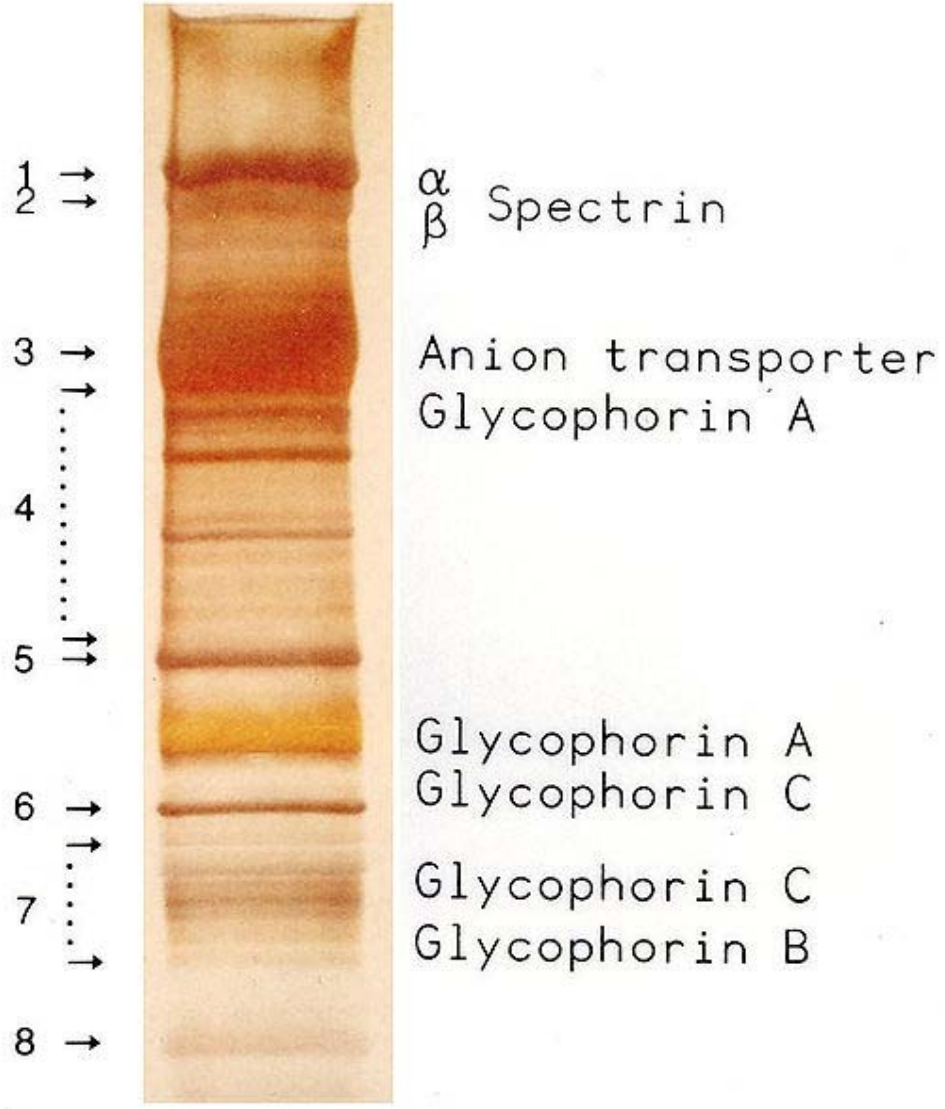
- An exposure of PS can potentiate adhesion of red cells to vascular endothelial cells, effectively preventing normal transit through the microvasculature. Thus it is important that PS is maintained only in the inner leaflet of the bilayer to ensure normal blood flow in microcirculation.

- 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

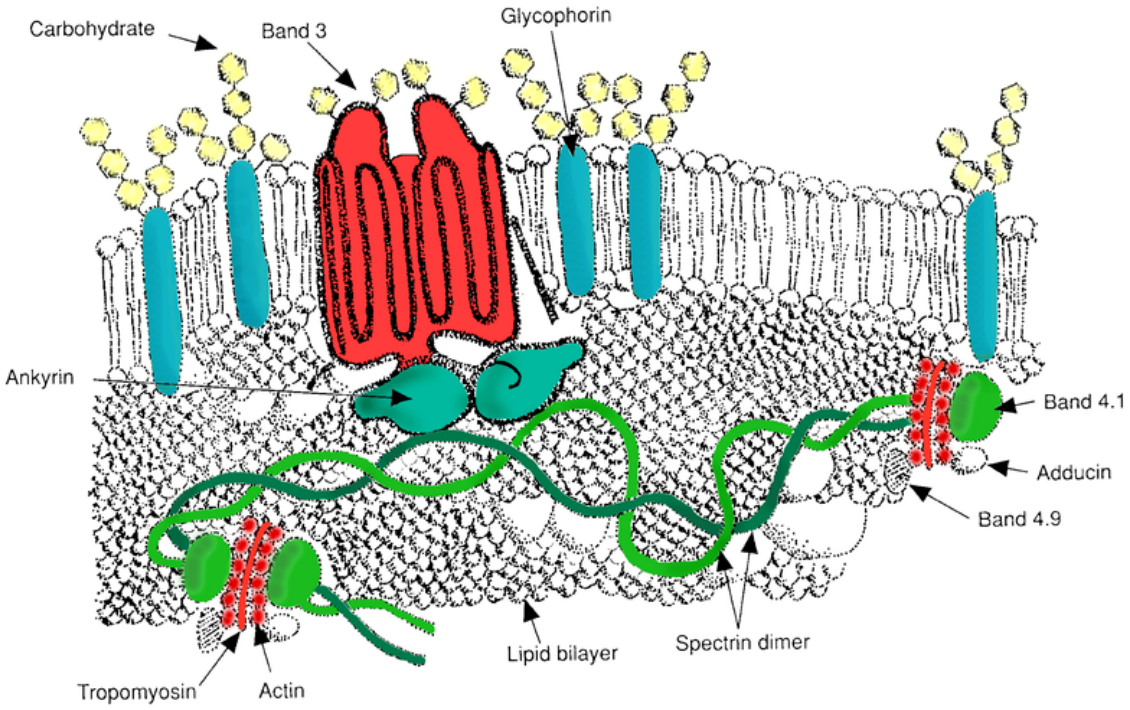


Red blood cell membrane proteins separated by SDS-Page and silverstained

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.

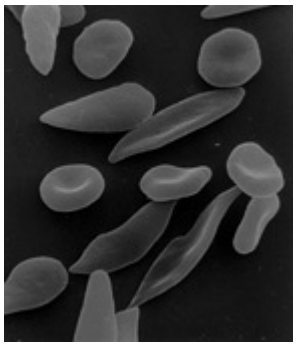
Separation and blood doping

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.

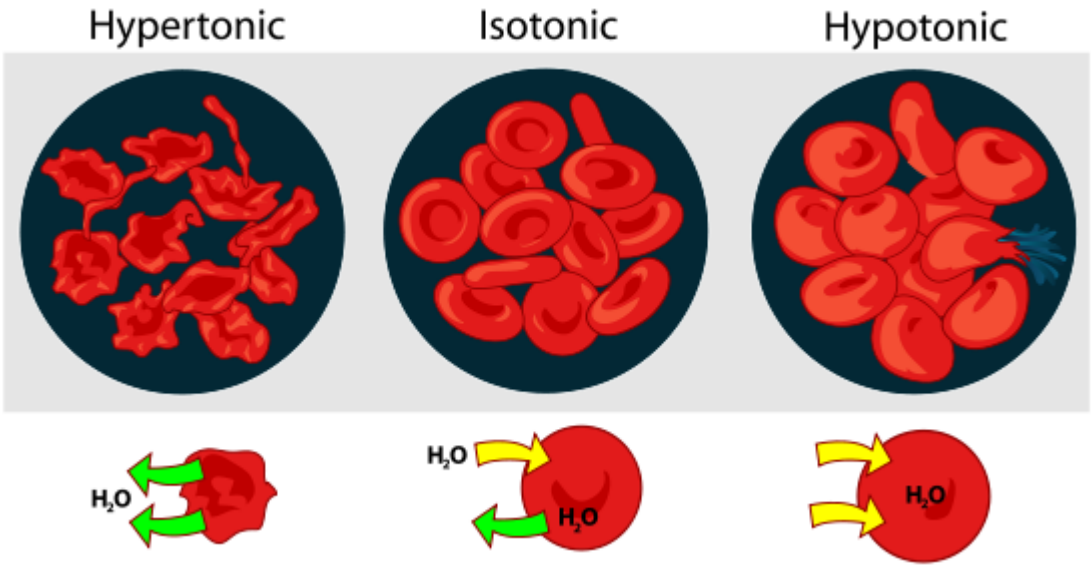
- Thalassemia is a genetic disease that results in the production of an abnormal ratio of hemoglobin subunits.

- 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.

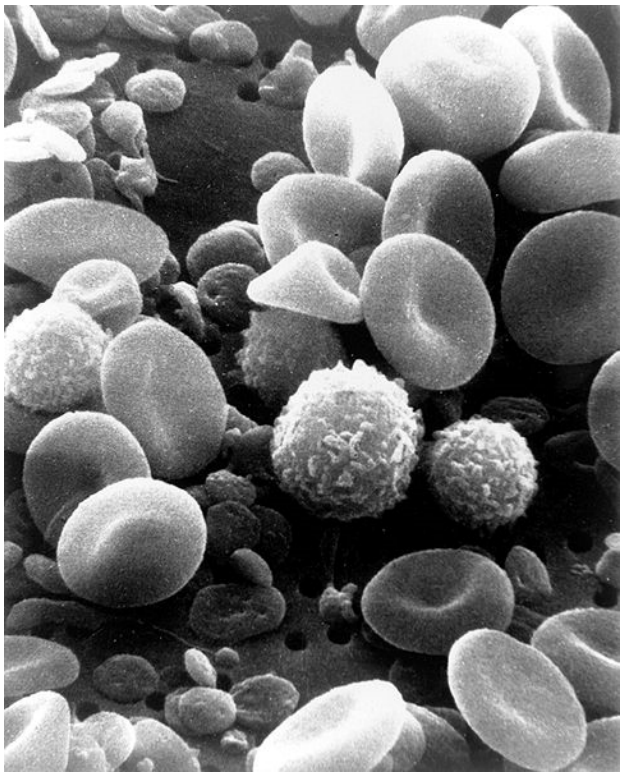
    - The Hereditary spherocytosis (HS) syndromes are a group of inherited disorders characterized by the presence of spherical-shaped erythrocytes on the peripheral blood smear. HS is found worldwide. It is the most common inherited anemia in individuals of northern European descent, affecting approximately 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

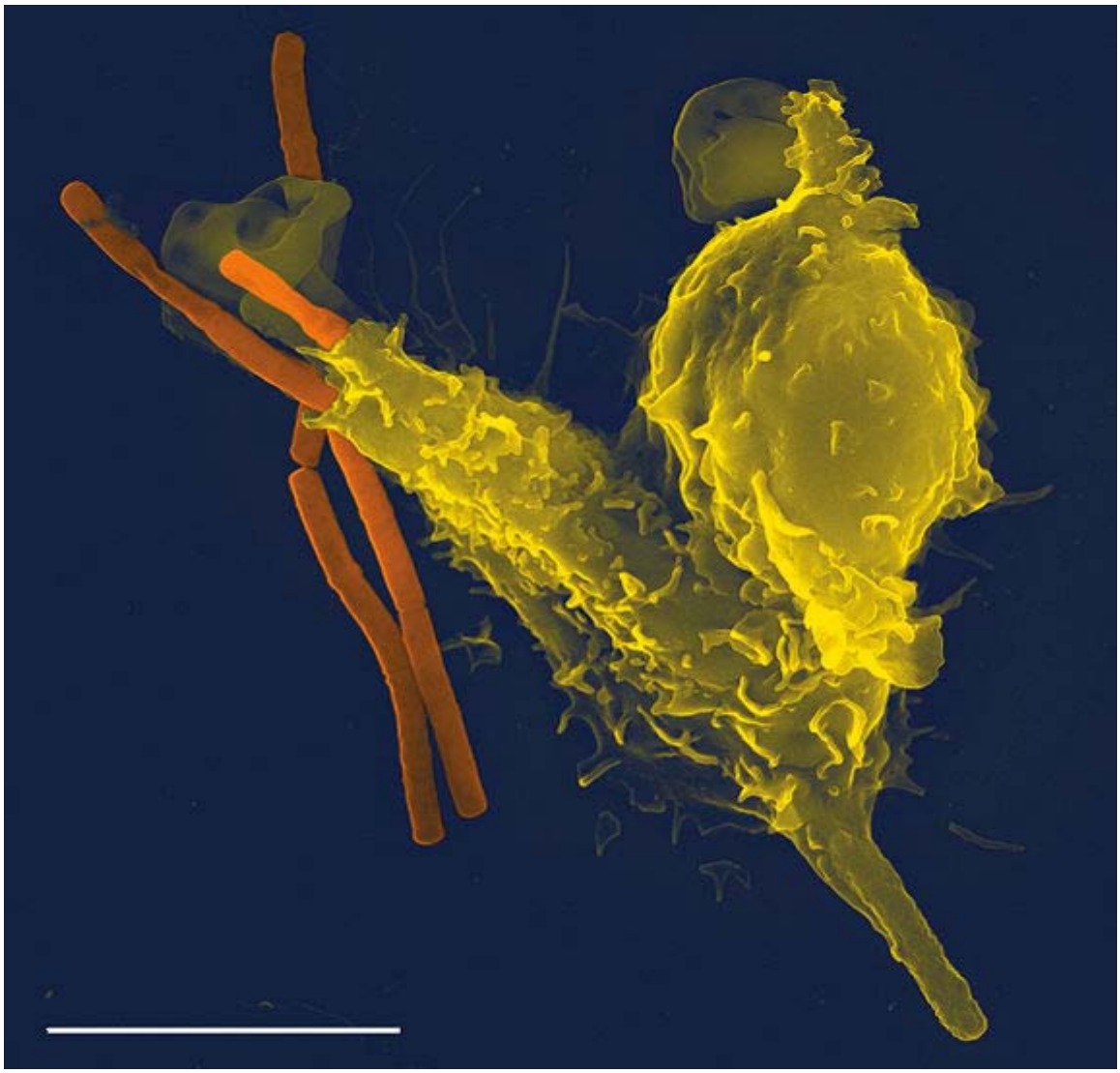
The name "white blood cell" derives from the fact that after centrifugation of a blood sample, the white cells are found in the *buffy coat*, a thin, typically white layer of nucleated cells between the sedimented red blood cells and the blood plasma. The scientific term *leukocyte* directly reflects 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.

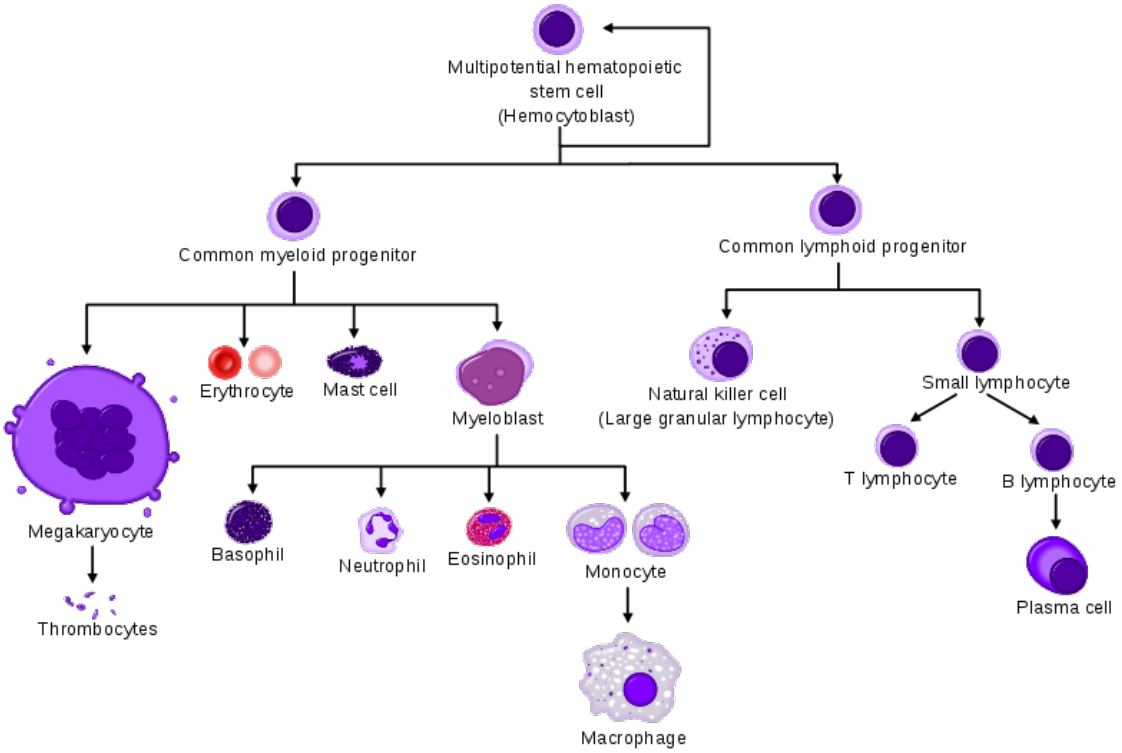
Medication causing leukopenia

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.

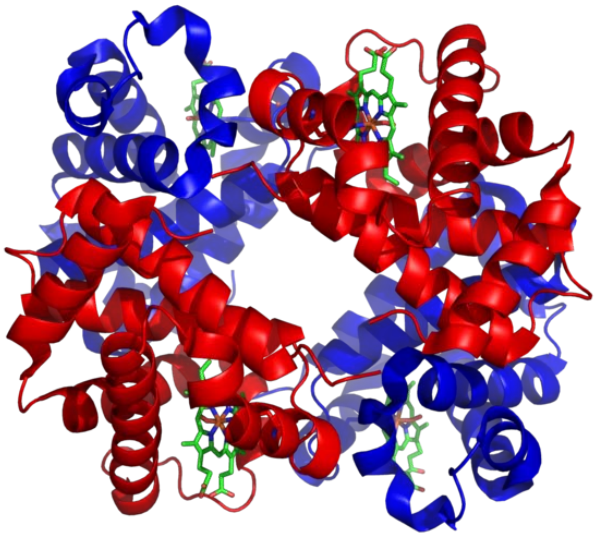
- Histiocytes
- Dendritic cells (Although these will often migrate to local lymph nodes upon ingesting antigens)
- Mast cells
- Microglia



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.

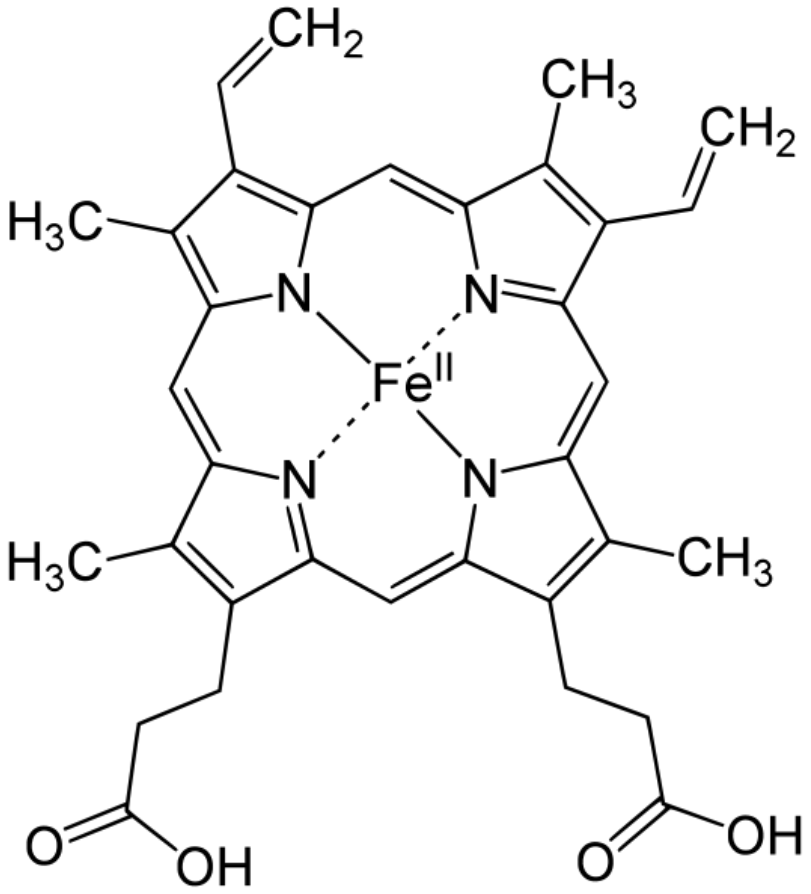
Variations in hemoglobin amino acid sequences, as with other proteins, may be adaptive. For example, recent studies have suggested genetic variants in deer mice that help explain how deer mice that live in the mountains are able to survive in the thin air that accompanies high altitudes. A researcher 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-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

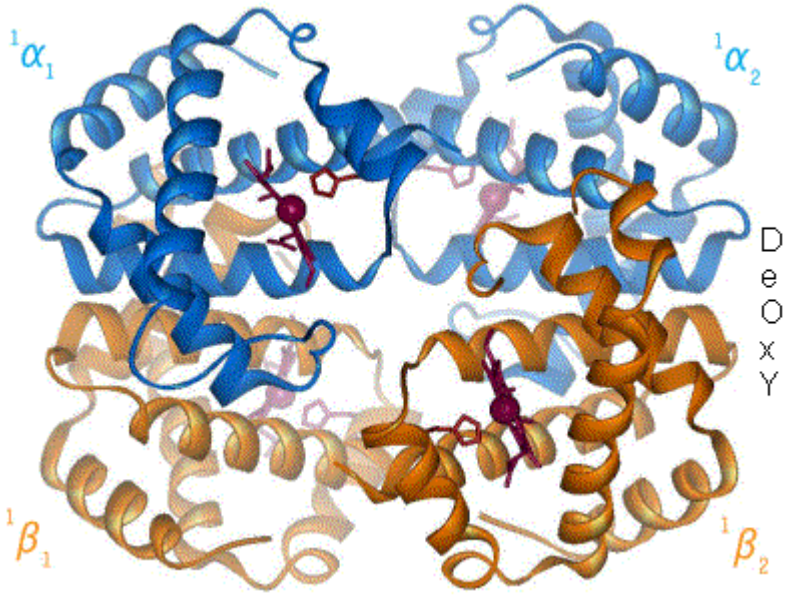
additional electron from iron by oxygen is required to explain both iron's smaller size and observed increased oxidation state, and oxygen's weaker bond.

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

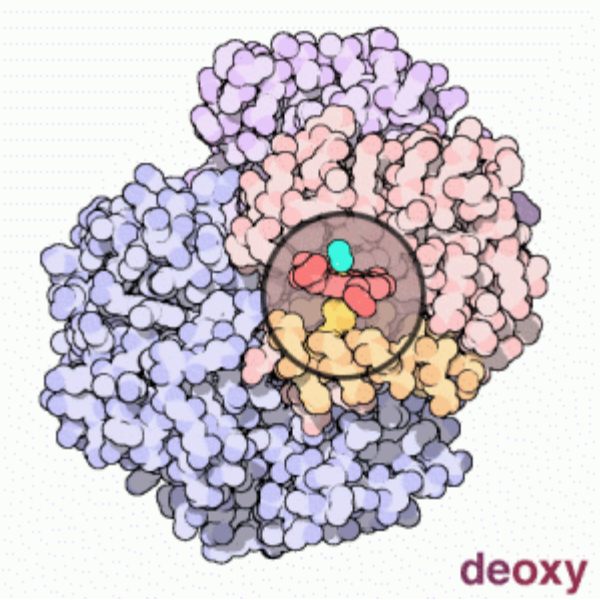
Besides the oxygen ligand, which binds to hemoglobin in a cooperative manner, hemoglobin ligands also include competitive inhibitors such as carbon monoxide (CO) and allosteric ligands such as carbon dioxide (CO_2) and nitric oxide (NO). The carbon dioxide is bound to amino groups of the globin proteins 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.

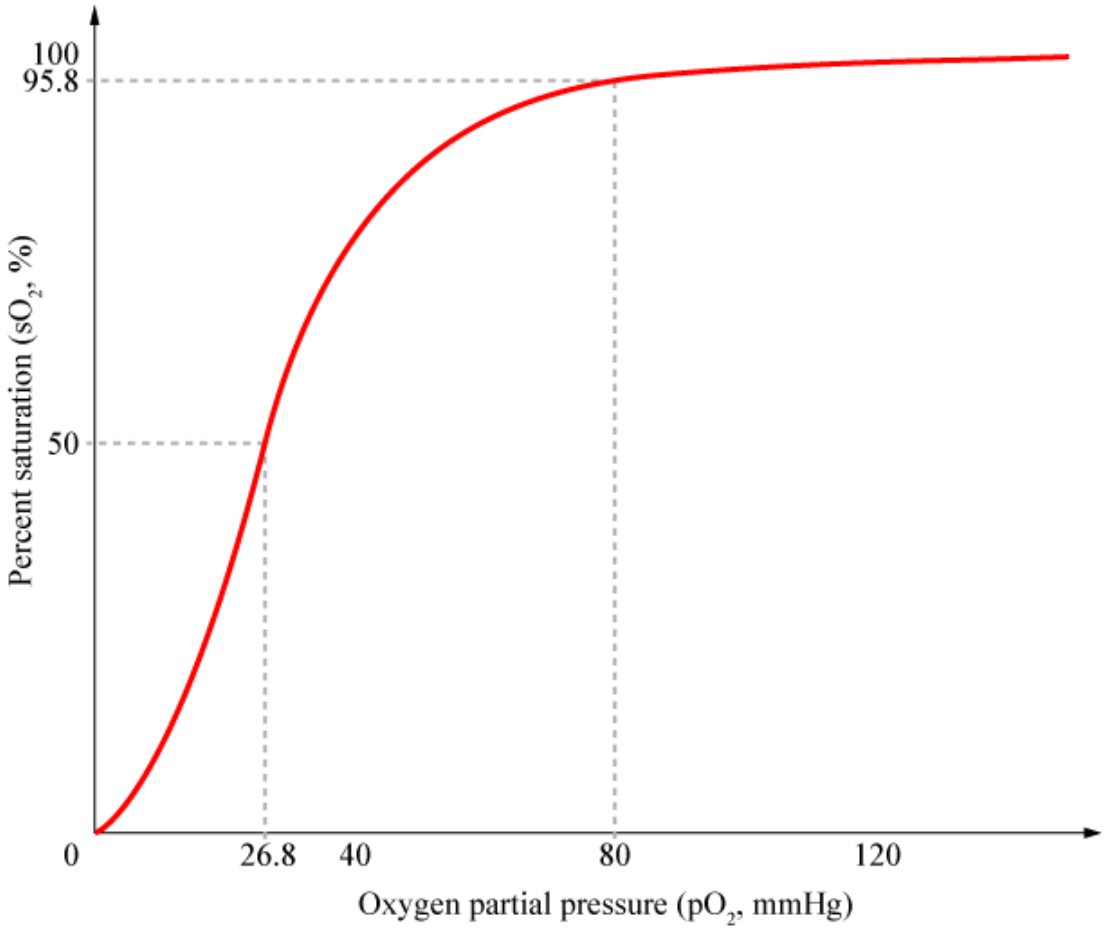
In similar fashion, hemoglobin also has competitive binding affinity for cyanide (CN^-), sulfur monoxide (SO), nitric oxide (NO), and sulfide(S^{2-}), including hydrogen sulfide (H_2S). All of these bind to iron in heme without changing its oxidation state, but they nevertheless inhibit oxygen-binding,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

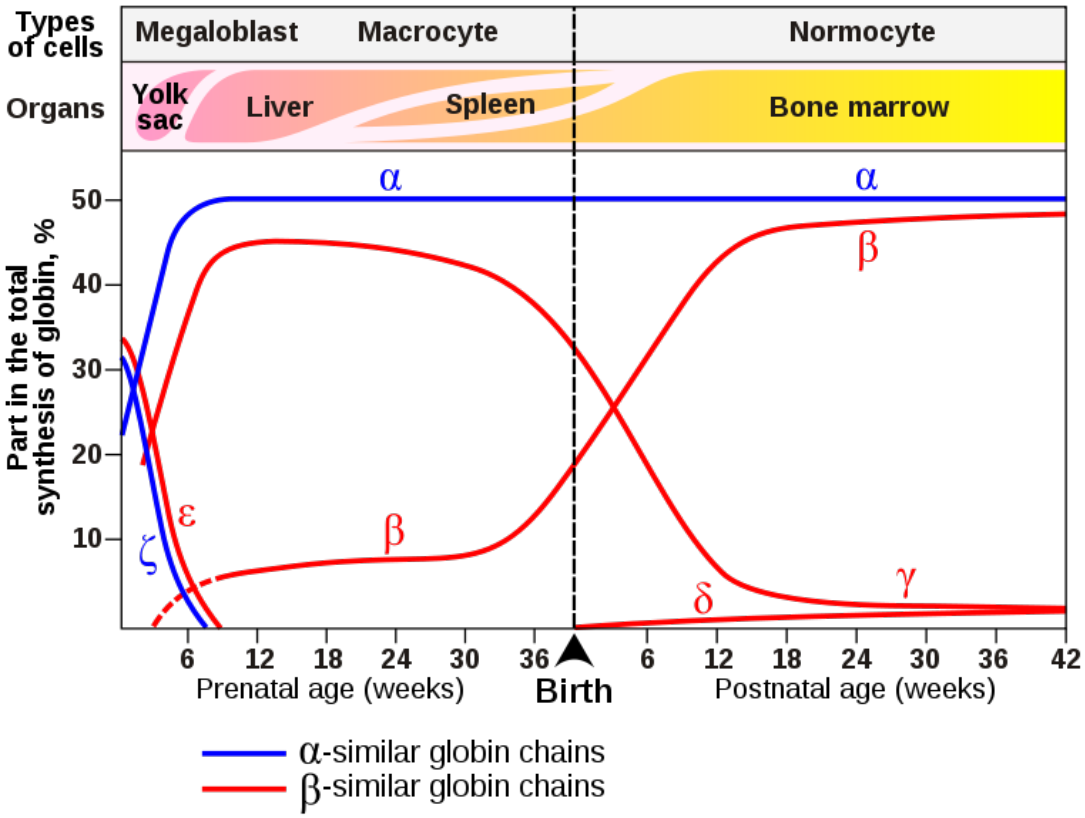
- Gower 1 ($\zeta_2\varepsilon_2$)
- Gower 2 ($\alpha_2\varepsilon_2$) (PDB 1A9W)
- Hemoglobin Portland ($\zeta_2\gamma_2$)

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.**)

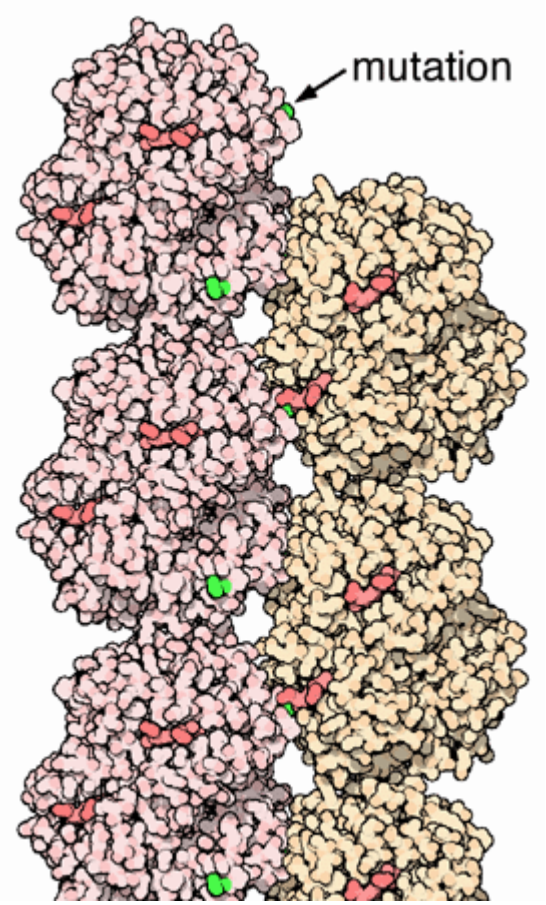
Variant forms that cause disease:

- 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.

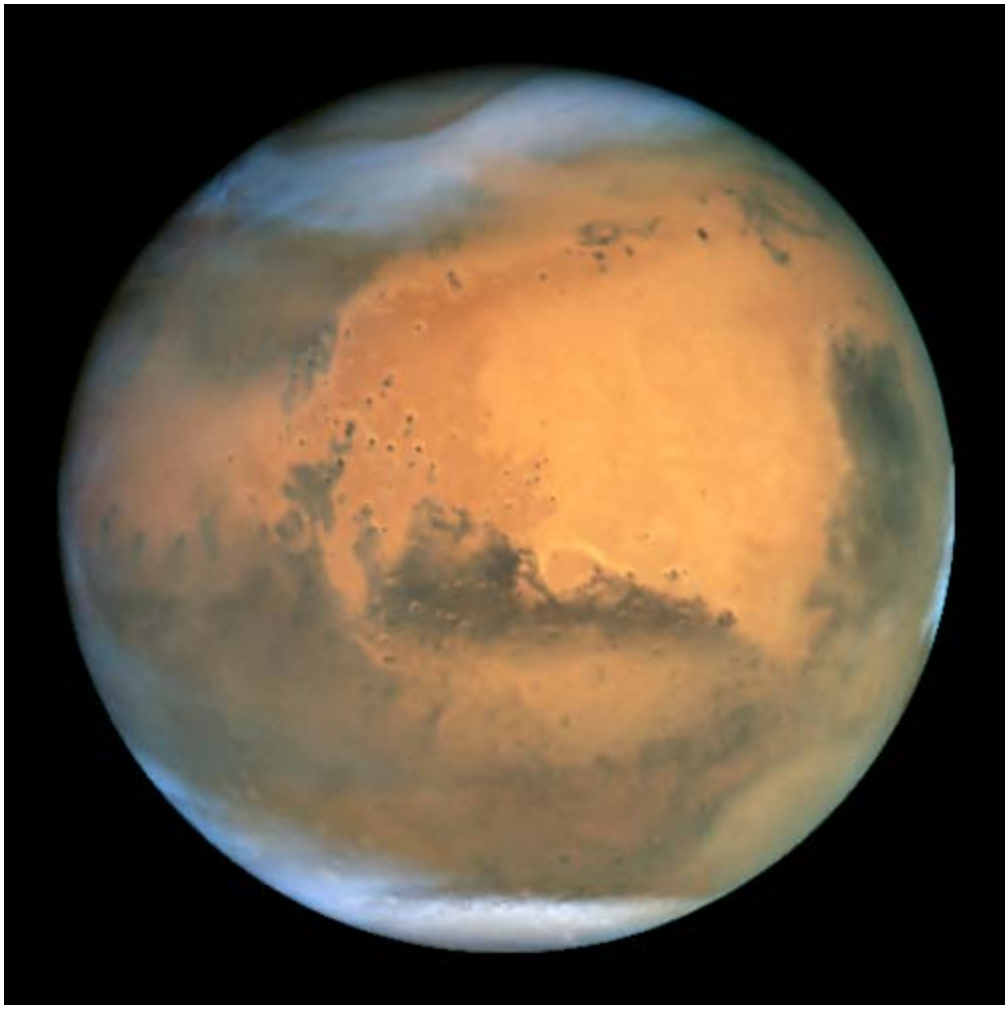
Coboglobin: A synthetic cobalt-based porphyrin. Coboprotein would appear colorless when oxygenated, but yellow when in veins.

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

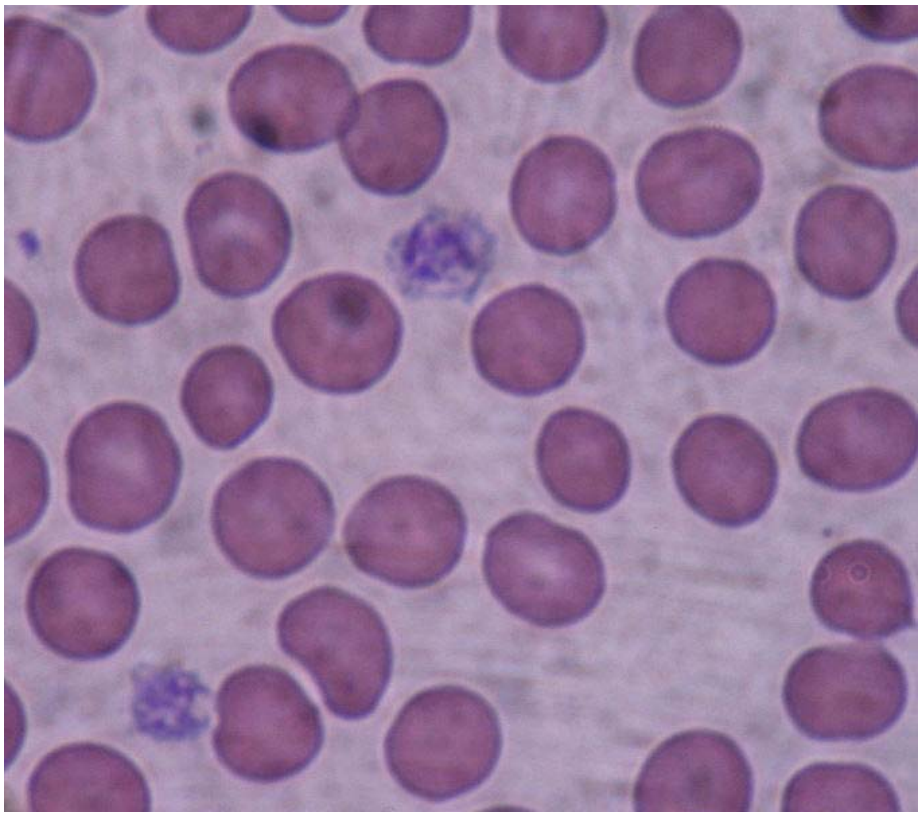


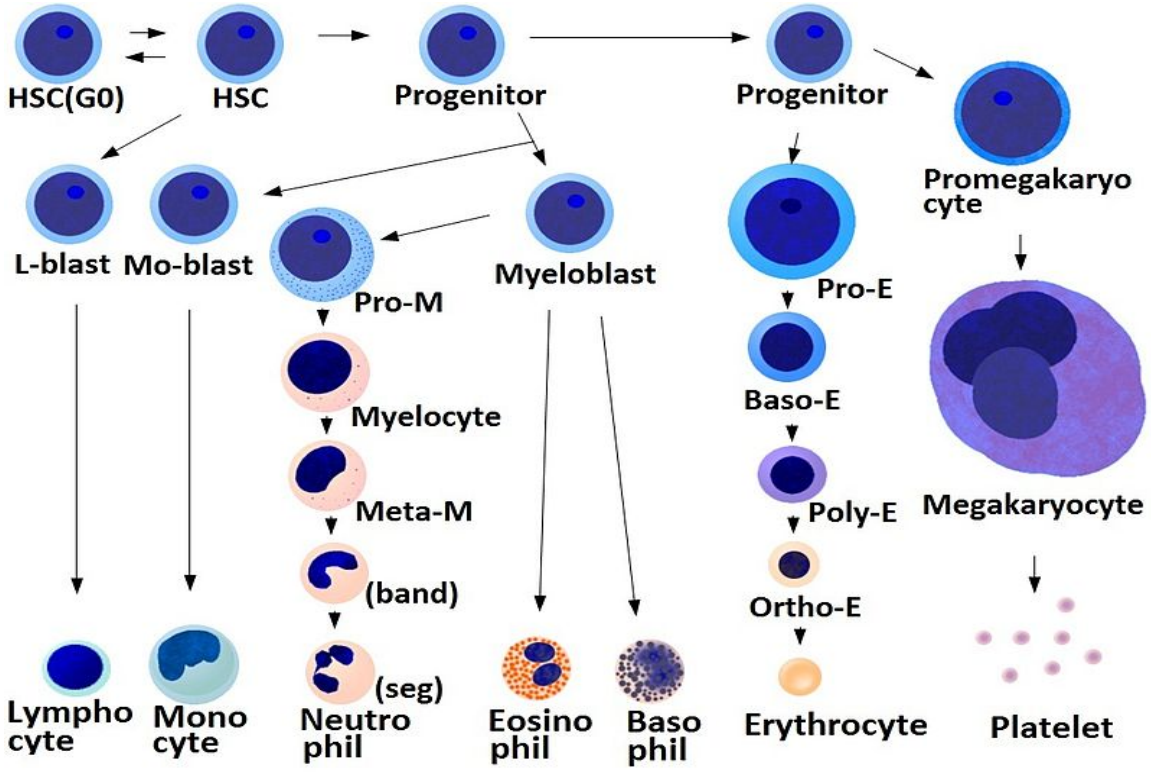
Image from a light microscope (40x) from a peripheral blood smear surrounded by red blood cells. One platelet can be seen in the upper left side of the image (purple) and is significantly smaller in size than the red blood cells (stained pink) and the two large platelets (stained purple).

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.

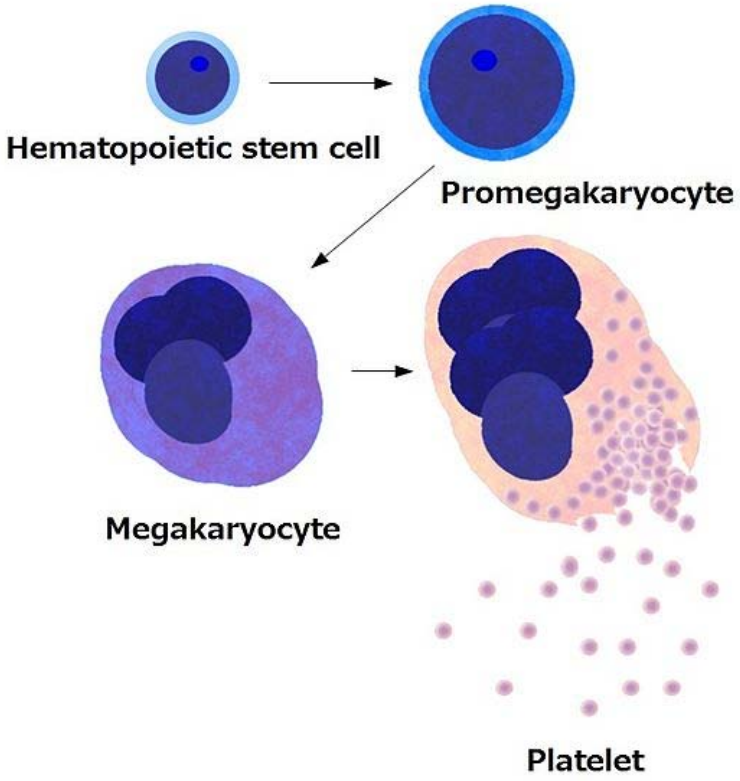
Platelets release a multitude of growth factors including Platelet-derived growth factor (PDGF), a potent chemotactic agent, and TGF beta, which stimulates the deposition of extracellular matrix. Both of these growth factors have been shown to play a significant role in the repair and regeneration of 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-

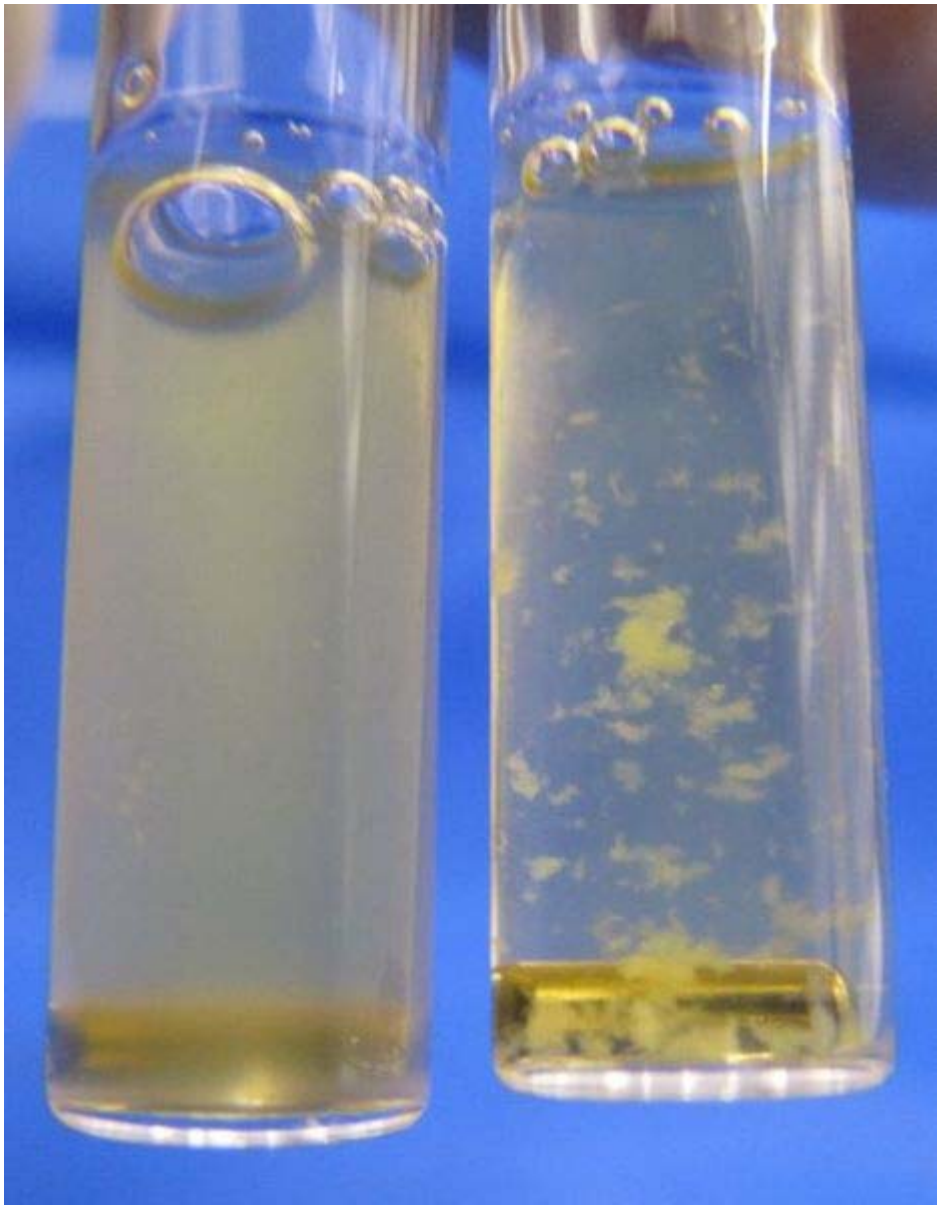
E=Proerythroblast, Baso-E=Basophilic erythroblast, poly-E=Polychromatic erythroblast, Ortho-E=Orthochromatic erythroblast, Erythrocyte, Promegakaryocyte, Megakaryocyte, Platlet



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

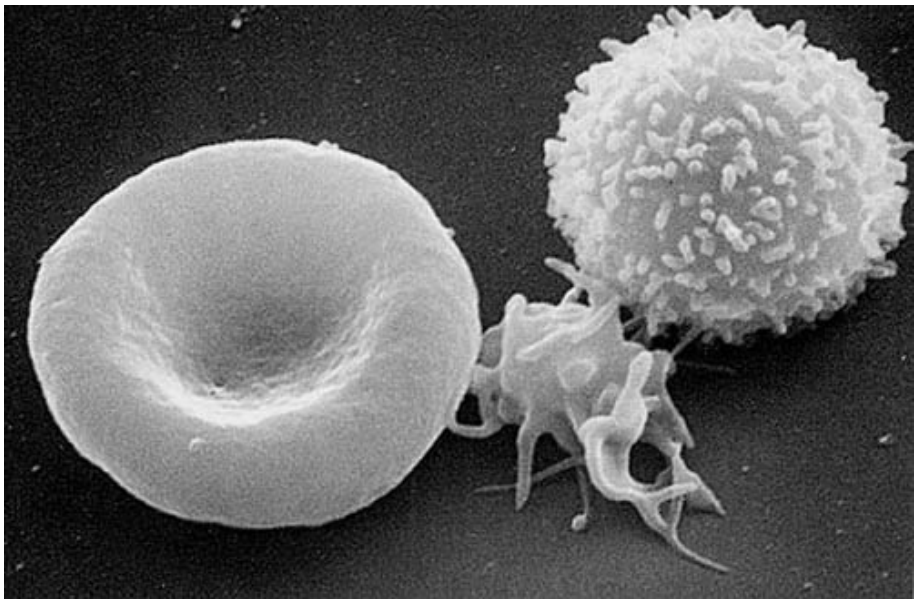
The inner surface of blood vessels is lined with a thin layer of endothelial cells that, in normal hemostasis, acts to inhibit platelet activation by producing nitric oxide, endothelial-ADPase, and PGI_2. Endothelial-ADPase clears away the platelet activator, ADP.

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 seen in amyloid purpura	prolonged	prolonged	unaffected	unaffected
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.

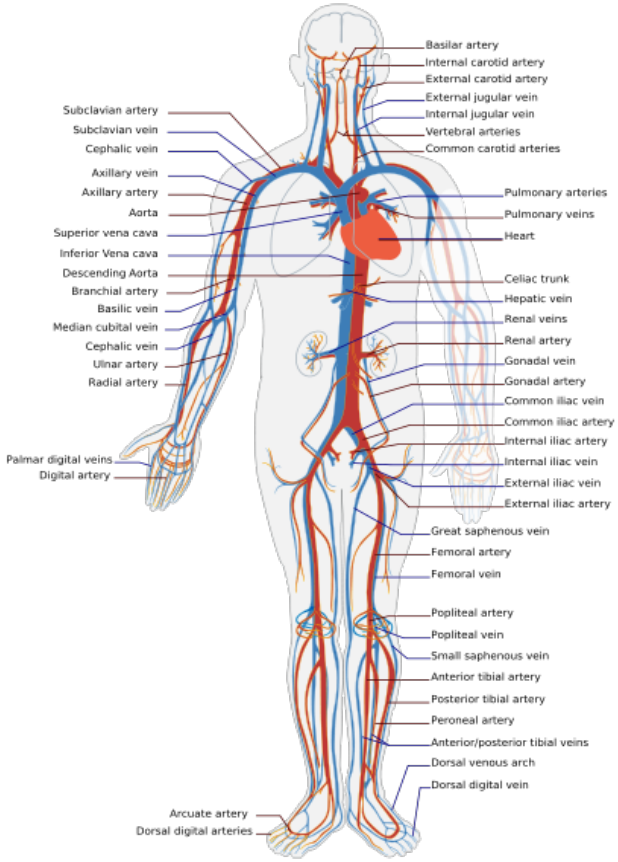
Other species

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.

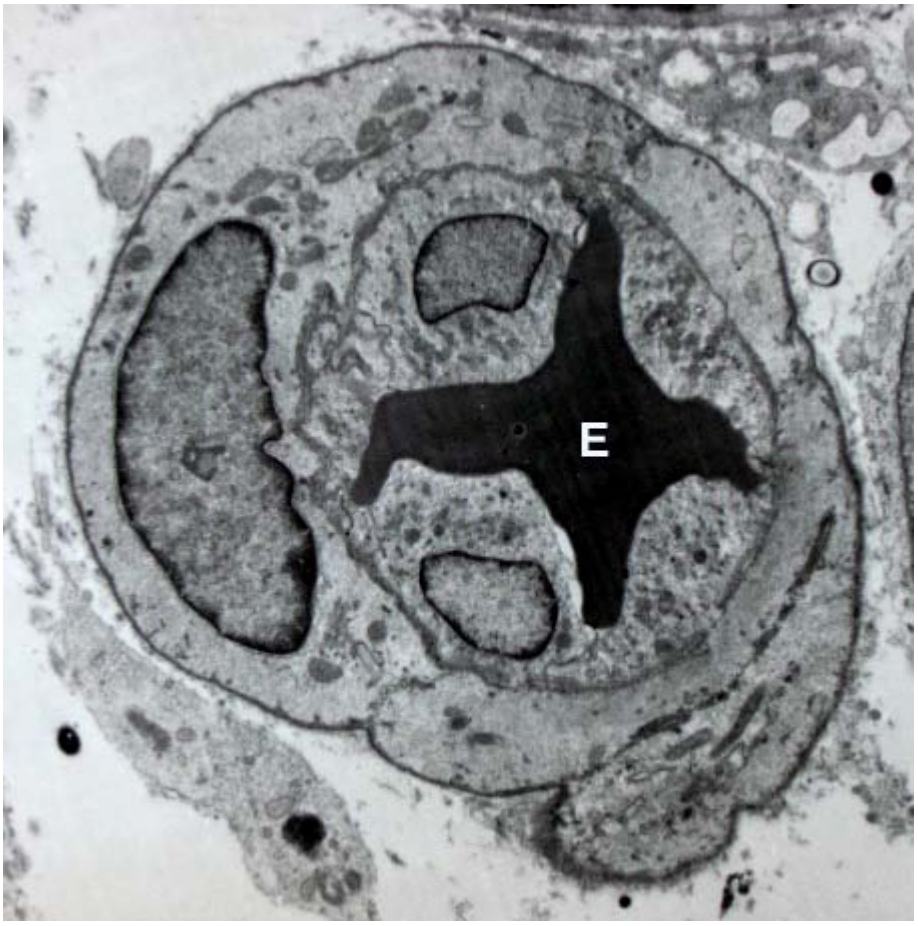
- *Tunica intima* (the thinnest layer): a single layer of simple squamous endothelial cells glued by a polysaccharide intercellular matrix, surrounded by a thin layer of subendothelial connective tissue interlaced with a number of circularly arranged elastic bands called the *internal elastic lamina*.
- *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

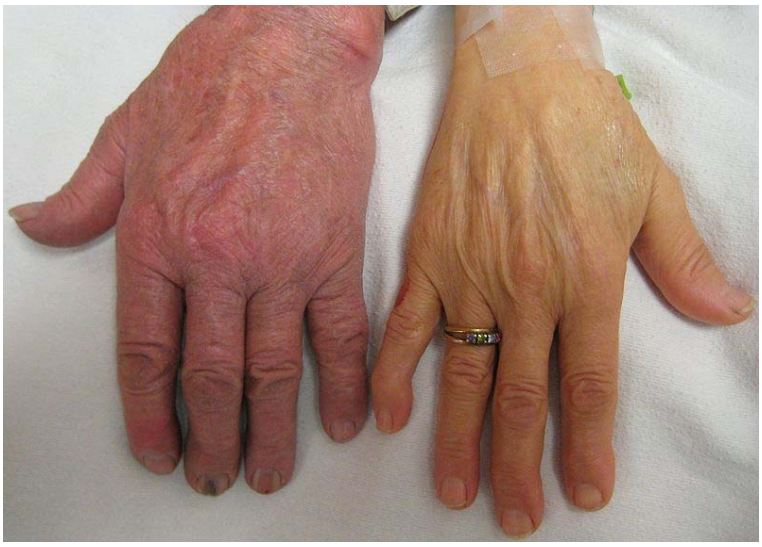
Blood vessels play a huge role in virtually every medical condition. Cancer, for example, cannot progress unless the tumor causes angiogenesis (formation of new blood vessels) to supply the malignant cells' metabolic demand. Atherosclerosis, the formation of lipid lumps (atheromas) in the blood vessel 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

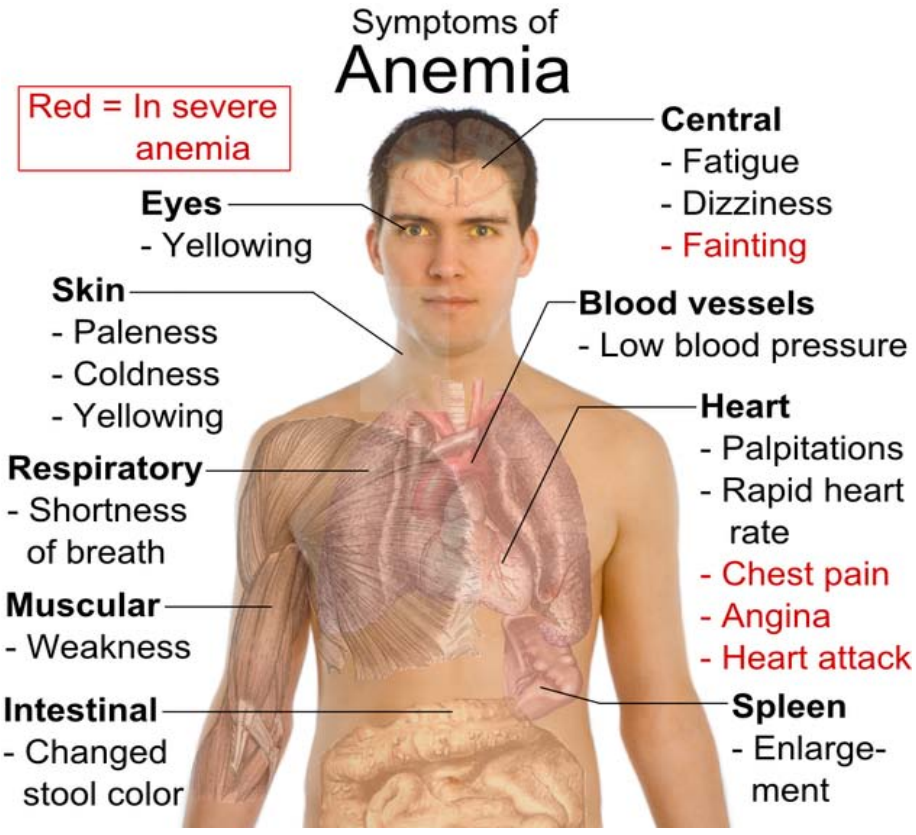
Anemia is a decrease in number of red blood cells (RBCs) or less than the normal quantity of hemoglobin in the blood. However, it can include decreased oxygen-binding ability of each hemoglobin molecule due to deformity or lack in numerical development as in some other types of hemoglobin deficiency.

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.

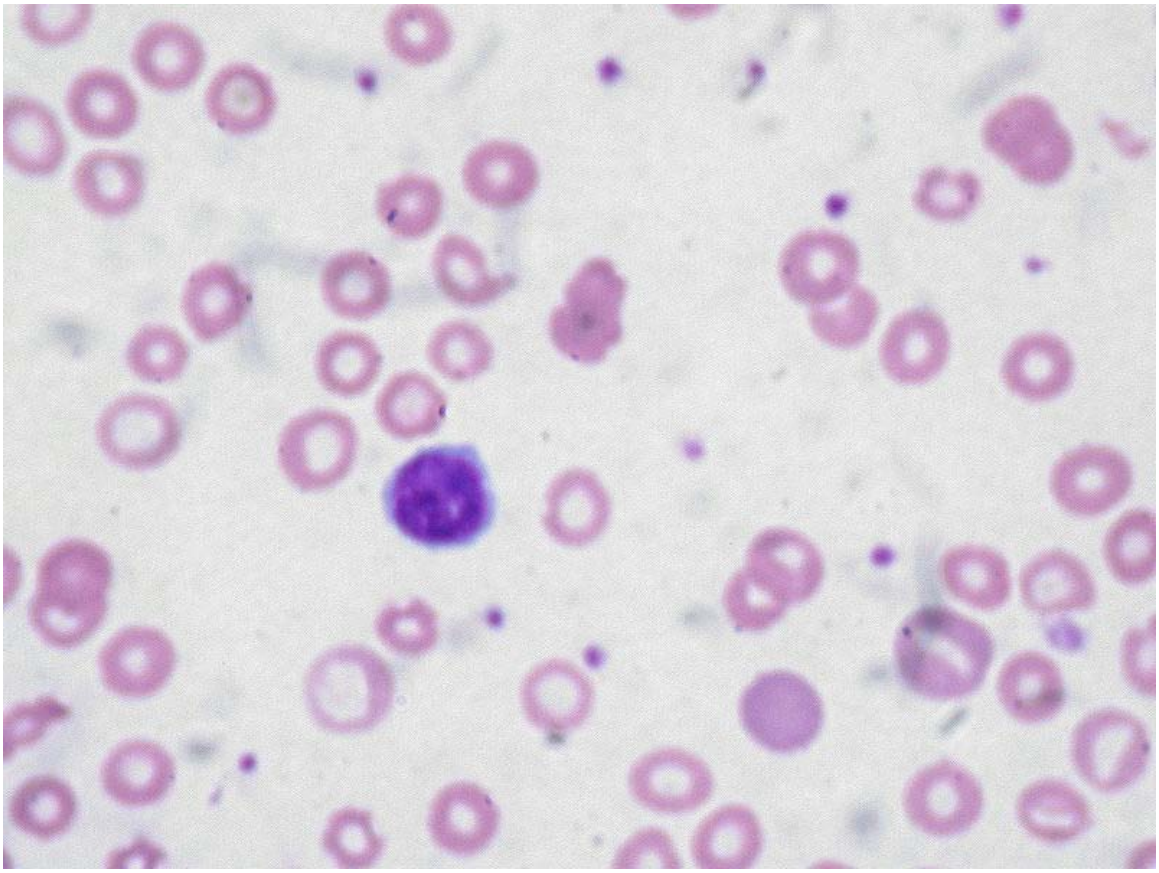
Pica, the consumption of non-food based items such as dirt, paper, wax, grass, ice, and hair, may be a symptom of iron deficiency, although it occurs often in those who have normal levels of hemoglobin.

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.

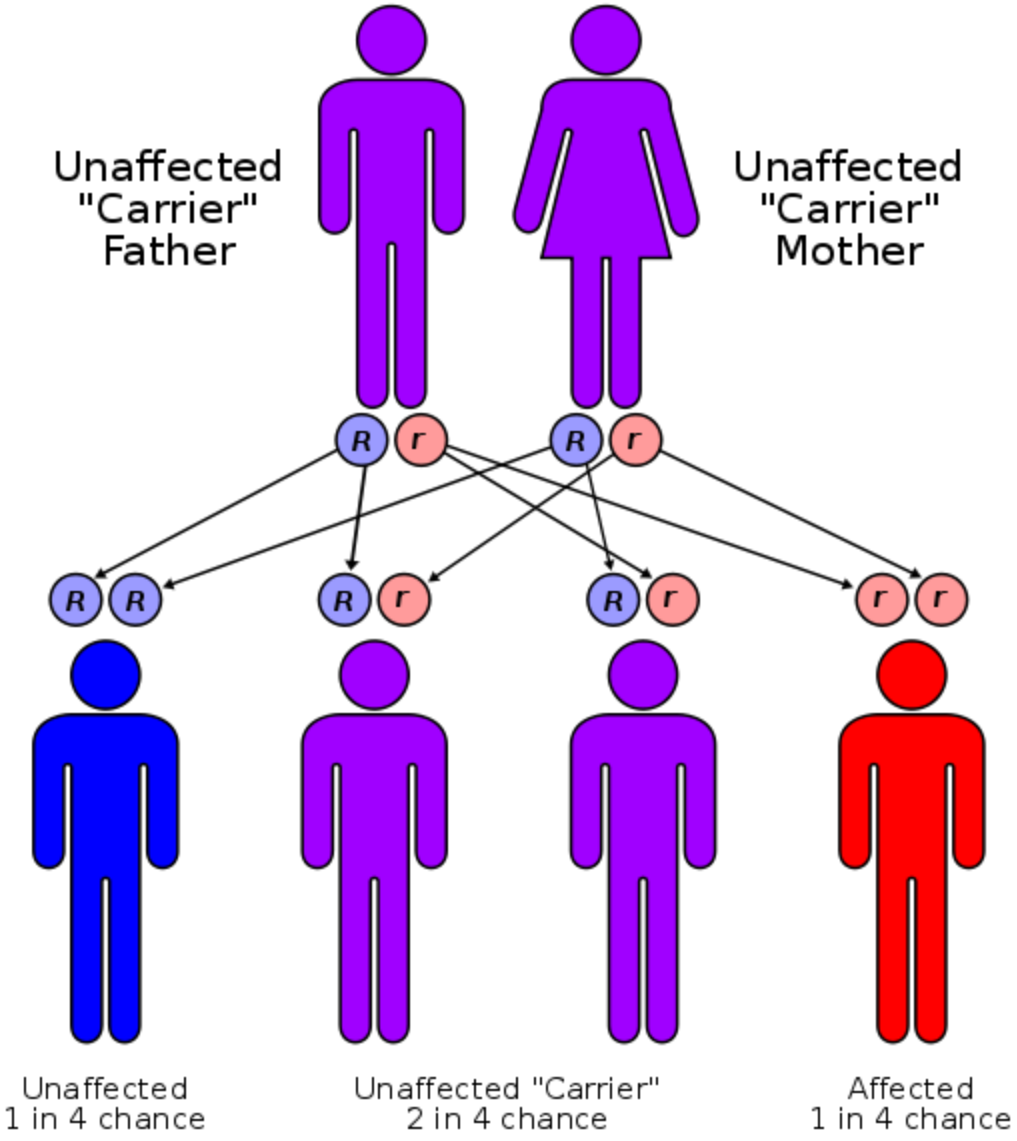
FA is the result of a genetic defect in a cluster of proteins responsible for DNA repair. As a result, 20% or more of FA patients develop cancer, most often acute myelogenous leukemia, and 90% develop bone marrow failure (the inability to produce blood cells) by age 40. About 60-75% of FA patients have 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/mm³ 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 granulocytopenia. 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), antibiotics (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.

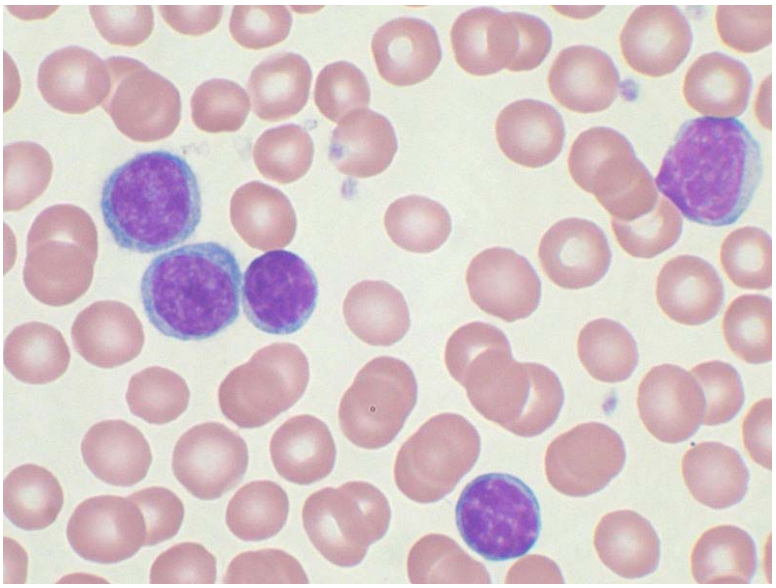
Infection in patients with low white blood cell counts is usually treated urgently, and usually includes a broad-spectrum penicillin or cephalosporin (piperacillin-tazobactam, ceftazidime or ticarcillin clavulanate), or meropenem in combination with gentamicin or amikacin.

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

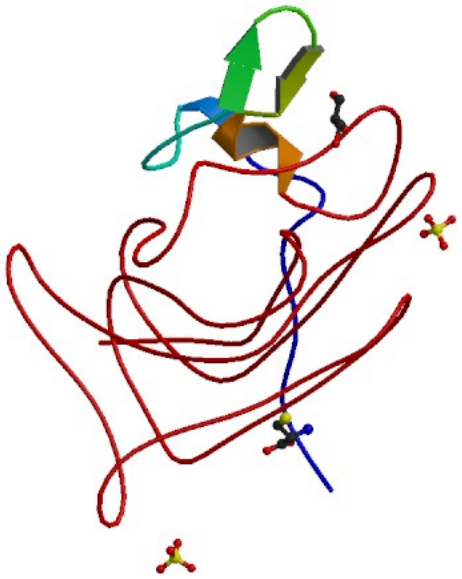
There is considerable research activity studying the many treatments individually or in combination with each other.

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

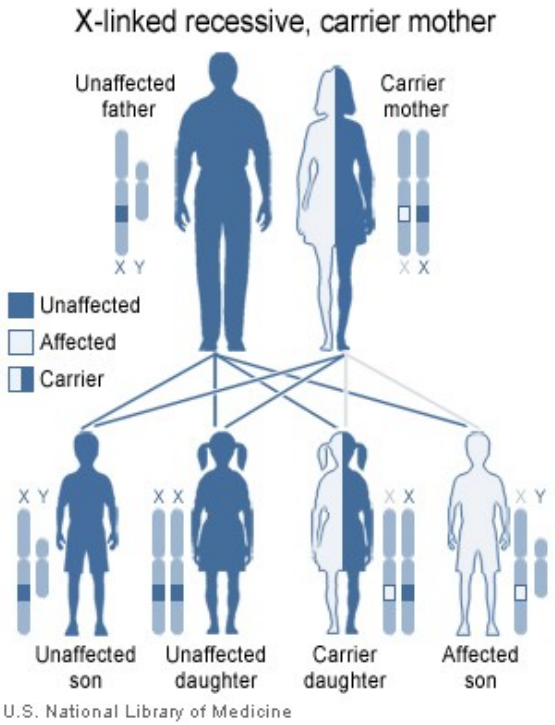
Like most aspects of the disorder, life expectancy varies with severity and adequate treatment. People with severe haemophilia who don't receive adequate, modern treatment have greatly shortened lifespans and often do not reach maturity. Prior to the 1960s when effective treatment became available, average 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.

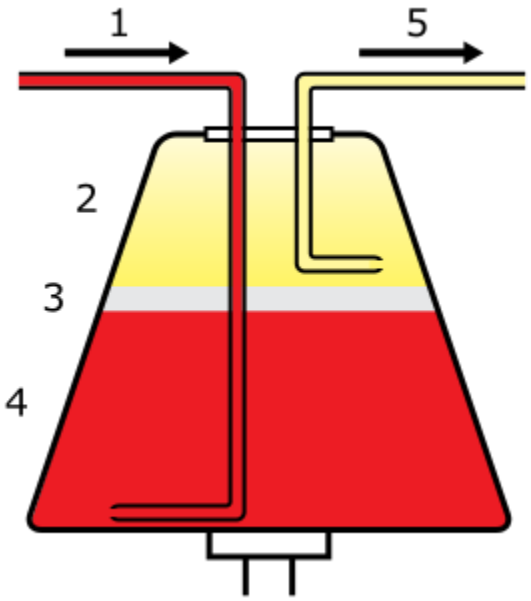
Blood contamination issues

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

Apheresis



Whole blood enters the centrifuge (1) and separates into plasma (2), leukocytes (3), and erythrocytes (4). Selected components are then drawn off (5).

Apheresis (*plural* **aphereses**; *also spelled* **aphaeresis**, **aphæresis**; from Ancient Greek ἀφαίρεσις (*aphairesis*, "a taking away")) is a medical technology in which the blood of a donor or patient is passed through an apparatus that separates out one particular constituent and returns the remainder to the circulation. It is thus an extracorporeal therapy.

Method

Depending on the substance that is being removed, different processes are employed in apheresis. If separation by Density is required, centrifugation is the most common method. Other methods involve absorption onto beads coated with an absorbent material and filtration.

The centrifugation method can be divided into two basic categories:

Continuous flow centrifugation (CFC)

Continuous flow centrifugation (CFC) historically required two venipunctures as the "continuous" means the blood is collected, spun, and returned simultaneously. Newer systems can use a single venipuncture. The main advantage of this system is the low extracorporeal volume (calculated by volume of the apheresis chamber, the donor's hematocrit, and total blood volume of the donor) used in the procedure, which may be advantageous in the elderly and for children.

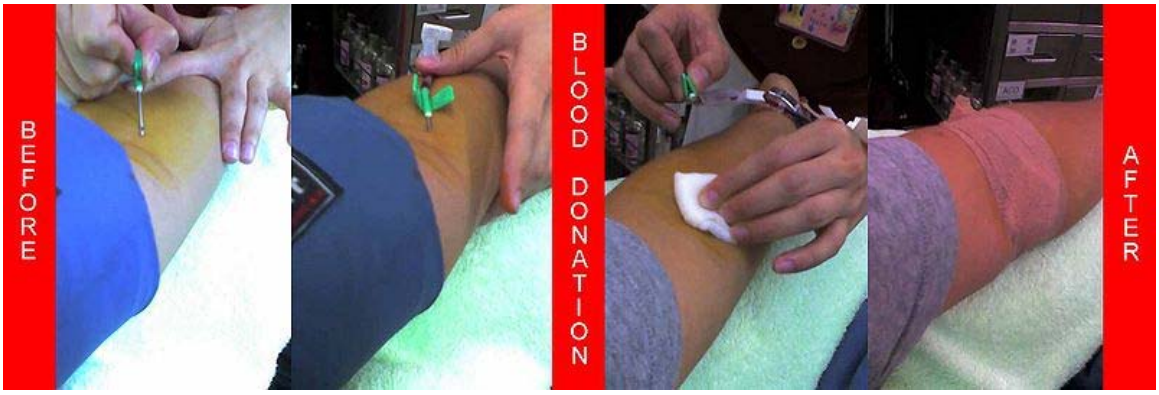
Intermittent flow centrifugation

Intermittent flow centrifugation works in cycles, taking blood, spinning/processing it and then giving back the necessary parts to the donor in a bolus. The main advantage is a single venipuncture site. To stop the blood from coagulating, anticoagulant is automatically mixed with the blood as it is pumped from the body into the apheresis machine.

Centrifugation Variables

The centrifugation process itself has four variables that can be controlled to selectively remove desired components. The first is spin speed and bowl diameter, the second is "sit time" in centrifuge, the third is solutes added, and the fourth is not as easily controllable: plasma volume and cellular content of the donor. The end product in most cases is the classic sedimented blood sample with the RBC's at the bottom, the "buffy coat" of platelets and WBC's (lymphocytes/granulocytes (PMN's, basophils, eosinophils/monocytes) in the middle and the plasma on top.

Types of apheresis



Disinfect, insert the cannula, pull out the cannula, dress the wound. The blue pressure cuff is controlled by the platelet apheresis machine in newer models.

There are numerous types of apheresis.

Donation

Blood taken from a healthy donor can be separated into its component parts during blood donation, where the needed component is collected and the "unused" components are returned to the donor. Fluid replacement is usually not needed in this type of collections. There are large categories of component collections:

- Plasmapheresis - blood plasma. Plasmapheresis is useful in collecting FFP (fresh frozen plasma) of a particular ABO group. Commercial uses aside from FFP for this procedure include immune globulin products, plasma derivatives, and collection of rare WBC and RBC antibodies.
- Erythrocytapheresis- red blood cells. Erythrocytapheresis is the separation of erythrocytes from whole blood. It is most commonly accomplished using the method of centrifugal sedimentation. This process is used for red blood cell diseases such as sickle cell crises or severe malaria. The automated red blood cell collection procedure for donating erythrocytes is referred to as 'Double Reds' or 'Double Red Cell Apheresis.'
- Plateletpheresis (thrombapheresis, thrombocytapheresis) - blood platelets. Plateletpheresis, like it sounds, is the collection of platelets by apheresis; while returning the RBC's, WBC's, and component plasma. The yield is normally the equivalent of between six and ten random platelet concentrates. Quality control demands the platelets from apheresis be equal to or greater than 3.0 x 10^11 in number and have a pH of equal to or greater than 6.2 in 90% of the products tested and must be used within five days.
- Leukapheresis - leukocytes (white blood cells). Leukopheresis is the removal of PMN's, basophils, eosinophils for transfusion into patients whose PMN's are ineffective or traditional therapy has failed. There is limited data to suggest the benefit of granulocyte infusion. The complications of this procedure are the difficulty in collection and short shelf life (24 hours at 20 to 24 C). Since the "buffy coat" layer sits directly atop the RBC layer, HES, a sedimenting agent, is employed to improve yield while minimizing RBC collection. Quality control demands the resultant concentrate be 1.0 x 10^10 granulocytes in 75% of the units tested and that the product be irradiated to avoid graft-versus-host disease (inactivate lymphocytes). Irradiation does not affect PMN function. Since there is usually a small amount of RBC's collected, ABO compatibility should be employed when feasible.
- Stem cell harvesting - circulating bone marrow cells are harvested to use in bone marrow transplantation.

Donor Safety

- Single use kits - Apheresis is done using single-use kits, so there is no risk of infection from blood-contaminated tubing or centrifuge.
- Immune system effects - "the immediate decreases in blood lymphocyte counts and serum immunoglobulin concentrations are of slight to moderate degree and

are without known adverse effects. Less information is available regarding long-term alterations of the immune system"

Kit Problems

Two apheresis kit recalls were:

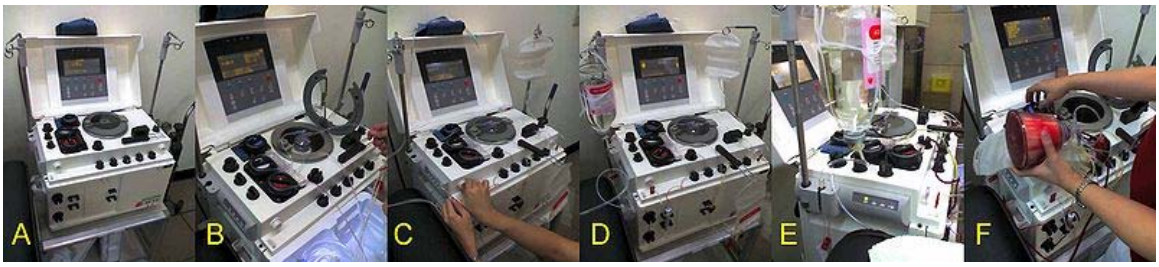
- Baxter Healthcare Corporation (2005) in which "pinhole leaks were observed at the two-omega end of the umbilicus (multilumen tubing), causing a blood leak. "
- Fenwal Incorporated (2007) in which there were "two instances where the anticoagulant citrate dextrose (ACD) and saline lines were reversed in the assembly process. The reversed line connections may not be visually apparent in the monitor box, and could result in excessive ACD infusion and severe injury, including death, to the donor."

Plasticizer exposure

Apheresis uses plastics and tubing, which come into contact with the blood. The plastics are made of PVC in addition to additives such as a plasticizer, often DEHP. DEHP leaches from the plastic into the blood, and people have begun to study the possible effects of this leached DEHP on donors as well as transfusion recipients.

- "current risk or preventive limit values for DEHP such as the RfD of the US EPA (20 µg/kg/day) and the TDI of the European Union (20-48 µg/kg/day) can be exceeded on the day of the plateletpheresis. . . . Especially women in their reproductive age need to be protected from DEHP exposures exceeding the above mentioned preventive limit values."
- "Commercial plateletpheresis disposables release considerable amounts of DEHP during the apheresis procedure, but the total dose of DEHP retained by the donor is within the normal range of DEHP exposure of the general population."
- The Baxter company manufactured blood bags without DEHP, but there was little demand for the product in the marketplace
- "Mean DEHP doses for both plateletpheresis techniques (18.1 and 32.3 µg/kg/day) were close to or exceeded the reference dose (RfD) of the US EPA and tolerable daily intake (TDI) value of the EU on the day of the apheresis. Therefore, margins of safety might be insufficient to protect especially young men and women in their reproductive age from effects on reproductivity. At present, discontinuous-flow devices should be preferred to avert conceivable health risks from plateletpheresis donors. Strategies to avoid DEHP exposure of donors during apheresis need to be developed."

Therapy



The assembly (A-D), operation (E) and disassembly (F) of the platelet apheresis machine which can be configured to separate other components as well.

The various apheresis techniques may be used whenever the removed constituent is causing severe symptoms of disease. Generally, apheresis has to be performed fairly often, and is an invasive process. It is therefore only employed if other means to control a particular disease have failed, or the symptoms are of such a nature that waiting for medication to become effective would cause suffering or risk of complications.

- Plasma exchange - removal of the liquid portion of blood to remove harmful substances. The plasma is replaced with a replacement solution.
- LDL apheresis - removal of low density lipoprotein in patients with familial hypercholesterolemia.
- Photopheresis - used to treat graft-versus-host disease, cutaneous T-cell lymphoma, and rejection in heart transplantation.
- Immunoadsorbtion with Staphylococcal protein A-agarose column - removal of allo- and autoantibodies (in autoimmune diseases, transplant rejection, hemophilia) by directing plasma through protein A-agarose columns. Protein A is a cell wall component produced by several strains of Staphylococcus aureus which binds to the Fc region of IgG.
- Leukocytapheresis - removal of malignant white blood cells in people with leukemia and very high white blood cell counts causing symptoms.
- Thrombocytapheresis - removal of platelets in people with symptoms from extreme elevations in platelet count such as those with Essential Thrombasthenia or Polycythemia vera

Evidence Based Guidelines for Therapeutic Apheresis

In 2010, the American Society for Apheresis published the 5th Special Edition(1) of evidence based guidelines for the practice of Apheresis Medicine. These guidelines are based upon a systematic review of available scientific literature. Clinical utility for a given disease is denoted by assignment of an **ASFA Category** (I – IV). The quality and strength of evidence are denoted by standard GRADE recommendations. ASFA Categories are defined as follows:**Category I** for disorders where therapeutic apheresis is accepted as a first line treatment,**Category II** for disorders where therapeutic apheresis is accepted as a second-line treatment, **Category III** for disorders where the optimal role of

therapeutic apheresis is not clearly established and **Category IV** for disorders where therapeutic apheresis is considered ineffective or harmful.

Fluid replacement during apheresis

It is important to remember that when the apheresis system is used for therapy the system is removing relatively small amounts of fluid (not more than 10.5 mL/kg body weight). That fluid must be replaced to keep correct intravascular volume. The fluid replaced is different at different institutions. If a crystalloid like normal saline is used, the infusion amount should be triple what is removed as the three to one ratio of NS for plasma is needed to keep up oncotic pressure. Some institutions use normal serum albumin, but it is costly and can be difficult to find. Some advocate using FFP or a similar blood product, but there are dangers including citrate toxicity (from the anticoagulant), ABO incompatibility, infection, and cellular antigens.

Chapter 11

Blood Donation

A man donating blood

A **blood donation** occurs when a person voluntarily has blood drawn and used for transfusions or made into medications by a process called fractionation.

In the developed world, most blood donors are unpaid volunteers who give blood for a community supply. In poorer countries, established supplies are limited and donors usually give blood when family or friends need a transfusion. Many donors donate as an

act of charity, but some are paid and in some cases there are incentives other than money such as paid time off from work. A donor can also have blood drawn for their own future use. Donating is relatively safe, but some donors have bruising where the needle is inserted or may feel faint.

Potential donors are evaluated for anything that might make their blood unsafe to use. The screening includes testing for diseases that can be transmitted by a blood transfusion, including HIV and viral hepatitis. The donor is also asked about medical history and given a short physical examination to make sure that the donation is not hazardous to his or her health. How often a donor can give varies from days to months based on what he or she donates and the laws of the country where the donation takes place. For example, in the United States donors must wait 8 weeks (56 days) between whole blood donations but only three days between plateletpheresis donations.

The amount of blood drawn and the methods vary. The collection can be done manually or with automated equipment that only takes specific portions of the blood. Most of the components of blood used for transfusions have a short shelf life, and maintaining a constant supply is a persistent problem.

Types of donation

A blood collection bus (bloodmobile) from Children's Hospital Boston at a manufacturing facility in Massachusetts. Blood banks sometimes use a modified bus or similar large vehicle to provide mobile facilities for donation.

Blood donations are divided into groups based on who will receive the collected blood. An *allogeneic* (also called *homologous*) donation is when a donor gives blood for storage at a blood bank for transfusion to an unknown recipient. A *directed* donation is when a person, often a family member, donates blood for transfusion to a specific individual. Directed donations are relatively rare. A *replacement donor* donation is a hybrid of the two and is common in developing countries such as Ghana. In this case, a friend or family member of the recipient donates blood to replace the stored blood used in a transfusion, ensuring a consistent supply. When a person has blood stored that will be transfused back to the donor at a later date, usually after surgery, that is called an *autologous* donation. Blood that is used to make medications can be made from allogeneic donations or from donations exclusively used for manufacturing.

The actual process varies according to the laws of the country, and recommendations to donors vary according to the collecting organization. The World Health Organization gives recommendations for blood donation policies, but in developing countries many of these are not followed. For example, the recommended testing requires laboratory facilities, trained staff, and specialized reagents, all of which may not be available or too expensive in developing countries.

An event where donors come to give allogeneic blood is sometimes called a *blood drive* or a *blood donor session*. These can occur at a blood bank but they are often set up at a location in the community such as a shopping center, workplace, school, or house of worship.

Screening

Donors are typically required to give consent for the process and this requirement means that minors cannot donate without permission from a parent or guardian. In some countries, answers are associated with the donor's blood, but not name, to provide anonymity; in others, such as the United States, names are kept to create lists of ineligible donors. If a potential donor does not meet these criteria, they are *deferred*. This term is used because many donors who are ineligible may be allowed to donate later.

The donor's race or ethnic background is sometimes important since certain blood types, especially rare ones, are more common in certain ethnic groups. Historically, donors were segregated or excluded on race, religion, or ethnicity, but this is no longer a standard practice.

Recipient safety

Donors are screened for health risks that might make the donation unsafe for the recipient. Some of these restrictions are controversial, such as restricting donations from men who have sex with men for HIV risk. Autologous donors are not always screened for recipient safety problems since the donor is the only person who will receive the blood. Donors are also asked about medications such as dutasteride since they can be dangerous to a pregnant woman receiving the blood.

Donors are examined for signs and symptoms of diseases that can be transmitted in a blood transfusion, such as HIV, malaria, and viral hepatitis. Screening may extend to questions about risk factors for various diseases, such as travel to countries at risk for malaria or variant Creutzfeldt-Jakob Disease (vCJD). These questions vary from country to country. For example, while blood centers in Québec, Poland, and many other places defer donors who lived in the United Kingdom for risk of vCJD, donors in the United Kingdom are only restricted for vCJD risk if they have had a blood transfusion in the United Kingdom.

Donor safety

The donor is also examined and asked specific questions about their medical history to make sure that donating blood is not hazardous to their health. The donor's hematocrit or hemoglobin level is tested to make sure that the loss of blood will not make them anemic, and this check is the most common reason that a donor is ineligible. Pulse, blood pressure, and body temperature are also evaluated. Elderly donors are sometimes also deferred on age alone because of health concerns. The safety of donating blood during pregnancy has not been studied thoroughly and pregnant women are usually deferred.

Blood testing

The donor's blood type must be determined if the blood will be used for transfusions. The collecting agency usually identifies whether the blood is type A, B, AB, or O and the donor's Rh (D) type and will screen for antibodies to less common antigens. More testing, including a crossmatch, is usually done before a transfusion. Group O is often cited as the "universal donor" but this only refers to red cell transfusions. For plasma transfusions the system is reversed and AB is the universal donor type.

Most blood is tested for diseases, including some STDs. The tests used are high-sensitivity screening tests and no actual diagnosis is made. Some of the test results are later found to be false positives using more specific testing. False negatives are rare, but donors are discouraged from using blood donation for the purpose of anonymous STD screening because a false negative could mean a contaminated unit. The blood is usually discarded if these tests are positive, but there are some exceptions, such as autologous donations. The donor is generally notified of the test result.

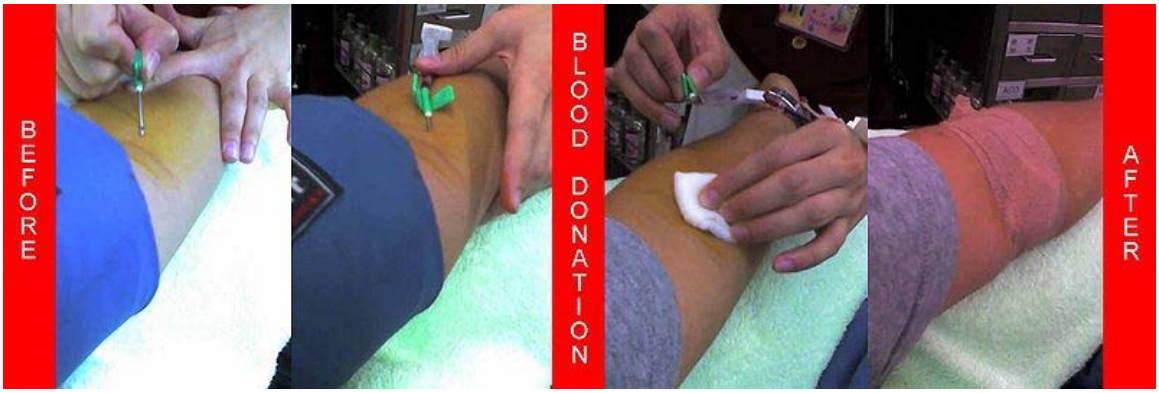
Donated blood is tested by many methods, but the core tests recommended by the World Health Organization are these four:

- Hepatitis B Surface Antigen
- Antibody to Hepatitis C
- Antibody to HIV, usually subtypes 1 and 2
- Serologic test for Syphilis

The WHO reported in 2006 that 56 out of 124 countries surveyed did not use these basic tests on all blood donations.

A variety of other tests for transfusion transmitted infections are often used based on local requirements. Additional testing is expensive, and in some cases the tests are not implemented because of the cost. These additional tests include other infectious diseases such as West Nile Virus. Sometimes multiple tests are used for a single disease to cover the limitations of each test. For example, the HIV antibody test will not detect a recently infected donor, so some blood banks use a p24 antigen or HIV nucleic acid test in addition to the basic antibody test to detect infected donors during that period. Cytomegalovirus is a special case in donor testing in that many donors will test positive for it. The virus is not a hazard to a healthy recipient, but it can harm infants and other recipients with weak immune systems.

Obtaining the blood



A donor's arm at various stages of donation. The two photographs on the left show a blood pressure cuff being used as a tourniquet.

There are two main methods of obtaining blood from a donor. The most frequent is simply to take the blood from a vein as whole blood. This blood is typically separated into parts, usually red blood cells and plasma, since most recipients need only a specific component for transfusions. A typical donation is 450 milliliters (or approximately one US pint) of whole blood, though 500 millileter donations are also common. Historically, blood donors in the People's Republic of China would donate only 200 milliliters, though larger 300 and 400 milliliter donations have become more common.

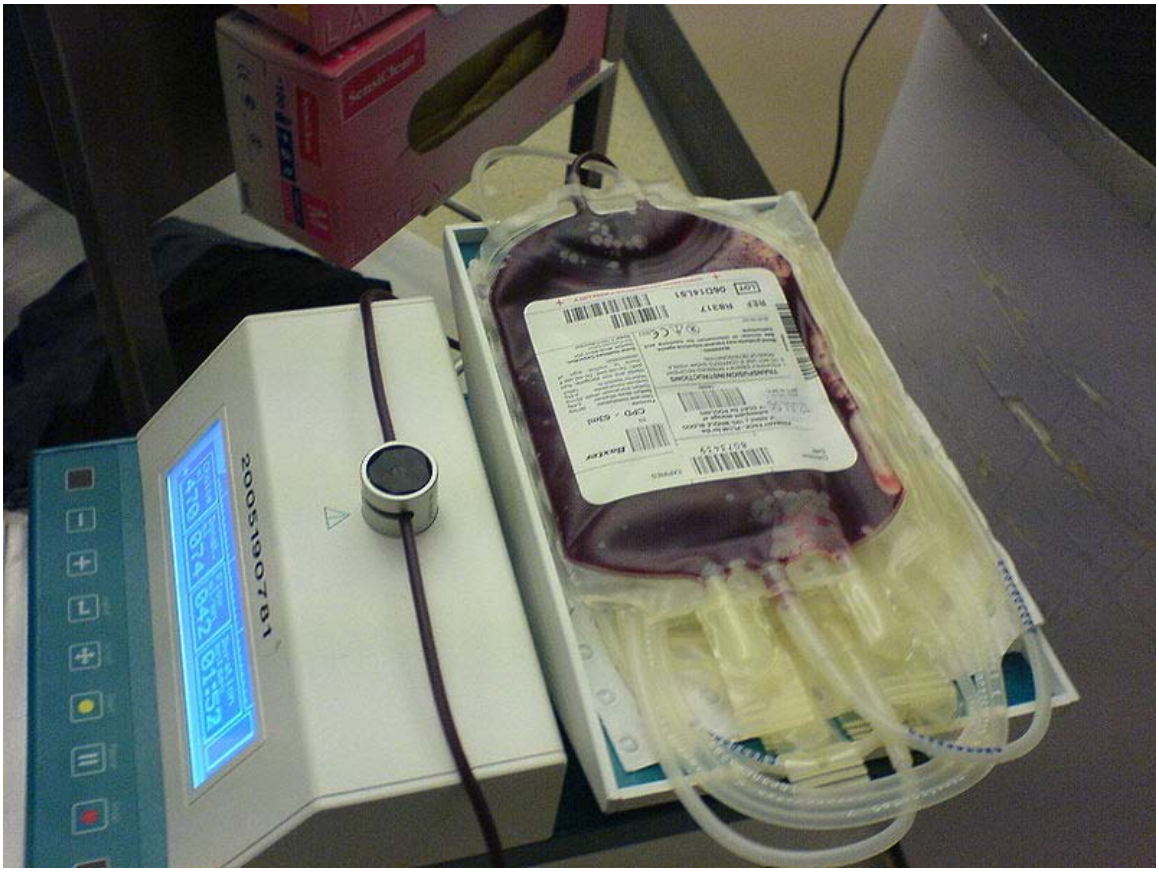
The other method is to draw blood from the donor, separate it using a centrifuge or a filter, store the desired part, and return the rest to the donor. This process is called apheresis, and it is often done with a machine specifically designed for this purpose. This process is especially common for plasma and platelets.

For direct transfusions a vein can be used but the blood may be taken from an artery instead. In this case, the blood is not stored, but is pumped directly from the donor into the recipient. This was an early method for blood transfusion and is rarely used in modern practice. It was phased out during World War II because of problems with logistics, and doctors returning from treating wounded soldiers set up banks for stored blood when they returned to civilian life.

Site preparation and drawing blood

The blood is drawn from a large arm vein close to the skin, usually the median cubital vein on the inside of the elbow. The skin over the blood vessel is cleaned with an antiseptic such as iodine or chlorhexidine to prevent skin bacteria from contaminating the collected blood and also to prevent infections where the needle pierced the donor's skin.

A large needle (16 to 17 gauge) is used to minimize shearing forces that may physically damage red blood cells as they flow through the needle. A tourniquet is sometimes wrapped around the upper arm to increase the pressure of the blood in the arm veins and speed up the process. The donor may also be prompted to hold an object and squeeze it repeatedly to increase the blood flow through the vein.



A mechanical tray agitates the bag to mix the blood with anticoagulants and prevent clotting.

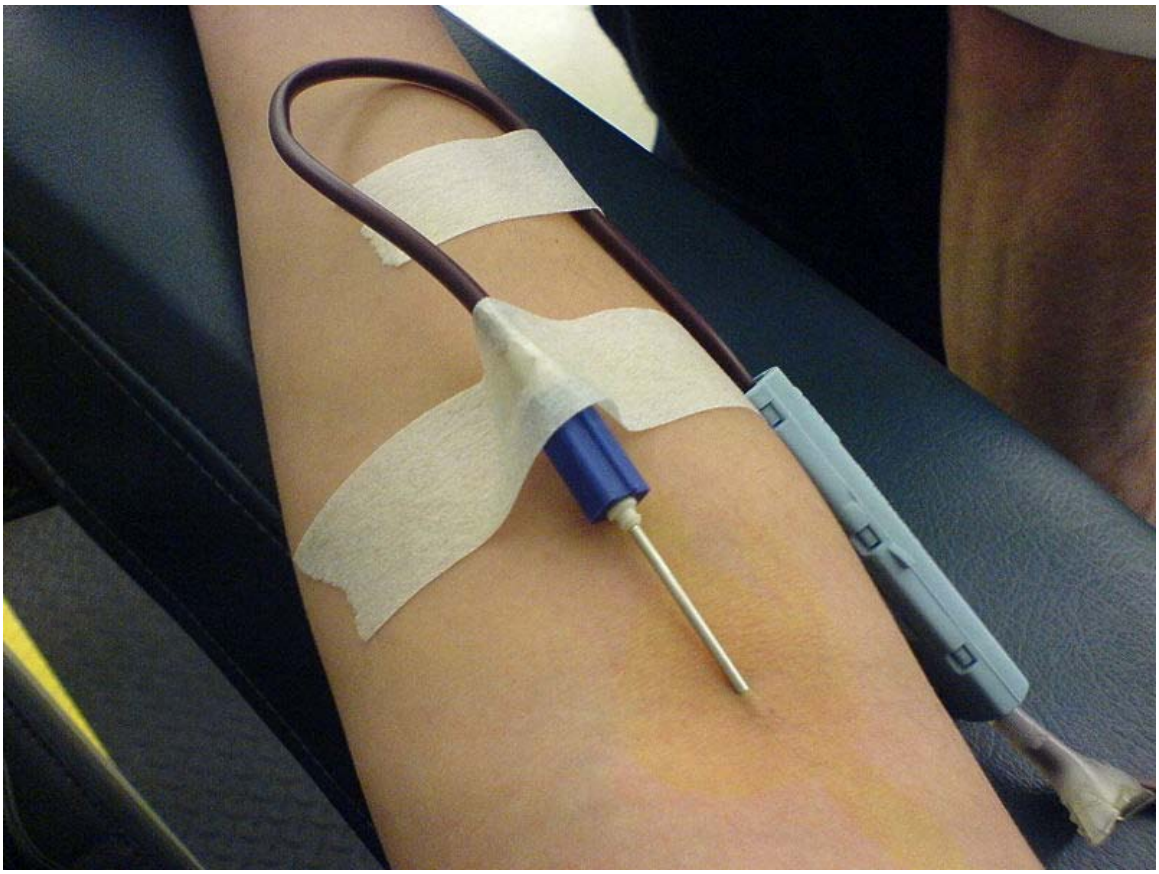
Whole blood

The most common method is collecting the blood from the donor's vein into a container. The amount of blood drawn varies from 200 milliliters to 550 milliliters depending on the country, but 450-500 milliliters is typical. The blood is usually stored in a flexible plastic bag that also contains sodium citrate, phosphate, dextrose, and sometimes adenine. This

combination keeps the blood from clotting and preserves it during storage. Other chemicals are sometimes added during processing.

The plasma from whole blood can be used to make plasma for transfusions or it can also be processed into other medications using a process called fractionation. This was a development of the dried plasma used to treat the wounded during World War II and variants on the process are still used to make a variety of other medications.

Apheresis



A relatively large needle is used for blood donations

Apheresis is a blood donation method where the blood is passed through an apparatus that separates out one particular constituent and returns the remainder to the donor. Usually the component returned is the red blood cells, the portion of the blood that takes the longest to replace. Using this method an individual can donate plasma or platelets much more frequently than they can safely donate whole blood. These can be combined, with a donor giving both plasma and platelets in the same donation.

Platelets can also be separated from whole blood, but they must be pooled from multiple donations. From three to ten units of whole blood are required for a therapeutic dose. Plateletpheresis provides at least one full dose from each donation.

Plasmapheresis is frequently used to collect source plasma that is used for manufacturing into medications much like the plasma from whole blood. Plasma collected at the same time as plateletpheresis is sometimes called *concurrent plasma*.

Apheresis is also used to collect more red blood cells than usual in a single donation and to collect white blood cells for transfusion.

Recovery and time between donations

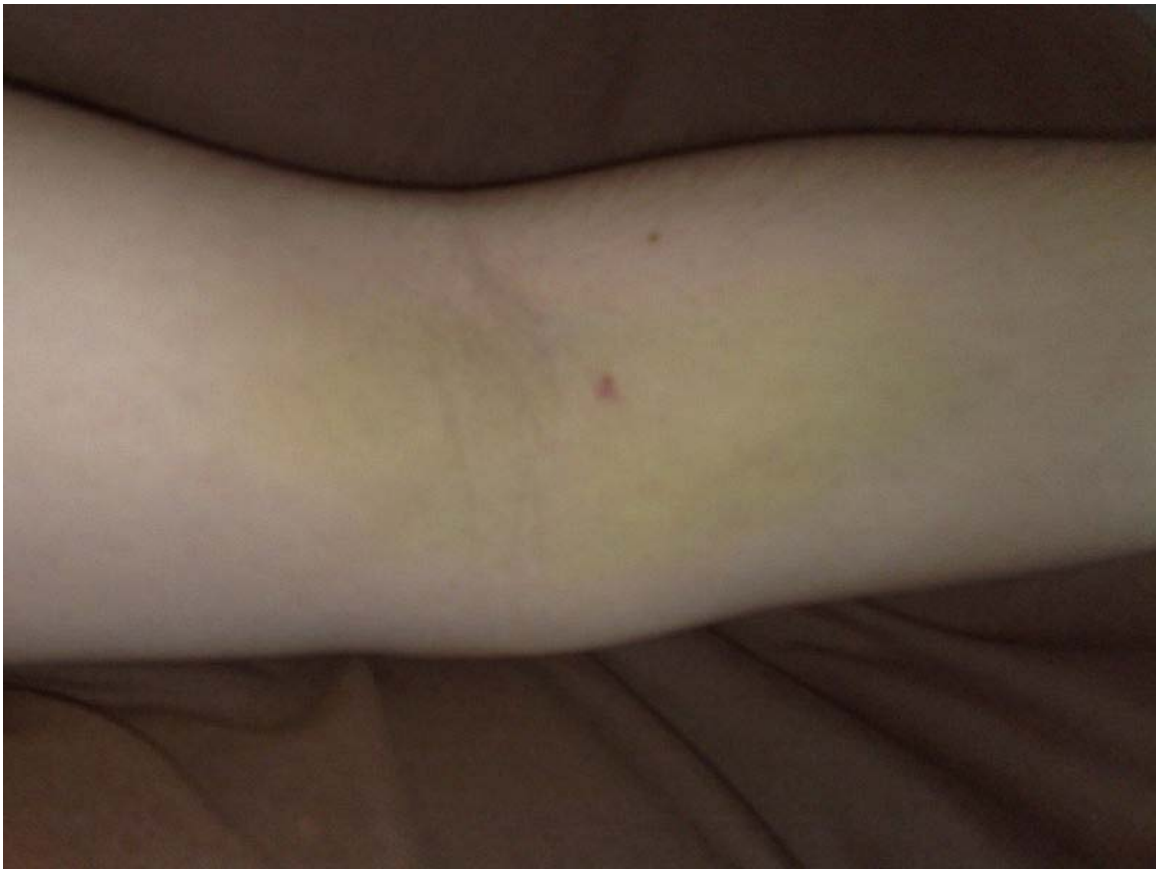
Donors are usually kept at the donation site for 10–15 minutes after donating since most adverse reactions take place during or immediately after the donation. Blood centers typically provide light refreshments or a lunch allowance to help the donor recover. The needle site is covered with a bandage and the donor is directed to keep the bandage on for several hours.

Donated plasma is replaced after 2–3 days. Red blood cells are replaced by bone marrow into the circulatory system at a slower rate, on average 36 days in healthy adult males. In one study, the range was 20 to 59 days for recovery. These replacement rates are the basis of how frequently a donor can give blood.

Plasmapheresis and plateletpheresis donors can give much more frequently because they do not lose significant amounts of red cells. The exact rate of how often a donor can donate differs from country to country. For example, plasmapheresis donors in the United States are allowed to donate large volumes twice a week and could nominally give 83 liters (about 22 gallons) in a year, whereas the same donor in Japan may only donate every other week and could only donate about 16 liters (about 4 gallons) in a year. Red blood cells are the limiting step for whole blood donations, and the frequency of donation varies widely. In Hong Kong it is from three to six months, in Australia it is twelve weeks, in Canada and the United States it is eight weeks and in the UK it is usually sixteen weeks but can be as little as twelve providing donation is not more frequently than three times per year.

Complications

Donors are screened for health problems that would put them at risk for serious complications from donating. First-time donors, teenagers, and women are at a higher risk of a reaction. One study showed that 2% of donors had an adverse reaction to donation. Most of these reactions are minor. A study of 194,000 donations found only one donor with long-term complications. In the United States, a blood bank is required to report any death that might possibly be linked to a blood donation. An analysis of all reports from October 2008 to September 2009 evaluated six events and found that five of the deaths were clearly unrelated to donation, and in the remaining case they found no evidence that the donation was the cause of death.



Bruising three days after donation

Hypovolemic reactions can occur because of a rapid change in blood pressure. Fainting is generally the worst problem encountered.

The process has similar risks to other forms of phlebotomy. Bruising of the arm from the needle insertion is the most common concern. One study found that less than 1% of donors had this problem. A number of less common complications of blood donation are known to occur. These include arterial puncture, delayed bleeding, nerve irritation, nerve injury, tendon injury, thrombophlebitis, and allergic reactions.

Donors sometimes have adverse reactions to the sodium citrate used in apheresis collection procedures to keep the blood from clotting. Since the anticoagulant is returned to the donor along with blood components that are not being collected, it can bind the calcium in the donor's blood and cause hypocalcemia. These reactions tend to cause tingling in the lips, but may cause convulsions or more serious problems. Donors are sometimes given calcium supplements during the donation to prevent these side effects.

In apheresis procedures, the red blood cells are often returned. If this is done manually and the donor receives the blood from a different person, a transfusion reaction can take place. Manual apheresis is extremely rare in the developed world because of this risk and automated procedures are as safe as whole blood donations.

The final risk to blood donors is from equipment that has not been properly sterilized. In most cases, the equipment that comes in direct contact with blood is discarded after use. Re-used equipment was a significant problem in China in the 1990s, and up to 250,000 blood plasma donors may have been exposed to HIV from shared equipment.

Storage, supply and demand

The collected blood is usually stored as separate components, and some of these have short shelf lives. There are no storage solutions to keep platelets for extended periods of time, though some are being studied as of 2008. The longest shelf life used for platelets is seven days. Red blood cells, the most frequently used component, have a shelf life of 35–42 days at refrigerated temperatures. This can be extended by freezing the blood with a mixture of glycerol but this process is expensive, rarely done, and requires an extremely cold freezer for storage. Plasma can be stored frozen for an extended period of time and is typically given an expiration date of one year and maintaining a supply is less of a problem.

The limited storage time means that it is difficult to have a stockpile of blood to prepare for a disaster. The subject was discussed at length after the September 11th attacks in the United States, and the consensus was that collecting during a disaster was impractical and that efforts should be focused on maintaining an adequate supply at all times. Blood centers in the U.S. often have difficulty maintaining even a three day supply for routine transfusion demands.

The World Health Organization recognizes World Blood Donor Day on 14th June each year to promote blood donation. This is the birthday of Karl Landsteiner, the scientist who discovered the ABO blood group system. As of 2008, the WHO estimated that more than 81 million units of blood were being collected annually.

Benefits and incentives

The World Health Organization set a goal in 1997 for all blood donations to come from unpaid volunteer donors, but as of 2006, only 49 of 124 countries surveyed had established this as a standard. Some Plasmapheresis donors in the United States are still paid for donations. A few countries rely on paid donors to maintain an adequate supply. Some countries, such as Tanzania, have made great strides in moving towards this standard, with 20 percent of donors in 2005 being unpaid volunteers and 80 percent in 2007, but 68 of 124 countries surveyed by WHO had made little or no progress. In some countries, for example Brazil, it is illegal to receive any compensation, monetary or otherwise, for the donation of blood or other human tissues.

In patients prone to iron overload, blood donation prevents the accumulation of toxic quantities. Blood banks in the United States may be required to label the blood if it is from a therapeutic donor, so some do not accept donations from donors with any blood disease. Others, such as the Australian Red Cross Blood Service, accept blood from donors with hemochromatosis. It is a genetic disorder that does not affect the safety of the

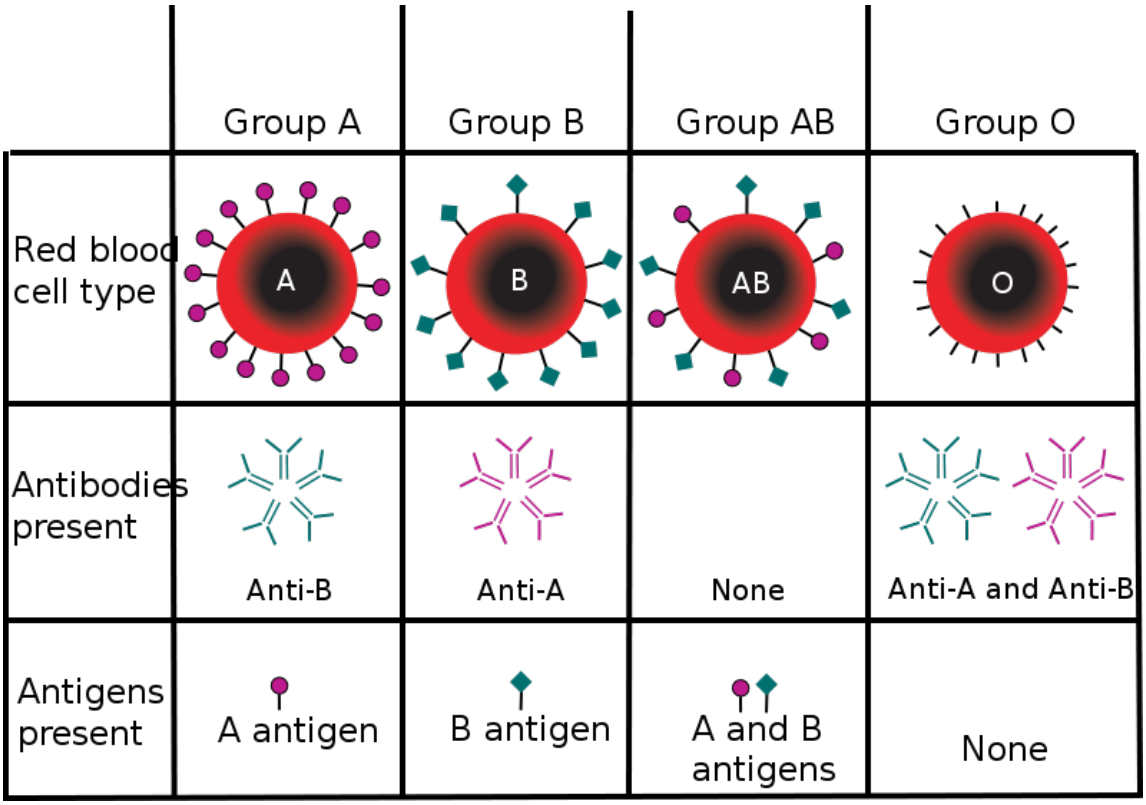
blood. Donating blood may reduce the risk of heart disease for men, but the link has not been firmly established.

In Italy, blood donors receive the donation day as a paid holiday from work. Other incentives are sometimes added by employers, usually time off for the purposes of donating. Blood centers will also sometimes add incentives such as assurances that donors would have priority during shortages, free T-shirts or other small trinkets (e.g., first aid kits, windshield scrapers, pens, etc.), or other programs such as prize drawings for donors and rewards for organizers of successful drives. Most allogeneic blood donors donate as an act of charity and do not expect to receive any direct benefit from the donation.

The sociologist Richard Titmuss, in his 1970 book *The Gift Relationship: From Human Blood to Social Policy*, compared the merits of the commercial and non-commercial blood donation systems of the USA and the UK. The book became a bestseller in the USA, resulting in legislation to regulate the private market in blood. The book is still referenced in modern debates about turning blood into a commodity. The book was republished in 1997 and the same ideas and principles are applied to analogous donation programs, such as organ donation and sperm donation.

Chapter 12

Blood Type



Blood type (or blood group) is determined, in part, by the ABO blood group antigens present on red blood cells.

A **blood type** (also called a **blood group**) is a classification of blood based on the presence or absence of inherited antigenic substances on the surface of red blood cells (RBCs). These antigens may be proteins, carbohydrates, glycoproteins, or glycolipids, depending on the blood group system. Some of these antigens are also present on the surface of other types of cells of various tissues. Several of these red blood cell surface antigens can stem from one allele (or very closely linked genes) and collectively form a blood group system. Blood types are inherited and represent contributions from both parents. A total of 30 human blood group systems are now recognized by the International Society of Blood Transfusion (ISBT).

Many pregnant women carry a fetus with a different blood type from their own, and the mother can form antibodies against fetal RBCs. Sometimes these maternal antibodies are IgG, a small immunoglobulin, which can cross the placenta and cause hemolysis of fetal RBCs, which in turn can lead to hemolytic disease of the newborn, an illness of low fetal blood counts that ranges from mild to severe.

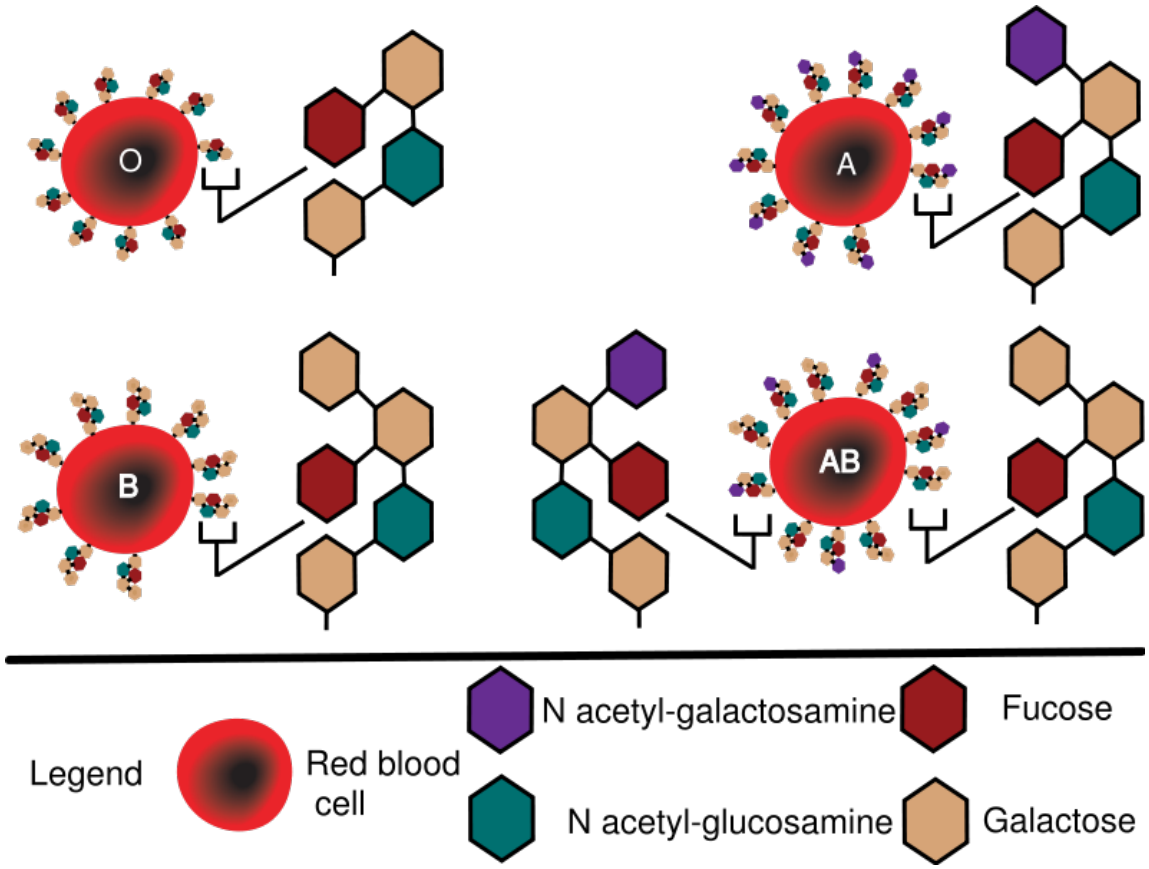
Blood group systems

A complete blood type would describe a full set of 30 substances on the surface of RBCs, and an individual's blood type is one of the many possible combinations of blood-group antigens. Across the 30 blood groups, over 600 different blood-group antigens have been found, but many of these are very rare and/or are mainly found in certain ethnic groups.

Almost always, an individual has the same blood group for life, but very rarely an individual's blood type changes through addition or suppression of an antigen in infection, malignancy, or autoimmune disease. An example of this rare phenomenon is the case of Demi-Lee Brennan, an Australian citizen, whose blood group changed after a liver transplant. Another more common cause in blood-type change is a bone marrow transplant. Bone-marrow transplants are performed for many leukemias and lymphomas, among other diseases. If a person receives bone marrow from someone who is a different ABO type (e.g., a type A patient receives a type O bone marrow), the patient's blood type will eventually convert to the donor's type.

Some blood types are associated with inheritance of other diseases; for example, the Kell antigen is sometimes associated with McLeod syndrome. Certain blood types may affect susceptibility to infections, an example being the resistance to specific malaria species seen in individuals lacking the Duffy antigen. The Duffy antigen, presumably as a result of natural selection, is less common in ethnic groups from areas with a high incidence of malaria.

ABO blood group system



ABO blood group system: diagram showing the carbohydrate chains that determine the ABO blood group

The **ABO system** is the most important blood-group system in human-blood transfusion. The associated anti-A and anti-B antibodies are usually *Immunoglobulin M*, abbreviated IgM, antibodies. ABO IgM antibodies are produced in the first years of life by sensitization to environmental substances such as food, bacteria, and viruses. The *O* in ABO is often called *0* (*zero*, or *null*) in other languages.

Phenotype	Genotype
A	AA or AO
B	BB or BO
AB	AB
O	OO

Rh blood group system

The Rh system is the second most significant blood-group system in human-blood transfusion with currently 50 antigens. The most significant Rh antigen is the D antigen, because it is the most likely to provoke an immune system response of the five main Rh

antigens. It is common for D-negative individuals not to have any anti-D IgG or IgM antibodies, because anti-D antibodies are not usually produced by sensitization against environmental substances. However, D-negative individuals can produce IgG anti-D antibodies following a sensitizing event: possibly a fetomaternal transfusion of blood from a fetus in pregnancy or occasionally a blood transfusion with D positive RBCs. Rh disease can develop in these cases. Rh negative blood types are much less in proportion of Asian populations (0.3%) than they are in White (15%). In the table below, the presence or absence of the Rh antigens is signified by the + or - sign, so that for example A- group does not have any of the Rh antigens.

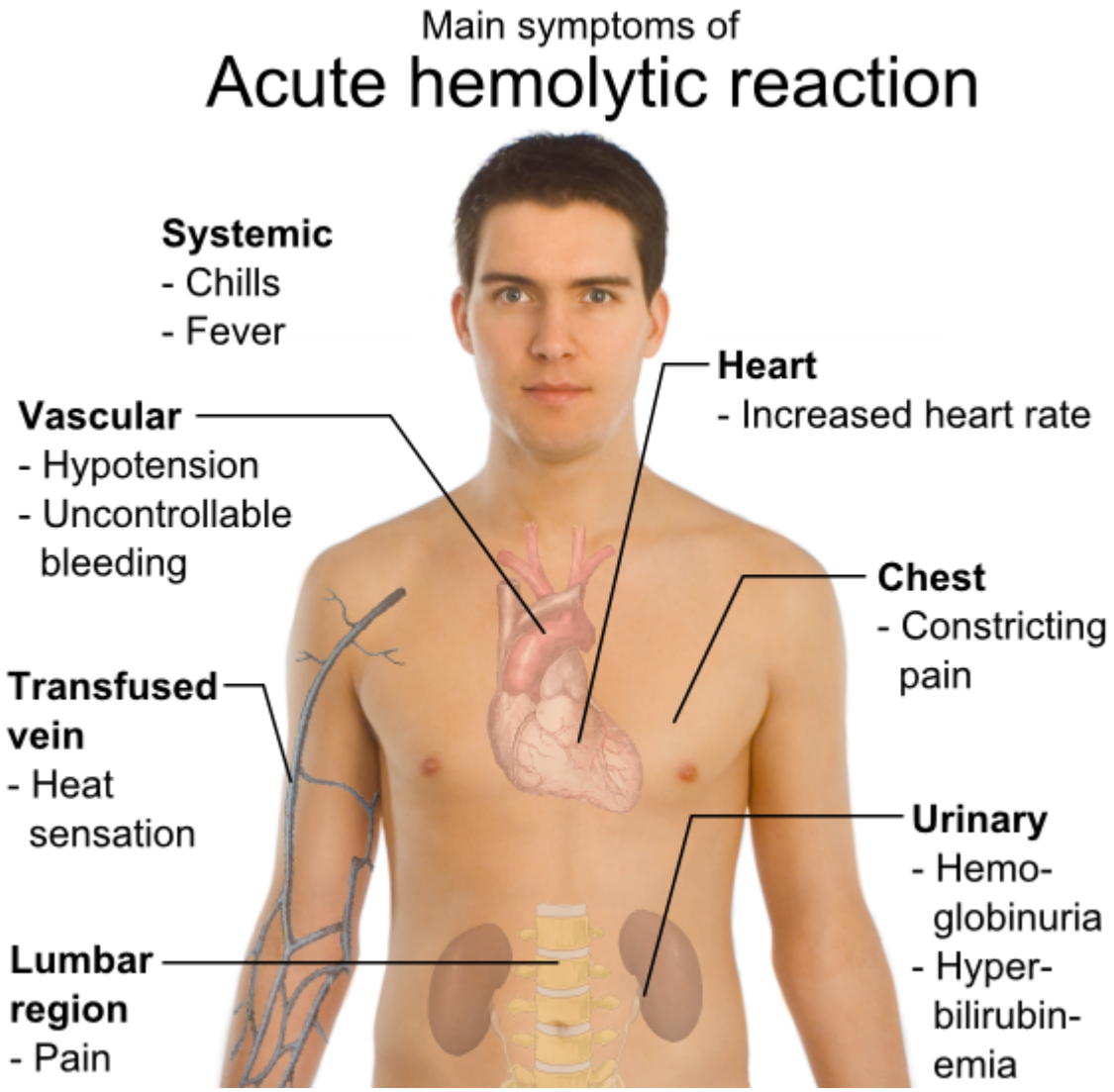
Other blood group systems

The International Society of Blood Transfusion currently recognizes 30 blood-group systems (including the ABO and Rh systems). Thus, in addition to the ABO antigens and Rh antigens, many other antigens are expressed on the RBC surface membrane. For example, an individual can be AB, D positive, and at the same time M and N positive (MNS system), K positive (Kell system), Le^a or Le^b negative (Lewis system), and so on, being positive or negative for each blood group system antigen. Many of the blood group systems were named after the patients in whom the corresponding antibodies were initially encountered.

Clinical significance

Blood transfusion

Transfusion medicine is a specialized branch of hematology that is concerned with the study of blood groups, along with the work of a blood bank to provide a transfusion service for blood and other blood products. Across the world, blood products must be prescribed by a medical doctor (licensed physician or surgeon) in a similar way as medicines.



Main symptoms of acute hemolytic reaction due to blood type mismatch

Much of the routine work of a blood bank involves testing blood from both donors and recipients to ensure that every individual recipient is given blood that is compatible and is as safe as possible. If a unit of incompatible blood is transfused between a donor and recipient, a severe acute hemolytic reaction with hemolysis (RBC destruction), renal failure and shock is likely to occur, and death is a possibility. Antibodies can be highly active and can attack RBCs and bind components of the complement system to cause massive hemolysis of the transfused blood.

Patients should ideally receive their own blood or type-specific blood products to minimize the chance of a transfusion reaction. Risks can be further reduced by cross-matching blood, but this may be skipped when blood is required for an emergency. Cross-matching involves mixing a sample of the recipient's serum with a sample of the donor's red blood cells and checking if the mixture *agglutinates*, or forms clumps. If agglutination is not obvious by direct vision, blood bank technicians usually check for

agglutination with a microscope. If agglutination occurs, that particular donor's blood cannot be transfused to that particular recipient. In a blood bank it is vital that all blood specimens are correctly identified, so labeling has been standardized using a barcode system known as ISBT 128.

The blood group may be included on identification tags or on tattoos worn by military personnel, in case they should need an emergency blood transfusion. Frontline German Waffen-SS had blood group tattoos during World War II.

Rare blood types can cause supply problems for blood banks and hospitals. For example Duffy-negative blood occurs much more frequently in people of African origin, and the rarity of this blood type in the rest of the population can result in a shortage of Duffy-negative blood for patients of African ethnicity. Similarly for RhD negative people, there is a risk associated with travelling to parts of the world where supplies of RhD negative blood are rare, particularly East Asia, where blood services may endeavor to encourage Westerners to donate blood.

Hemolytic disease of the newborn (HDN)

A pregnant woman can make IgG blood group antibodies if her fetus has a blood group antigen that she does not have. This can happen if some of the fetus' blood cells pass into the mother's blood circulation (e.g. a small fetomaternal hemorrhage at the time of childbirth or obstetric intervention), or sometimes after a therapeutic blood transfusion. This can cause Rh disease or other forms of hemolytic disease of the newborn (HDN) in the current pregnancy and/or subsequent pregnancies. If a pregnant woman is known to have anti-D antibodies, the Rh blood type of a fetus can be tested by analysis of fetal DNA in maternal plasma to assess the risk to the fetus of Rh disease. One of the major advances of twentieth century medicine was to prevent this disease by stopping the formation of Anti-D antibodies by D negative mothers with an injectable medication called Rho(D) immune globulin. Antibodies associated with some blood groups can cause severe HDN, others can only cause mild HDN and others are not known to cause HDN.

Blood products

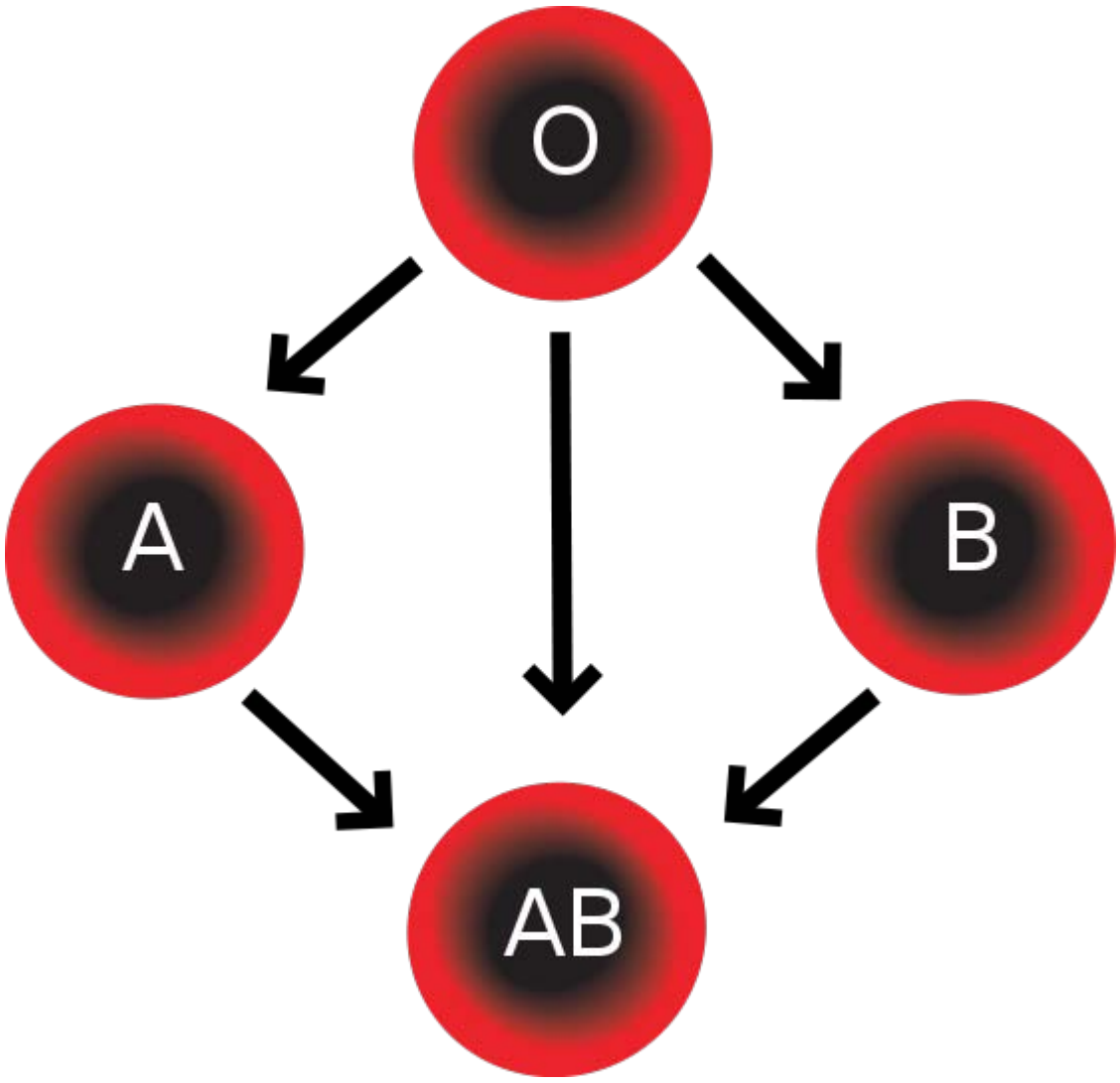
To provide maximum benefit from each blood donation and to extend shelf-life, blood banks fractionate some whole blood into several products. The most common of these products are packed RBCs, plasma, platelets, cryoprecipitate, and fresh frozen plasma (FFP). FFP is quick-frozen to retain the labile clotting factors V and VIII, which are usually administered to patients who have a potentially fatal clotting problem caused by a condition such as advanced liver disease, overdose of anticoagulant, or disseminated intravascular coagulation (DIC).

Units of packed red cells are made by removing as much of the plasma as possible from whole blood units.

Clotting factors synthesized by modern recombinant methods are now in routine clinical use for hemophilia, as the risks of infection transmission that occur with pooled blood products are avoided.

Red blood cell compatibility

- **Blood group AB** individuals have both A and B antigens on the surface of their RBCs, and their blood serum does not contain any antibodies against either A or B antigen. Therefore, an individual with type AB blood can receive blood from any group (with AB being preferable), but can donate blood only to another type AB individual.
- **Blood group A** individuals have the A antigen on the surface of their RBCs, and blood serum containing IgM antibodies against the B antigen. Therefore, a group A individual can receive blood only from individuals of groups A or O (with A being preferable), and can donate blood to individuals with type A or AB.
- **Blood group B** individuals have the B antigen on the surface of their RBCs, and blood serum containing IgM antibodies against the A antigen. Therefore, a group B individual can receive blood only from individuals of groups B or O (with B being preferable), and can donate blood to individuals with type B or AB.
- **Blood group O** (or blood group zero in some countries) individuals do not have either A or B antigens on the surface of their RBCs, but their blood serum contains IgM anti-A and anti-B antibodies against the A and B blood group antigens. Therefore, a group O individual can receive blood only from a group O individual, but can donate blood to individuals of any ABO blood group (i.e., A, B, O or AB). If anyone needs a blood transfusion in an emergency, and if the time taken to process the recipient's blood would cause a detrimental delay, O Negative blood can be issued.



RBC Compatibility chart

In addition to donating to the same blood group; type O blood donors can give to A, B and AB; blood donors of types A and B can give to AB.

Red blood cell compatibility table

Recipient	Donor							
	O−	**O+**	**A−**	**A+**	**B−**	**B+**	**AB−**	**AB+**
O−	✓	✗	✗	✗	✗	✗	✗	✗
O+	✓	✓	✗	✗	✗	✗	✗	✗
A−	✓	✗	✓	✗	✗	✗	✗	✗
A+	✓	✓	✓	✓	✗	✗	✗	✗
B−	✓	✗	✗	✗	✓	✗	✗	✗
B+	✓	✓	✗	✗	✓	✓	✗	✗
AB−	✓	✗	✓	✗	✓	✗	✓	✗

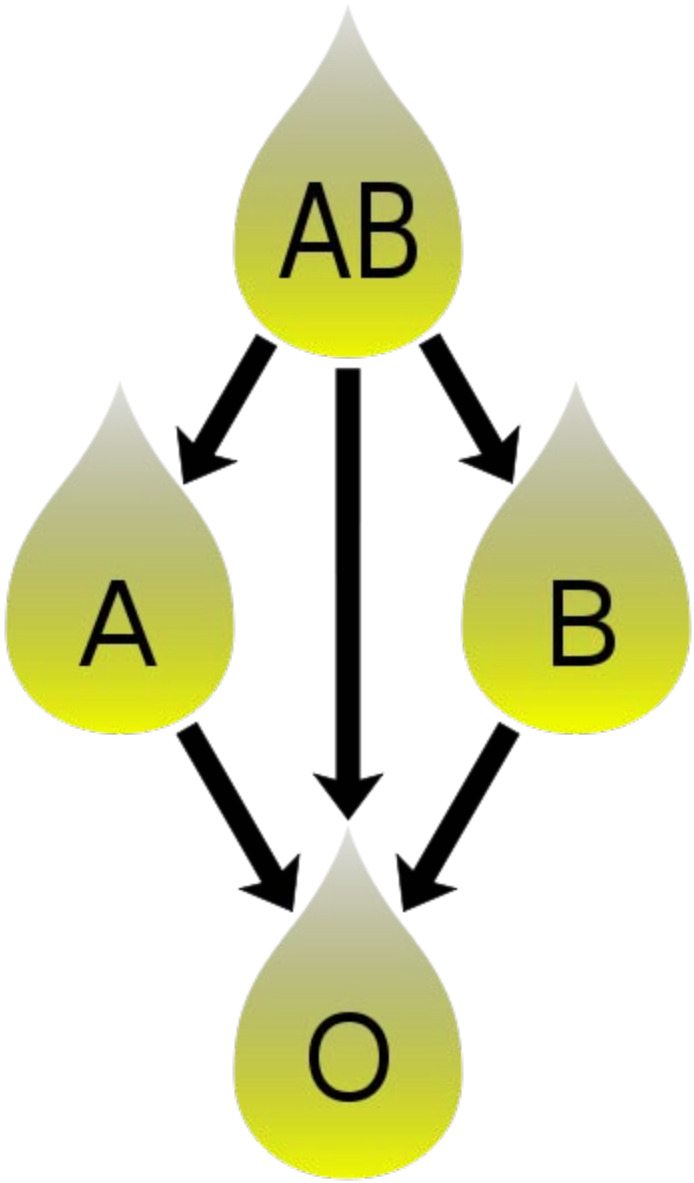
AB+	✓	✓	✓	✓	✓	✓	✓	✓

Table note
1. Assumes absence of atypical antibodies that would cause an incompatibility between donor and recipient blood, as is usual for blood selected by cross matching.

An Rh D-negative patient who does not have any anti-D antibodies (never being previously sensitized to D-positive RBCs) can receive a transfusion of D-positive blood once, but this would cause sensitization to the D antigen, and a female patient would become at risk for hemolytic disease of the newborn. If a D-negative patient has developed anti-D antibodies, a subsequent exposure to D-positive blood would lead to a potentially dangerous transfusion reaction. Rh D-positive blood should never be given to D-negative women of child bearing age or to patients with D antibodies, so blood banks must conserve Rh-negative blood for these patients. In extreme circumstances, such as for a major bleed when stocks of D-negative blood units are very low at the blood bank, D-positive blood might be given to D-negative females above child-bearing age or to Rh-negative males, providing that they did not have anti-D antibodies, to conserve D-negative blood stock in the blood bank. The converse is not true; Rh D-positive patients do not react to D negative blood.

This same matching is done for other antigens of the Rh system as C, c, E and e and for other blood group systems with a known risk for immunization such as the Kell system in particular for females of child-bearing age or patients with known need for many transfusions.

Plasma compatibility



Plasma compatibility chart
In addition to donating to the same blood group; plasma from type AB can be given to A, B and O; plasma from types A, B and AB can be given to O.

Recipients can receive plasma of the same blood group, but otherwise the donor-recipient compatibility for blood plasma is the converse of that of RBCs: plasma extracted from type AB blood can be transfused to individuals of any blood group; individuals of blood group O can receive plasma from any blood group; and type O plasma can be used only by type O recipients.

Plasma compatibility table

Recipient	Donor			
	O	A	B	AB
O	✓	✓	✓	✓
A	✗	✓	✗	✓
B	✗	✗	✓	✓
AB	✗	✗	✗	✓

Table note
1. Assumes absence of strong atypical antibodies in donor plasma

Rh D antibodies are uncommon, so generally neither D negative nor D positive blood contain anti-D antibodies. If a potential donor is found to have anti-D antibodies or any strong atypical blood group antibody by antibody screening in the blood bank, they would not be accepted as a donor (or in some blood banks the blood would be drawn but the product would need to be appropriately labeled); therefore, donor blood plasma issued by a blood bank can be selected to be free of D antibodies and free of other atypical antibodies, and such donor plasma issued from a blood bank would be suitable for a recipient who may be D positive or D negative, as long as blood plasma and the recipient are ABO compatible.

Universal donors and universal recipients

A hospital corpsman with the Blood Donor Team from Portsmouth Naval Hospital takes samples of blood from a donor for testing

With regard to transfusions of whole blood or packed red blood cells, individuals with type O Rh D negative blood are often called universal donors, and those with type AB Rh D positive blood are called universal recipients; however, these terms are only generally true with respect to possible reactions of the recipient's anti-A and anti-B antibodies to transfused red blood cells, and also possible sensitization to Rh D antigens. One exception is individuals with hh antigen system (also known as the Bombay blood group) who can only receive blood safely from other hh donors, because they form antibodies against the H substance.

Blood donors with particularly strong anti-A, anti-B or any atypical blood group antibody are excluded from blood donation. The possible reactions of anti-A and anti-B antibodies present in the transfused blood to the recipients RBCs need not be considered, because a relatively small volume of plasma containing antibodies is transfused.

By way of example: considering the transfusion of O Rh D negative blood (universal donor blood) into a recipient of blood group A Rh D positive, an immune reaction between the recipient's anti-B antibodies and the transfused RBCs is not anticipated. However, the relatively small amount of plasma in the transfused blood contains anti-A antibodies, which could react with the A antigens on the surface of the recipients RBCs, but a significant reaction is unlikely because of the dilution factors. Rh D sensitization is not anticipated.

Additionally, red blood cell surface antigens other than A, B and Rh D, might cause adverse reactions and sensitization, if they can bind to the corresponding antibodies to generate an immune response. Transfusions are further complicated because platelets and white blood cells (WBCs) have their own systems of surface antigens, and sensitization to platelet or WBC antigens can occur as a result of transfusion.

With regard to transfusions of plasma, this situation is reversed. Type O plasma, containing both anti-A and anti-B antibodies, can only be given to O recipients. The antibodies will attack the antigens on any other blood type. Conversely, AB plasma can be given to patients of any ABO blood group due to not containing any anti-A or anti-B antibodies.

Blood group genotyping

In addition to the current practice of serologic testing of blood types, the progress in molecular diagnostics allows the increasing use of blood group genotyping. In contrast to serologic tests reporting a direct blood type phenotype, genotyping allows the prediction of a phenotype based on the knowledge of the molecular basis of the currently known antigens. This allows a more detailed determination of the blood type and therefore a better match for transfusion, which can be crucial in particular for patients with needs for many transfusions to prevent allo-immunization.

Conversion

In April 2007, a method was discovered to convert blood types A, B, and AB to O, using enzymes. This method is still experimental and the resulting blood has yet to undergo human trials. The method specifically removes or converts antigens on the red blood cells, so other antigens and antibodies would remain. This does not help plasma compatibility, but that is a lesser concern since plasma has much more limited clinical utility in transfusion and is much easier to preserve.

History

The two most significant blood group systems were discovered by Karl Landsteiner during early experiments with blood transfusion: the ABO group in 1901 and in co-operation with Alexander S. Wiener the Rhesus group in 1937. Development of the Coombs test in 1945, the advent of transfusion medicine, and the understanding of hemolytic disease of the newborn led to discovery of more blood groups, and now 30 human blood group systems are recognized by the International Society of Blood Transfusion (ISBT), and across the 30 blood groups, over 600 different blood group antigens have been found, many of these are very rare or are mainly found in certain ethnic groups. Blood types have been used in forensic science and were formerly used to demonstrate impossibility of paternity (e.g., a type AB father cannot be the father of a type O infant), but both of these uses are being replaced by genetic fingerprinting, which provides greater certainty.

Chapter 13

Autotransfusion

Autotransfusion is a process when a person receives their own blood for a transfusion, instead of banked donor blood. Blood can be pre-donated before a surgery, or can be collected during and after the surgery using a device commonly known as the Cell Saver. The Cell Saver is utilized in surgeries where there is expected a large volume blood loss. For example, aneurysm, total joint replacements and spinal surgeries. There are also small reservoir devices designed to collect the patients blood for return, such as the Orthopat and Constavacs.

The first documented use of the procedure was in 1818, and interest in the practice continued until the Second World War, at which point blood supply became less of an issue, due to the increased number of blood donors. Later, interest in the procedure returned with new automated machinery being developed for it. Autotransfusion is used in a number of orthopedic, trauma and cardiac cases, amongst others, and it carries advantages, including the reduction of infection risk and the provision of more functional cells.

History

There is some evidence that in 1785 that Philip Physic of Philadelphia transfused a post-partum patient. However the first documented use of autologous blood transfusion was in 1818 when an Englishman, Blundell salvaged vaginal blood from patients with postpartum hemorrhage. By swabbing the blood from the bleeding site and rinsing the swabs with saline, he found that he could re-infuse the result of the washings. This unsophisticated method resulted in a 75% mortality rate, but it marked the start of autologous blood transfusion.

During the American Civil War Union Army physicians are said to have administered four transfusions. In 1886, J. Duncan used autotransfusion during the amputation of limbs by removing blood from the amputated limb and returning it to the patient by femoral injection. This method was apparently fairly successful. A German, M.J. Theis, reported the first successful use of intraoperative autotransfusion in 1914, with a ruptured ectopic pregnancy. The earliest report in the American literature on the use of autotransfusion was by Lockwood in 1917 who used the technique during a splenectomy for Banti syndrome. Interest in unrefined technique of autotransfusion continued through to the early 1940s, and was applied to various procedures including treatment of ectopic

pregnancy hemothorax, ruptured spleen, perforating abdominal injuries, and neurosurgical procedures.

The interest in autotransfusion dwindled during World War Two, when there was a large pool of donors. After the war, blood testing, typing and crossmatching techniques were improved making blood banks the answer to the increased demand for blood. In the 1960s, interest in autotransfusion revived. With the advances in all fields of surgery, new companies developed autotransfusion devices. Problems still arose, however, with air embolism, coagulopathy and hemolysis. The devices used during the Korean and Vietnam War collected and provided gross filtration of blood before it was reinfused. With the introduction of cardiopulmonary bypass in 1952, autotransfusion became an area of study. Klebanoff began a new era of autotransfusion by developing the first commercially available autotransfusion unit in 1968. His system, the Bentley Autotransfusion System aspirated, collected, filtered and reinfused autologous whole blood shed from the operative field. The problems with the Bentley system included the requirement of systemic anticoagulation of the patient, introduction of air embolism, and renal failure resulting from unfiltered particulate in the reinfused blood.

As the Bentley system lost favor Wilson and associates proposed the use of a discontinuous flow centrifuge process for autotransfusion which would wash the red cells with normal saline solution. In 1976, this system was introduced by Haemonetics Corporation and is known commonly as "Cell Saver". More recently in 1995 Fresenius introduced a continuous autotransfusion system.

There are three types of systems: un-washed filtered blood; discontinuous flow centrifugal; and continuous flow centrifugal. The unwashed systems are popular because of their perceived inexpense and simplicity. However unwashed systems can cause increase potential for clinical complications. The washed system requires a properly trained and clinically skilled operator. It returns only red blood cells suspended in saline and is rarely associated with any clinical complications. The use of Autotransfusion process described in this documented reflects the washed discontinuous centrifugal system. This type of autotransfusion can practically eliminate the need for exposure to homologous blood in elective surgical patients and can greatly reduce the risk of exposure to emergency surgical patients. Autotransfusion represents a measure of blood conservation and reduction of exposure risk to homologous blood.

Literature

Literature relating to autologous blood is extensive - numerous articles and letters have been written. The subject heading of "Blood Transfusion, Autologous" first appeared in 1971 in the Cumulated Index Medicus. The Cumulative Index to Nursing & Allied Health began the same title in 1983, and also added a new heading of "Blood Salvage" in 1993. The Hospital Literature Index began the heading "Blood Transfusion, Autologous" in 1985.

The concepts of bloodless medicine and surgery have recently been emerging in literature, books and course programs.

Indications for autotransfusion

Autotransfusion is intended for use in situations characterized by the loss of one or more units of blood and may be particularly advantageous for use in cases involving rare blood groups, risk of infectious disease transmission, restricted homologous blood supply or other medical situations for which the use of homologous blood is contraindicated. Autotransfusion is commonly used intraoperatively and postoperatively. Intraoperative autotransfusion refers to recovery of blood lost during surgery or the concentration of fluid in an extracorporeal circuit. Postoperative autotransfusion refers to the recovery of blood in the extracorporeal circuit at the end of surgery or from aspirated drainage.

Common cases

Orthopedic

Spinal Instrumentation, Spinal Fusion, Discectomy, Laminectomy, Total shoulder replacement, Total hip replacement, Total knee replacement, Femur Fractures, Open Reduction Internal Fixation Pelvic Fractures, IM Rodding

Trauma

Subdural Hematoma, Chest Injuries, Liver Fractures, Kidney Fractures, Major Vessel Lacerations, Aneurysms, Gun Shot Wounds, Stab Wounds, Extremity Reimplantations, Splenectomy, Blunt Trauma (Thoracic or Abdominal)

Cardiac

Cardiothoracic, Coronary Artery Bypass, Cardiac Valvular Repair / Replacement, Aortic Arch Aneurysms, Thoracic Trauma, Cardiac Transplantation

Other

Ectopic Pregnancy, Liver Resection (Non-Malignant), Porto-Caval Shunts, Liver Transplant, Nephrectomy (Non-Malignant), Speno-Renal Shunts, Abdominal Aortic Aneurysm, Aorto-Femoral Reconstruction, Major Vessel Resection, Hysterectomy (Non-Malignant), Cerebral Aneurysms, Craniotomy (Non-Malignant), Thoracotomy (Non-Malignant)

Advantages

- High levels of 2,3 DPG
- Normothermic
- pH relatively normal

- Lower risk of Infectious Diseases
- Functionally superior cells
- Lower Potassium (compared to stored blood)
- Quickly available

Substances washed out

- Plasma
- Platelets
- White Cells
- Anticoagulant Solution
- Plasma free Hemoglobin
- Cellular stroma
- Activated clotting factors
- Intracellular Enzymes
- Potassium
- Plasma bound Antibiotics

Disadvantages

The disadvantage of autotransfusion is the depletion of plasma and platelets. The washed autotransfusion system removes the plasma and platelets, to eliminate activated clotting factors and activated platelets which would cause coagulopathy if they were reinfused to the patient. This disadvantage is only evident when very large blood losses occur. The autotransfusionist monitors blood loss and will recommend the transfusion of fresh frozen plasma (FFP) and platelets when the blood loss and return of autotransfusion blood increases. Typically the patient will require FFP and platelets as the estimated blood loss exceeds half of the patient's blood volume. When possible diagnostic tests should be performed to determine the need for any blood products (i.e. PRBCs, FFP and Platelets).

Contraindications

The use of blood recovered from the operative field is contraindicated in the presence of bacterial contamination or malignancy. The use of autotransfusion in the presence of such contamination may result in the dissemination of pathologic microorganisms and / or malignant cells. The following statements reflect current clinical concerns involving autotransfusion contraindications.

Contamination of the surgical site

Any abdominal procedure poses the risk of enteric contamination of shed blood. The surgical team must be diligent in observing for signs of bowel contamination of the blood. If there is a question of possible contamination the blood may be held until the surgeon determines whether or not bowel contents are in the surgical field. If the blood is contaminated the entire contents should be discarded. If the patient's life depends upon

this blood supply it may be reinfused with the surgeon's consent, while washing with large amounts of NaCl .9% will reduce the bacterial contamination of the blood, it will not be totally eliminated.

Malignancy

There is a possibility of the reinfusion of cancer cells from the surgical site. There are possible exceptions to this contraindication:

- The surgeon feels complete removal of an encapsulated tumor is possible. Blood may be aspirated from the surgical site, processed and reinfused with the surgeon's consent.
- If an inadequate supply of blood exists, the washed red cells may be used to support the patient's vital signs with the surgeon's consent.

The use of Leukocyte reduction filters is recommended.

Obstetrics

Autotransfusion is not normally used in C-Sections, because the possibility of an amniotic fluid embolism exists. Emerging literature suggests that amniotic fluid is being cleared during the wash cycle. It is possible that the utilization of autotransfusion in obstetrics may increase as more research is completed. However, if a patient is at risk for blood loss and is a Jehovah's witness, for example, the cell saver can be used with strict guidelines of irrigating profusely to remove amniotic fluid and then suctioning the blood that is being lost.

Emergency

In life saving situations with the consent of the surgeon, autotransfusion can be utilized in the presence of the previous stated contraindications i.e. sepsis, bowel contamination and malignancy.

Collection and processing of blood

Utilizing a special double lumen suction tubing, fluid is aspirated from the operative field, and is mixed with an anticoagulant solution. Collected fluid is filtered in a sterile cardiotomy reservoir. The reservoir contains filter and has a capacity of between two and three liters of fluid. When a volume adequate to fill the wash bowl has been collected, processing may begin. The volume required to fill the bowl is dependent on the concentration of red cells collected (hematocrit) and size of the centrifuge wash bowl. But if the patients HCT (hematocrit) is normal, the amount needed to process a unit is roughly two times the bowl volume. Therefore, if the centrifuge processing bowl size is 250 ml, you will need roughly 500 ml of blood.

When aspirating the blood it is important to utilize the following technique whenever possible:

- Suction blood from pools rather than skimming.
- Keep the suction tip below the level of the air-blood interface.
- Avoid occluding the suction tip (i.e. using suction as a retractor).

Following these techniques will help reduce hemolysis of the red cells and will help increase the amount of red cells that will be salvaged.

Special considerations

Antibiotic irrigation

Antibiotics which are plasma bound can be removed during the autotransfusion wash cycle, however topical antibiotics which are typically not plasma bound may not be washed out during autotransfusion, and may actually become concentrated to the point of being nephrotoxic.

Topical coagulant products

When Avitene, Hemopad, Instat, or collagen type products are used, autotransfusion should be interrupted and a waste or wall suction source must be used. Autotransfusion can be resumed once these products are flushed from the surgical site. If Gelfoam, Surgicel, Thrombogen or Thrombostat are used, autotransfusion can continue, however direct suctioning of these products should be avoided. The clinically experienced autotransfusionist is familiar with the commonly used products and will know whether the autotransfusion must be interrupted.

Orthopaedic bone cement

Cement is often used or encountered during primary or revision total joint replacement surgery. The cement when in the liquid or soft state should not be introduced into the autotransfusion system. When cement is being applied a waste or wall suction source must be used, however when the cement hardens autotransfusion may be resumed. The use of ultrasonic equipment during revision of total joints changes the cement to a liquid or soft state, which precludes the use of autotransfusion during the use of such equipment. Autotransfusion can only continue when the cement has hardened.

Processing

Prime phase

When the "prime" button is pressed, the centrifuge begins rotation and accelerates to the speed selected on the centrifuge speed control, typically 5,600 rpm. Simultaneously, the pump begins counterclockwise rotation, enabling the transfer of the reservoir contents to

the wash bowl. The application of centrifugal force separates the components of the fluid according to their weight. The wash bowl filling continues until the buffy-coat reaches the shoulder of the wash bowl. The buffy-coat is the accumulation of platelets and white cells which appears during centrifugation as an interface between the red cells and supernatant. Some autotransfusion devices have automatic features including a buffy-coat sensor, which is calibrated to detect a full bowl and advance the process to the wash phase automatically.

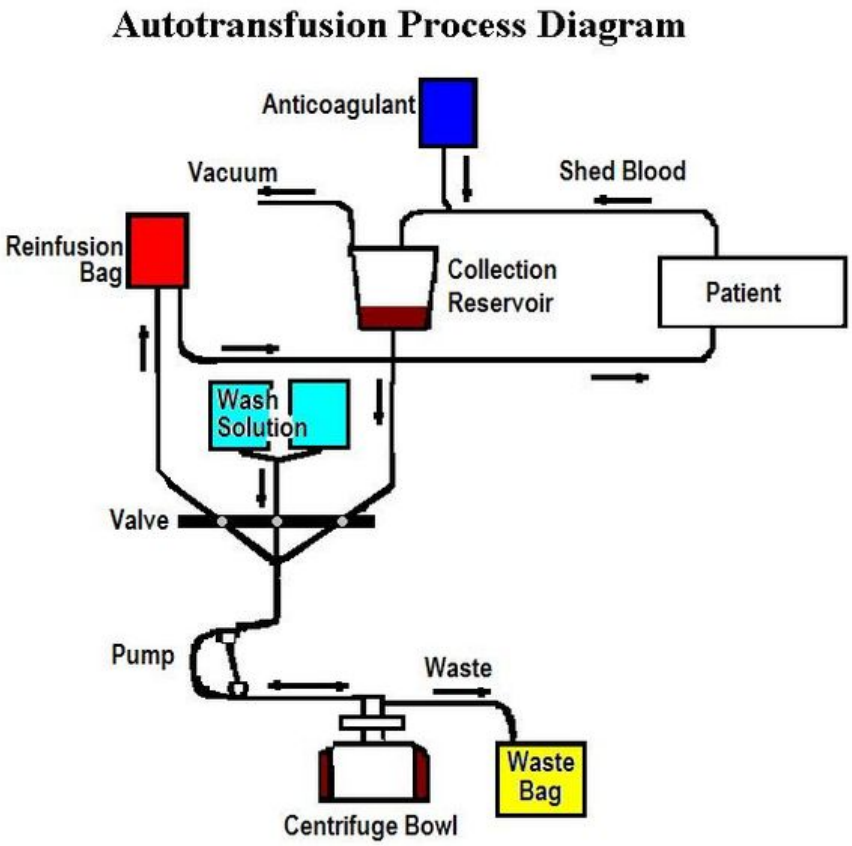
Wash phase

The wash phase begins when the wash bowl is appropriately filled with red cells. The autotransfusion devices with automatic features, when operated in the automatic mode will advance to the wash phase without action from the autotransfusionist. The pump continues a counterclockwise rotation and clamps adjust, enabling the transfer of wash solution to the wash bowl. The washing phase removes cellular stromata, plasma free hemoglobin, anticoagulant solution, activated clotting factors, any plasma bound antibiotics, intracellular enzymes, plasma, platelets, and white cells. The unwanted fluid passes out of the wash bowl and into a waste reservoir bag. Washing continues until the reinfuse button is depressed and the appropriate amount of wash solution has been delivered to the wash bowl. The wash phase is terminated when one to two liters of wash solution has been transferred, or the fluid transferred to the waste bag appears transparent (or both). An autotransfusion device with automatic features will pump the wash solution which was set by the autotransfusionist and then automatically proceed to the reinfuse phase.

Empty phase

When the Empty function is selected, the centrifuge braking begins. The clamps change positions, enabling the transfer of (emptying) the wash bowl contents to the reinfusion bag. An autotransfusion device with automatic features will pump the blood into the reinfusion bag and stop when the bowl is emptied. The centrifuge bowl must come to a complete stop before the pump begins a clockwise rotation to empty the bowl. Once the bowl is emptied, the "stop" button can be pressed to complete the cycle, or the "prime" button can be pressed to start a new cycle. The reinfusion bag attached to the autotransfusion wash set should not be used for high pressure infusion back to the patient. The reinfusion bag contains a significant amount of air, careful monitoring should take place during reinfusion to avoid the potential of air embolism. Therefore, it is recommended to use a separate blood bag attached to the reinfusion bag. This second bag can then be disconnected, air purged from it, and then tied off before giving to anesthesia for reinfusion. Thus reducing the chances of an air embolism. In accordance with Guidelines set by the American Association of Blood Banks the blood should be reinfused within 4 hours from washing.

Process Diagram



Postoperative autotransfusion

Postoperative autotransfusion is performed by connecting the double lumen autotransfusion suction line directly to the drain line placed at the conclusion of surgery. Postoperative autotransfusion begins in the operating room when the drain line is placed and the surgical site is closed. Typical postoperative cases are total knee and hip replacements. Autotransfusion is continued and is effective while the patient actively bleeds during the immediate postoperative phase of recovery. Autotransfusion is ended when bleeding is stopped or is significantly slow, autotransfusion is discontinued by connecting an ordinary self draining device such as a Hemovac container to the drain line(s). Available for postoperative autotransfusion are universal bifurcated connectors which can accommodate two drain lines of any size, these connectors can be attached to the standard ten foot double lumen suction line for postoperative use.

Soaking sponges

In some institutions to maximize the effectiveness of autotransfusion and provide the best conservation and return of red cells the soaking of sponges is employed. During the surgical procedure the blood soaked sponges are collected and placed in a sterile basin by

the surgical team, sterile heparinized saline is add to the basin to prevent clotting and facilitate the release of red cells. The sponges are periodically wrung out and removed from the basin, the remaining solution can be suction into the autotransfusion reservoir so that the red cells can be recovered.

The ratio of heparinized saline is 5,000 units of Heparin per 1,000 ml of 0.9% Sodium Chloride. The heparin is removed during the autotransfusion process.

Autotransfusion and religion

Individuals of the Jehovah's Witness religion in particular refuse to accept Homologous and Autologous pre-donated blood. However some individual members may accept the use of autotransfusion by means of the Cell Saver. The process of autotransfusion using the Cell Saver is modified to maintain a continuous circuit of blood which maintains continuous contact with the body. This process when carefully explained to the patient may be acceptable when a patient refuses based on religious beliefs.

Platelet sequestration and autologous platelet gel

Many of the newest autotransfusion machines are programmable to provide separation of blood into three groups; Red Cells, Platelet Poor Plasma, and Platelet Rich Plasma. Blood can be drawn from the patient just prior to surgery and then separated. The separated blood components which have been sequestered can be stored during the surgical procedure. The Red Cells and Platelet Poor Plasma can be given back to the patient through Intravenous transfusion during or after surgery. The Platelet Rich Plasma can be mixed with Calcium and Thrombin to create a product known as autologous platelet gel (APG). This is an Autologous product which can be used for a variety of techniques including use as a hemostatic aid, a dural sealant and an aid to fusion of bone. Its applications are being widely studied and reported in the literature on a regular basis recently.

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; she was accused as well. 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 Bram Stoker's novel "Dracula", published in 1897, various incidences of blood transfusion were deliberated upon.

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.

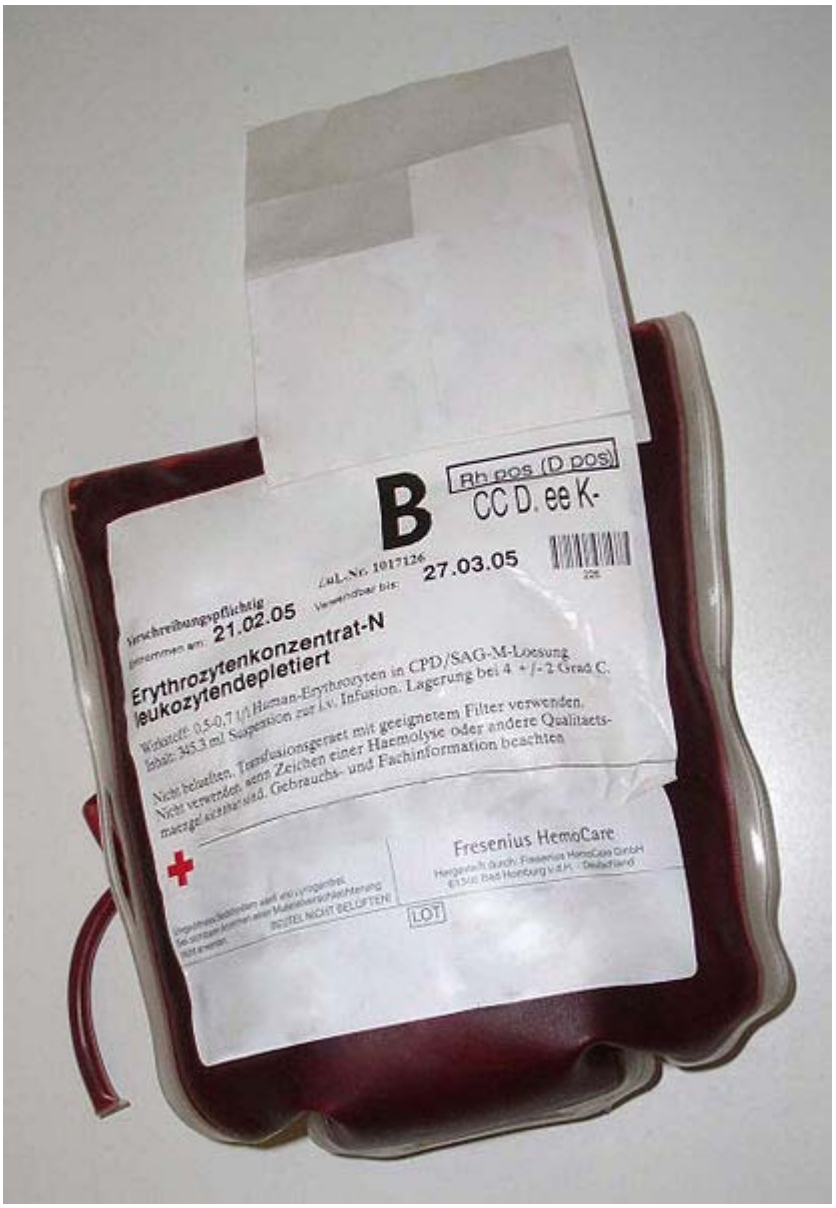
The modern era

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

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.

Chapter 15

Coombs Test

Coombs test (also known as **Coombs' test**, **antiglobulin test** or **AGT**) refers to two clinical blood tests used in immunohematology and immunology. The two Coombs tests are the **direct Coombs test** (also known as **direct antiglobulin test** or **DAT**), and the **indirect Coombs test** (also known as **indirect antiglobulin test** or **IAT**).

The more commonly used test, the Direct Coombs test, is used to test for autoimmune hemolytic anemia.

In certain diseases or conditions an individual's blood may contain IgG antibodies that can specifically bind to antigens on the red blood cell (RBC) surface membrane, and their circulating red blood cells (RBCs) can become coated with IgG alloantibodies and/or IgG autoantibodies. Complement proteins may subsequently bind to the bound antibodies. The **direct Coombs test** is used to detect these antibodies or complement proteins that are bound to the surface of red blood cells; a blood sample is taken and the RBCs are washed (removing the patient's own plasma) and then incubated with antihuman globulin (also known as "Coombs reagent"). If this produces agglutination of RBCs, the direct Coombs test is positive, a visual indication that antibodies (and/or complement proteins) are bound to the surface of red blood cells.

The **indirect Coombs test** is used in prenatal testing of pregnant women, and in testing blood prior to a blood transfusion. It detects antibodies against RBCs that are present unbound in the patient's serum. In this case, serum is extracted from the blood, and the serum is incubated with RBCs of known antigenicity. If agglutination occurs, the indirect Coombs test is positive.

Mechanism

The two Coombs tests are based on the fact that anti-human antibodies, which are produced by immunizing non-human species with human serum, will bind to human antibodies, commonly IgG or IgM. Animal anti-human antibodies will also bind to human antibodies that may be fixed onto antigens on the surface of red blood cells (also referred to as RBCs), and in the appropriate test tube conditions this can lead to agglutination of RBCs. The phenomenon of agglutination of RBCs is important here, because the resulting clumping of RBCs can be visualised; when clumping is seen the test is positive and when clumping is not seen the test is negative.

Common clinical uses of the Coombs test include the preparation of blood for transfusion in cross-matching, screening for atypical antibodies in the blood plasma of pregnant women as part of antenatal care, and detection of antibodies for the diagnosis of immune-mediated haemolytic anemias.

Coombs tests are done on serum from venous blood samples which are taken from patients by venepuncture. The venous blood is taken to a laboratory (or blood bank), where trained scientific technical staff do the Coombs tests. The clinical significance of the result is assessed by the physician who requested the Coombs test, perhaps with assistance from a laboratory-based hematologist.

Direct Coombs test

The direct Coombs test (also known as the **direct antiglobulin test** or DAT) is used to detect if antibodies or complement system factors have bound to RBC surface antigens *in vivo*. The DAT is not currently required for pre-transfusion testing but may be included by some laboratories.

Examples of diseases that give a positive direct Coombs test

The direct Coombs test is used clinically when immune-mediated hemolytic anemia (antibody-mediated destruction of RBCs) is suspected. A positive Coombs test indicates that an immune mechanism is attacking the patient's own RBC's. This mechanism could be autoimmunity, alloimmunity or a drug-induced immune-mediated mechanism.

Examples of alloimmune hemolysis

- Hemolytic disease of the newborn (also known as HDN or erythroblastosis fetalis)
    - Rh D hemolytic disease of the newborn (also known as Rh disease)
    - ABO hemolytic disease of the newborn (the indirect Coombs test may only be weakly positive)
    - Anti-Kell hemolytic disease of the newborn
    - Rh c hemolytic disease of the newborn
    - Rh E hemolytic disease of the newborn
    - Other blood group incompatibility (RhC, Rhe, Kidd, Duffy, MN, P and others)
- Alloimmune hemolytic transfusion reactions

Examples of autoimmune hemolysis

- Warm antibody autoimmune hemolytic anemia
    - Idiopathic
    - Systemic lupus erythematosus
    - Evans' syndrome (antiplatelet antibodies and hemolytic antibodies)

- Cold antibody autoimmune hemolytic anemia
    - Idiopathic cold hemagglutinin syndrome
    - Infectious mononucleosis
    - Paroxysmal cold hemoglobinuria (rare)

Drug-induced immune-mediated hemolysis

- Methyldopa (IgG mediated type II hypersensitivity)
- Penicillin (high dose)
- Quinidine (IgM mediated activation of classical complement pathway and Membrane attack complex, MAC)

(A memory device to remember that the *DAT* tests the RBCs and is used to test infants for *haemolytic disease of the newborn* is: ***R**h **D**isease*; ***R*** = RBCs, ***D*** = DAT.)

Laboratory method

The patient's red blood cells (RBCs) are washed (removing the patient's own serum) and then incubated with antihuman globulin (also known as Coombs reagent). If immunoglobulin or complement factors have been fixed on to the RBC surface in-vivo, the antihuman globulin will agglutinate the RBCs and the direct Coombs test will be positive. (A visual representation of a positive direct Coombs test is shown in the upper half of the schematic).

Indirect Coombs test

The indirect Coombs test (also known as the **indirect antiglobulin test** or IAT) is used to detect in-vitro antibody-antigen reactions. It is used to detect very low concentrations of antibodies present in a patient's plasma/serum prior to a blood transfusion. In antenatal care, the IAT is used to screen pregnant women for antibodies that may cause hemolytic disease of the newborn. The IAT can also be used for compatibility testing, antibody identification, RBC phenotyping, and titration studies.

Examples of clinical uses of the indirect Coombs test

Blood transfusion preparation

The indirect Coombs test is used to screen for antibodies in the preparation of blood for blood transfusion. The donor's and recipient's blood must be ABO and Rh D compatible. Donor blood for transfusion is also screened for infections in separate processes.

- Antibody screening

A blood sample from the recipient and a blood sample from every unit of donor blood are screened for antibodies with the indirect Coombs test. Each sample is incubated against a

wide range of RBCs that together exhibit a full range of surface antigens (i.e. blood types).

- Cross matching

The indirect Coombs test is used to test a sample of the recipient's serum against a sample of the blood donor's RBCs. This is sometimes called cross-matching blood.

Antenatal antibody screening

The indirect Coombs test is used to screen pregnant women for IgG antibodies that are likely to pass through the placenta into the fetal blood and cause haemolytic disease of the newborn.

Laboratory method

The IAT is a two-stage test. (A cross match is shown visually in the lower half of the schematic as an example of an indirect Coombs test).

First stage

Washed test red blood cells (RBCs) are incubated with a test serum. If the serum contains antibodies to antigens on the RBC surface, the antibodies will bind onto the surface of the RBCs.

Second stage

The RBCs are washed three or four times with isotonic saline and then incubated with antihuman globulin. If antibodies have bound to RBC surface antigens in the first stage, RBCs will agglutinate when incubated with the antihuman globulin (also known Coombs reagent) in this stage, and the indirect Coombs test will be positive.

Titrations

By diluting a serum containing antibodies the quantity of the antibody in the serum can be gauged. This is done by using doubling dilutions of the serum and finding the maximum dilution of test serum that is able to produce agglutination of relevant RBCs.

Coombs reagent

Coombs reagent (also known as **Coombs antiglobulin** or **antihuman globulin**) is used in both the direct Coombs test and the indirect Coombs test. Coombs reagent is antihuman globulin. It is made by injecting human globulin into animals, which produce polyclonal antibodies specific for human immunoglobulins and human complement system factors. More specific Coombs reagents or monoclonal antibodies can be used.

Enhancement media

Both IgM and IgG antibodies bind strongly with their antigens. IgG antibodies are most reactive at 37°C. IgM antibodies are easily detected in saline at room temperature as IgM antibodies are able to bridge between RBC's owing to their large size, efficiently creating what is seen as agglutination. IgG antibodies are smaller and require assistance to bridge well enough to form a visual agglutination reaction. Reagents used to enhance IgG detection are referred to as potentiators. RBCs have a net negative charge called zeta potential which causes them to have a natural repulsion for one another. Potentiators reduce the zeta potential of RBC membranes. Common potentiators include low ionic strength solution (LISS), albumin, polyethylene glycol (PEG), and proteolytic enzymes.

History of the Coombs test

The Coombs test was first described in 1945 by Cambridge immunologists Robin Coombs (after whom it is named), Arthur Mourant and Rob Race. Historically, it was done in test tubes. Today, it is commonly done using microarray and gel technology.

Chapter 16

Intraoperative Blood Salvage and Leukapheresis

Intraoperative blood salvage

Intraoperative blood salvage, also known as **autologous blood salvage**, is a medical procedure involving recovering blood lost during surgery and re-infusing it into the patient.

It has been used for many years and gained greater attention over time as risks associated with allogenic blood transfusion have seen greater publicity and more fully appreciated. Several medical devices have been developed to assist in salvaging the patient's own blood in the perioperative setting. These are used frequently in cardiothoracic and vascular surgery, in which blood usage has traditionally been high. With a greater effort to avoid adverse events due to transfusion there has also been an emphasis on blood conservation.

Background

Providing safe blood for transfusion remains a challenge despite advances in preventing transmission of hepatitis B, hepatitis C, AIDS/HIV, West Nile virus(WNV), and transfusion-transmitted bacterial infection. Human errors such as misidentifying patients and drawing blood samples from the wrong person present much more of a risk than transmissible diseases.

Additional risks include transfusion related acute lung injury (TRALI), a potentially life-threatening condition with symptoms such as dyspnea, fever, and hypotension occurring within hours of transfusion, and also transfusion-associated immunomodulation, which may suppress the immune response and cause adverse effects such a small increase in the risk of postoperative infection.

Other risks such as variant Creutzfeldt-Jakob disease (vCJD), an invariably fatal disease, remain worrisome. Blood centers worldwide have instituted criteria to reject donors who may have been exposed to vCJD. Screening for transmissible diseases and deferral policies for vCJD designed to improve safety have contributed to shrinking the donor

pool. Blood shortages exist in the United States and worldwide. In many industrialized countries 5% or less of the eligible population are blood donors.

As a result, the global medical community has increasingly moved from allogenic blood (blood collected from another person) towards autologous infusion, in which patients receive their own blood. Another impetus for autologous transfusion is the position of Jehovah's Witnesses on blood transfusions. For religious reasons, Jehovah's Witnesses will not accept any allogeneic transfusions from a volunteer's blood donation, but may accept the use of autologous blood salvaged during surgery to restore their blood volume and homeostasis during the course of an operation, although not autologous blood donated beforehand.

Bloodless options

Ways to avoid the adverse events associated with allogenic transfusion are often grouped under the umbrella term bloodless surgery. There are several so-called bloodless options. These include:

- Minimally invasive surgical techniques
- Erythropoietin (a hormone that stimulates peripheral stem cells in the bone marrow to produce red blood cells)
- Blood substitutes such as blood volume expanders and oxygen carriers (the latter as yet unlicensed in North America)
- Autologous blood donation, including pre-operative donation (suitable only for scheduled surgery in which transfusion is anticipated) and intraoperative autologous donation and blood salvage.

Intraoperative blood salvage has been used for many years, especially in cardiothoracic and vascular surgery, where blood usage has traditionally been high.

Blood salvage procedures

Several processes have been developed to assist in salvaging the patient's own whole blood in the perioperative setting. These can be categorized into three general types of salvage procedures:

1. Cell processors and salvage devices that wash and save red blood cells, i.e., "cell washers" or RBC-savers
2. Direct transfusion
3. Ultrafiltration of whole blood

Regardless of manufacturer, there are many types of cell processors. Cell processors are red cell washing devices that collect anticoagulated shed or recovered blood, wash and separate the red blood cells (RBCs) by centrifugation, and reinfuse the RBCs. RBC washing devices can help remove byproducts in salvaged blood such as activated cytokines, anaphylatoxins, and other waste substances that may have been collected in

the reservoir suctioned from the surgical field. However, they also remove viable platelets, clotting factors, and other [plasma proteins] essential to whole blood and homeostasis. The various RBC-savers also yield RBC concentrates with different characteristics and quality.

Direct transfusion is a blood salvaging method associated with cardiopulmonary bypass (CPB) circuits or other extracorporeal circuits (ECC) that are used in surgery such as coronary artery bypass grafts (CABG), valve replacement, or surgical repair of the great vessels. Following bypass surgery the ECC circuit contains a significant volume of diluted whole blood that can be harvested in transfer bags and re-infused into patients. Residual CPB blood is fairly dilute ([Hb] = 6–9 g/dL; 60–90 g/L) compared to normal values (12–18 g/dL; 120–180 g/L) and can also contain potentially harmful contaminants such as activated cytokines, anaphylatoxins, and other waste substances that have been linked to organ edema and organ dysfunction and need a diuretic to reverse.

Hemofiltration or ultrafiltration devices constitute the third major type of blood salvage appearing in operating rooms. In general, ultrafiltration devices filter the patient's anticoagulated whole blood. The filter process removes unwanted excess non-cellular plasma water, low molecular weight solutes, platelet inhibitors and some particulate matter through hemoconcentration, including activated cytokines, anaphylatoxins, and other waste substances making concentrated whole blood available for reinfusion. Hemofilter devices return the patient's whole blood with all the blood elements and fractions including platelets, clotting factors, and plasma proteins with a substantial Hb level. Presently, the only whole blood ultrafiltration device in clinical use is the Hemobag. These devices do not totally remove potentially harmful contaminants that can be washed away by most RBC-savers. However, the contaminants that are potentially reduced by using RBC-savers, as shown by data from in vitro laboratory tests, are transient and reversible in vivo with hemostatic profiles returning to baselines within hours. The key is that coagulation and homeostasis are immediately improved with the return of concentrated autologous whole blood.

Over the years numerous studies have been done to compare these methods of blood salvage in terms of safety, patient outcomes, and cost effectiveness, often with equivocal or contradictory results.

Leukapheresis

Leukapheresis is a laboratory procedure in which white blood cells are separated from a sample of blood. It is a specific type of apheresis, the more general term for separating out one particular constituent of blood and returning the remainder to the circulation.

Leukapheresis may be performed to decrease a very high white blood cell count, to obtain autologous (i.e., the patient's own) blood cells for later transplant back into the patient, or to obtain cells for research purposes. In the case of hematological malignancies such as acute leukemias, white blood cell counts may be high enough to cause hemostasis and "sludging" in the capillaries. This can affect retinal vasculature leading to vision changes, pulmonary vasculature leading to shortness of breath from decreased efficiency in oxygen exchange, as well as other organ systems such as the brain which would become clinically apparent with neurological deterioration of a patient from cerebrovascular compromise.

Leukapheresis may also be performed to obtain the patient's own blood cells for later transplant. White blood cells may be removed to protect them from damage before high-dose chemotherapy, then transfused back into the patient, in the treatment of advanced breast cancer. Another novel use of cells obtained through leukapheresis is to stimulate a patient’s immune system to target prostate cancer cells.

Alternatively, only granulocytes, macrophages and monocytes can be removed, leaving the lymphocyte count largely unchanged. This is used as a treatment for autoimmune diseases such as ulcerative colitis and rheumatoid arthritis , where these cells play an active part in the inflammation process.

Leukapheresis, typically for granulocytes, is a rarely performed blood donation process. The product is collected by automated apheresis and is used for systemic infections in patients with neutropenia. The donor is typically a blood relative who has received stimulating medications (a directed donation), and the product is irradiated to prevent GVHD. The product generally has a 24 hour shelf life from collection and is often transfused before infectious disease testing is completed. It is a therapy of last resort, and its use is controversial and rare.

Chapter 17

Leukoreduction and Mixed-Field Agglutination

Leukoreduction

Leukoreduction is the removal of white blood cells (or *leukocytes*) from the blood or blood components supplied for blood transfusion. After the removal of the leukocytes, the blood product is said to be *leukoreduced*.

Benefits and costs

It is theorized that transfusions that contain white blood cells may cause adverse effects through multiple mechanisms. White blood cells may themselves harbor infectious disease and some pathogens will be more concentrated in white blood cells than the rest of the blood product. It is also theorized that the donor white blood cells may suppress the recipient's immune system by interacting with it.

An April 2007 meta-analysis by Dr. Neil Blumberg and others and covering 3093 patients who received leukoreduced blood was published in the scientific journal Transfusion. According to the meta-analysis, use of leukoreduced blood reduced the frequency of post-transfusion infection by 50%. In a previous study, Blumberg and others reported that a change to universal use of leukoreduced blood at Strong Memorial Hospital at University of Rochester reduced post-transfusion infection by 33-45%.

However, other scientific studies question the effectiveness of leukoreduction. A March 2007 study by researchers at University of South Alabama Medical Center found no reduction of mortality or length of hospital stay in 439 trauma patients who received leukoreduced transfusions compared to 240 patients who did not. University of Washington researchers reported in October 2006 that a study of 286 transfused injury patients showed no reduction in mortality or length of stay, although a 16% reduction in rate of infection was shown with marginal statistical significance.

Leukoreduction has the inadvertent effect of removing approximately 10% of red blood cells from a processed unit of Red Blood Cells. Because blood from persons who possess the sickle cell mutation is difficult to filter, leukoreduction is often not performed on

donors who may have the sickle cell gene, which is most common in people of African descent.

Dr. Blumberg, the lead author of the meta-analysis covering 3093 patients, stated in the press that the cost savings due to universal leukoreduction exceeds the cost of performing the leukoreduction. The cost of leukoreduction is an increase of approximately US$30 per unit of blood product.

History of availability

Universal leukoreduction is currently not practiced in all countries.

As of 2008, most developed nations have adopted universal leukoreduction of transfusions (defined as the routine application of this blood-processing step to all units of whole blood, red blood cells, and platelets prior to storage) with the notable exception of the United States. Canada, Britain and France adopted universal leukoreduction in the late 1990s. Leukoreduced products are commonly available in the United States and some hospitals use only leukoreduced blood while others only use leukoreduced products in certain patient populations. For example, Strong Memorial Hospital began universal use of leukoreduced blood in July 2000; University of South Alabama Medical Center began use in January 2002. Woodlands Medical Centre is beginning a randomised controlled trial to look into the benefits of transfusing leukoreduced whole blood for the ICCU patients.

Mixed-field agglutination

In transfusion medicine, **mixed-field agglutination** refers to mixed reactions during cell typing where two distinct cell populations are present: agglutinated cells admixed with many unagglutinated cells. The presence of two or more cell population is known as chimerism. Mixed-field agglutination is an important cause of ABO typing and genotype discrepancies. The cause of mixed field agglutinations should be sought prior to setting up blood for transfusion.

Causes

False chimerism

By far the most common cause of mixed-field agglutination is false chimerism artificially induced through transfusion of identical donor red cells or through a stem cell transplant. For example, a type B individual who has received massive transfusion of group O donor red cells may show mixed field agglutination with anti-B sera whereby his own group B

red cells are agglutinated, while the group O donor red cells in his circulation are unagglutinated.

True chimerism

A true chimerism is a rare sporadic phenomenon whereby an individual has a dual cell population derived from more than one zygote. This may result from intrauterine exchange of erythrocyte precursors between twins (twin chimerism) or two fertilized eggs fuse into one individual. Twin chimerism results from mixing of blood between two twin fetuses through placental blood vessel anastomoses, leading to engraftment of hematopoietic stem cells from one twin within the marrow of the other. Each twin ends up with two distinct cell populations of varying proportions.

Chapter 18

Plasmapheresis

Plasmapheresis (from the Greek *πλάσμα - plasma*, something molded, and *ἀφαίρεσις - aphairesis*, taking away) is the removal, treatment, and return of (components of) blood plasma from blood circulation. It is thus an extracorporeal therapy (a medical procedure which is performed outside the body). The method can also be used to collect plasma for further manufacturing into a variety of medications.

The procedure is used to treat a variety of disorders, including those of the immune system, such as Myasthenia gravis Guillain-Barré syndrome, lupus, and thrombotic thrombocytopenic purpura. Dr. D. J. Wallace states that Michael Rubinstein was the first person to use plasmapheresis to treat an immune-related disorder when he "saved the life of an adolescent boy with thrombotic thrombocytopenic purpura (TTP) at the old Cedars of Lebanon Hospital in Los Angeles in 1959". Also according to Wallace, the modern plasmapheresis process itself originated in the "[U.S.] National Cancer Institute between 1963 and 1968, [where] investigators drew upon an old dairy creamer separation technology first used in 1878 and refined by Edwin Cohn's centrifuge marketed in 1953.

As therapy

During plasmapheresis, blood is initially taken out of the body through a needle or previously implanted catheter. Plasma is then removed from the blood by a cell separator. Three procedures are commonly used to separate the plasma from the blood cells:

- Discontinuous flow centrifugation: One venous catheter line is required. Typically, a 300 ml batch of blood is removed at a time and centrifuged to separate plasma from blood cells.
- Continuous flow centrifugation: Two venous lines are used. This method requires slightly less blood volume to be out of the body at any one time as it is able to continuously spin out plasma.
- Plasma filtration: Two venous lines are used. The plasma is filtered using standard hemodialysis equipment. This continuous process requires less than 100 ml of blood to be outside the body at one time.

Each method has its advantages and disadvantages. After plasma separation, the blood cells are returned to the person undergoing treatment, while the plasma, which contains the antibodies, is first treated and then returned to the patient in traditional

plasmapheresis. (In plasma exchange, the removed plasma is discarded and the patient receives replacement donor plasma, albumin, or a combination of albumin and saline (usually 70% albumin and 30% saline). Rarely, other replacement fluids, such as hydroxyethyl starch, may be used in individuals who object to blood transfusion but these are rarely used due to severe side-effects. Medication to keep the blood from clotting (an anticoagulant) is given to the patient during the procedure. Plasmapheresis is used as a therapy in particular diseases. It is an uncommon treatment in the United States, but it is more common in Europe and particularly Japan.

An important use of plasmapheresis is in the therapy of autoimmune disorders, where the rapid removal of disease-causing autoantibodies from the circulation is required in addition to other medical therapy. It is important to note that plasma exchange therapy in and of itself is useful to temper the disease process, where simultaneous medical and immunosuppressive therapy is required for long-term management. Plasma exchange offers the quickest short-term answer to removing harmful autoantibodies; however, the production of autoantibodies by the immune system must also be suppressed, usually by the use of medications such as prednisone, cyclophosphamide, cyclosporine, mycophenolate mofetil, rituximab or a mixture of these.

Other uses are the removal of blood proteins where these are overly abundant and cause hyperviscosity syndrome.

Examples of diseases that can be treated with plasmapheresis:

- Guillain-Barré syndrome
- Chronic inflammatory demyelinating polyneuropathy
- Goodpasture's syndrome
- Hyperviscosity syndromes:
    - Cryoglobulinemia
    - Paraproteinemia
    - Waldenström macroglobulinemia
- Myasthenia gravis
- Thrombotic thrombocytopenic purpura (TTP)/hemolytic uremic syndrome
- Wegener's granulomatosis
- Lambert-Eaton Syndrome
- Antiphospholipid Antibody Syndrome (APS or APLS)
- Microscopic polyangiitis
- Recurrent focal and segmental glomerulosclerosis in the transplanted kidney
- HELLP syndrome
- Refsum disease
- Behcet syndrome
- HIV-related neuropathy
- Graves' disease in infants and neonates
- Pemphigus vulgaris
- Multiple sclerosis
- Rhabdomyolysis

Complications of plasmapheresis therapy

Though plasmapheresis is helpful in certain medical conditions, like any other therapy, there are potential risks and complications. Insertion of a rather large intravenous catheter can lead to bleeding, lung puncture (depending on the site of catheter insertion), and, if the catheter is left in too long, it can get infected.

Aside from placing the catheter, the procedure itself has complications. When patient blood is outside of the body passing through the plasmapheresis machine, the blood has a tendency to clot. To reduce this tendency, in one common protocol, citrate is infused while the blood is running through the circuit. Citrate binds to calcium in the blood, calcium being essential for blood to clot. Citrate is very effective in preventing blood from clotting; however, its use can lead to life-threateningly low calcium levels. This can be detected using the Chvostek's sign or Trousseau's sign. To prevent this complication, calcium is infused intravenously while the patient is undergoing the plasmapheresis; in addition, calcium supplementation by mouth may also be given.

Other complications include:

- Potential exposure to blood products, with risk of transfusion reactions or transfusion transmitted diseases
- Suppression of the patient's immune system
- Bleeding or hematoma from needle placement

As a manufacturing process

Donating plasma is similar in many ways to whole blood donation, though the end product is used for different purposes. Most plasmapheresis is for fractionation into other products, other blood donations are transfused with relatively minor modifications. Plasma that is collected solely for further manufacturing is called Source Plasma.

Plasma donors undergo a screening process to ensure both the donor's safety and the safety of the collected product. Factors monitored include blood pressure, pulse, temperature, total protein, protein electrophoresis, health history screening similar to that for whole blood, as well as an annual physical exam with a licensed physician or an approved physician substitute under the supervision of the physician. Donors are screened at each donation for viral diseases that can be transmitted by blood, sometimes by multiple methods. For example, donors are tested for HIV by EIA, which will show if they have ever been exposed to the disease, as well as by nucleic acid methods (PCR or similar) to rule out recent infections that might be missed by the EIA test. Industry standards require at least two sets of negative test results before the collected plasma is used for injectable products. The plasma is also treated in processing multiple times to inactivate any virus that was undetected during the screening process.

Plasma donors are typically paid cash for their donations, though this is not universal. For example, donors in the UK, Australia and New Zealand are not given financial

incentives. Since the products are heavily processed and treated to remove infectious agents, the higher risk is considered acceptable. Standards for donating plasma are set by national regulatory agencies such as the U.S. Food and Drug Administration (FDA), the European Union, and by a professional organization, the Plasma Protein Therapeutics Association (or PPTA), which audits and accredits collection facilities. A National Donor Deferral Registry (NDDR) is also maintained by the PPTA for use in keeping donors with prior positive test results from donating at any facility.

Almost all plasmapheresis in the US is performed by automated methods such as the Plasma Collection System (PCS2) made by Haemonetics or the Autopheresis-C (Auto-C) made by Fenwal, a division of Baxter International. In some cases, automated plasmapheresis is used to collect plasma products like Fresh frozen plasma for direct transfusion purposes, often at the same time as plateletpheresis.

Manual method

> For the manual method, approximately the same as a whole blood donation is collected from the donor. The collected blood is then separated by centrifuge machines in separate rooms, the plasma is pressed out of the collection set into a satellite container, and the red blood cells are returned to the donor. Since returning red cells causes the plasma to be replaced more rapidly by the body, a donor can provide up to a liter of plasma at a time and can donate with only a few days between donations, unlike the 56-day deferral for blood donation. The amount allowed in a donation varies vastly from country to country, but generally does not exceed two donations, each as much as a liter, per 7-day period.

The danger with this method was that if the wrong red blood cells were returned to the donor, a serious and potentially fatal transfusion reaction could occur. Requiring donors to recite their names and ID numbers on returned bags of red cells minimized this risk. This procedure has largely become obsolete in favor of the automated method.

Automated method

> The automated method uses a very similar process. The difference is that the collection, separation, and return are all performed inside a machine which is connected to the donor through a needle placed in the arm, typically the antecubital vein. There is no risk of receiving the wrong red cells. The devices used are very similar to the devices used for therapeutic plasmapheresis, and the potential for citrate toxicity is similar. The potential risks are explained to prospective donors at the first donation, and most donors tolerate the procedure well.

If a significant amount of red blood cells cannot be returned, the donor may not donate for 56 days, just as if they had donated a unit of blood. Depending on the collection system and the operation, the removed plasma may be replaced by saline. The body will typically replace the collected volume within 24 hours, and donors typically donate up to twice a week, though this varies by country.

The collected plasma is promptly frozen at lower than -20 °C (-4 °F) and is typically shipped to a processing facility for fractionation. This process separates the collected plasma into specific components, such as albumin and immunoglobulins, most of which are made into medications for human use. Sometimes the plasma is thawed and transfused as Fresh Frozen Plasma (FFP), much like the plasma from a normal blood donation.

Donors are sometimes immunized against agents such as tetanus or hepatitis B so that their plasma contains the antibodies against the toxin or disease. In other donors, an intentionally incompatible unit of blood is transfused to produce antibodies to the antigens on the red cells. The collected plasma then contains these components, which are used in manufacturing of medications. Donors who are already ill may have their plasma collected for use as a positive control for laboratory testing.

Chapter 19

Plateletpheresis

A 250 ml bag of newly collected platelets

Plateletpheresis (more accurately called **thrombocytapheresis** or **thrombapheresis**, though these names are rarely used) is the process of collecting thrombocytes, more commonly called platelets, a component of blood involved in hemostasis (blood clotting). Thrombapheresis can be a life-saving procedure in preventing or treating serious complications from bleeding and hemorrhage in patients who have disorders manifesting

as thrombocytopenia (low platelet count) or platelet dysfunction. This process may also be used therapeutically to treat disorders resulting in extraordinarily high platelet counts such as Essential Thrombocytosis.

Indications for transfusion

Platelet transfusions are traditionally given to those undergoing chemotherapy for leukemia, multiple myeloma, those with aplastic anemia, AIDS, hypersplenism, ITP, sepsis, bone marrow transplant, radiation treatment, organ transplant or surgeries such as cardiopulmonary bypass. Platelet transfusions should be avoided in those with TTP because it can worsen neurologic symptoms and acute renal failure, presumably due to creation of new thrombi as the platelets are consumed. It should also be avoided in those with heparin-induced thrombocytopenia (HIT) or disseminated intravascular coagulation (DIC).

Thrombocytopenia due to underproduction. Patients in this category include those undergoing chemotherapy, those with myelophthisic anemia, AIDS, or with aplastic anemia. If indicated, transfusions (one thrombapheresis concentrate) should be given until recovery of platelet function, generally approximately twice weekly. Surgical bleeding due solely to thrombocytopenia occurs when platelets < 50,000/μL while spontaneous bleeding occurs when platelets < 10,000/μL. Thrombocytopenic patients can develop "dry" bleeding, that is, petechiae and ecchymoses only. They will not suffer fatal hemorrhagic events unless they first have extensive mucosal bleeding, or "wet" bleeding. Therefore, in those with no bleeding or only "dry" bleeding, the threshold for transfusion should be between 5,000 to 10,000/μL. A more conservative threshold of 20,000/μL should be used in those with a fever or other risk factors for bleeding. Those with active bleeding or prior to surgery should have a threshold of 50,000/μL. An unconfirmed, but helpful, way to determine whether a patient is recovering from chemotherapy-induced thrombocytopenia is to measure "reticulated" platelets, or young RNA-containing platelets, which signifies that the patient is starting to make new platelets.

Immune thrombocytopenia. Patients in this category include those with ITP or drug-induced thrombocytopenia. Platelet transfusions are generally not recommended for this group of patients because the underlying cause involves antibodies that destroy platelets, therefore any newly transfused platelets will also be destroyed. More studies need to be done.

Altered platelet functions. Disorders of platelet function can be congenital or acquired. Most of these disorders are mild and may respond to therapy with desmopressin (dDAVP). Transfusion is not necessarily required. However, with some more severe disorders such as Glanzmann thrombasthenia, transfusions with large amount of platelets may be needed. The number of transfusions may be reduced if these patients are given recombinant human factor VIIa since the underlying cause are antibodies to platelet glycoproteins IIb/IIIa.

Cardiopulmonary bypass surgery. This surgery can result in destruction of a large proportion of the patient's platelets and may render the remaining viable platelets to be dysfunctional. The indications for transfusion in such patients is controversial. General guidelines recommend not transfusing patients prophylactically but only when they are bleeding excessively, while also giving desmopressin.

Drug-induced platelet dysfunction. The most common of these is aspirin, and its similar drug class, the NSAIDs. Other antiplatelet drugs are commonly prescribed for patients with acute coronary syndromes such as clopidogrel and ticlopidine. When surgery is undertaken following the administration of these drugs, bleeding can be serious. Transfusion under these circumstances is not clear-cut and one has to use clinical judgment in these cases.

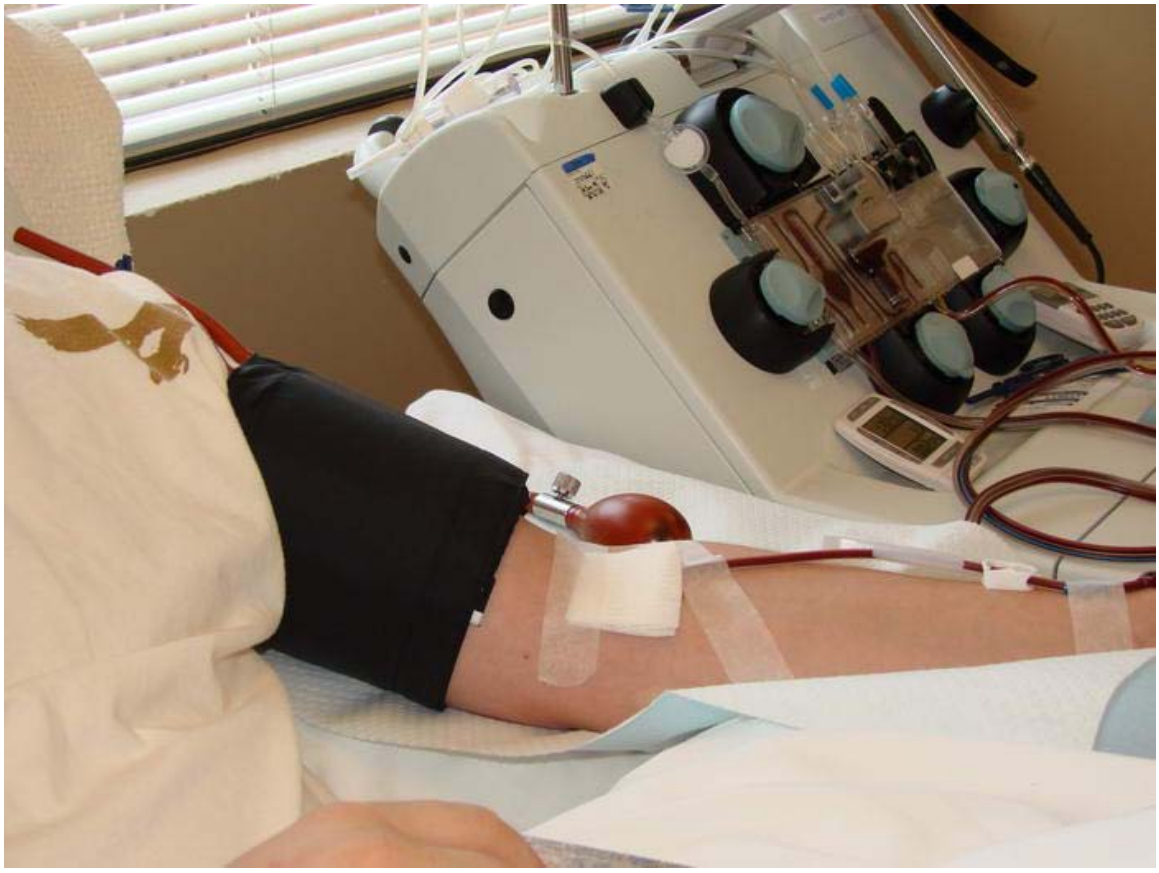
Expected platelet increase after transfusion

Platelet count increase as well as platelet survival after transfusion is related to the dose of platelets infused and to the patient's body surface area (BSA). Usually these values are less than what would be expected.

- Corrected platelet count increment (CCI) = platelet increment at one hr x BSA (m^2) / # platelets infused x 10^{11}
- Expected platelet increase (per μL) = platelets infused x CCI / BSA (m^2)

The theoretical value of the CCI is 20,000/μL but clinically, the value is more close to 10,000/μL. If the CCI is less than 5,000/μL, patients are said to have "refractoriness" to platelet transfusion.

Platelet collection



a single line cartridge based, centrifuge machine. Collecting a 'double unit' in this instance

The separation of individual blood components is done with a specialized centrifuge. The earliest manual forms of thrombapheresis are done by the separation of platelets from multiple bags of whole blood collected from donors or blood sellers. Since each blood bag (usually 250 ml or 500 ml) contains a relatively small number of platelets, it can take as many as a dozen blood bags (usually from 5 to 10 bags, depending on the size of the blood bags and each donor's platelet count) to accumulate a single unit of platelets (enough for one patient). This greatly increases the risks of the transfusion. Each unit of platelets separated from donated whole blood is called a "platelet concentrate".

Modern automatic thrombapheresis allows blood donors to give a portion of their platelets, while keeping their red blood cells and at least a portion of blood plasma. Therefore, no more than three units of platelets are generally harvested in any one sitting from a donor. Most donors will donate a "single" or "double" unit, however the occurrence of "triples" has been increasing as more suitable donors are recruited.

Because platelets have a life-span of just 5 days, more platelet donors are always needed. Some centers are experimenting with 7 day platelets, but this requires additional testing

and the lack of any preservative solutions means that the product is far more effective when fresh.

Even though red blood cells can also be collected in the process, most blood donation organizations do not do so because it takes much longer for the human body to replenish their loss. If the donor donates both red blood cells and platelets, it takes months, rather than days or weeks, before they are allowed to donate again (the guidelines regarding blood donation intervals are country-specific).

In most cases, blood plasma is returned to the donor as well. However, in locations that have plasma processing facilities, a part of the donor's plasma can also be collected in a separate blood bag.

Leukoreduction

Due to their higher relative density, white blood cells are collected as an unwanted component with the platelets. Since it takes up to 3 liters of whole blood (the amount of a dozen blood bags) to generate a dose of platelets, white blood cells from one or several donors will also be collected along with the platelets. A 70 kg (154 lb) man has only about 6 liters of blood. If all of the incidentally collected white blood cells are transfused with the platelets, substantial rejection problems can occur. Therefore, it is standard practice to filter out white blood cells before transfusion by the process of leukoreduction.

Early platelet transfusions used a filter to remove white blood cells at the time of transfusion. It takes a trained person about 10 minutes to assemble the equipment, and this is not the safest or most efficient means of filtration because living white blood cells can release cytokines during storage and dead white blood cells can break up into smaller fragments that can still stimulate a dangerous response from the immune system. In addition, simple filtration can lead to increased risks of infection and loss of valuable platelets. Newer, more advanced thrombapheresis machines can filter white blood cells during separation.

For example, with marginally acceptable whole blood (white blood cells: $< 10{,}000/mm^3$; platelets: $> 150{,}000/mm^3$), a dose (3×10^{11}) of platelets comes with about 2×10^{10} white blood cells. This can seriously damage the patient's health. A dose of single-donor platelets prepared using latest filters can contain as little as 5×10^{6} white blood cells.

Apheresis

There are two types of manual platelet apheresis. Platelet-rich plasma (PRP) is widely used in North America and Buffy coat (BC) is more widely used in Europe.

Platelets are the clotting factor of the blood, and when donated, frequently go to cancer patients, because due to chemotherapy many cancer patients are unable to generate enough platelets of their own.

The basic principles of automatic platelet apheresis are the same as in the manual procedure, but the whole procedure is performed by a computer-controlled machine. Since the donor's blood is processed in a sterile single-use centrifuge, the unwanted components can be returned to the donor safely. This allows the apheresis machine to repeat the draw-centrifuge-return cycle to obtain more platelets. The bulk of the machine and the length of the donation process means most platelet donations are done in blood centers instead of mobile blood drives.

Each country has its own rules to protect the safety of both donor and recipient. In a typical set of rules, a platelet donor must weigh at least 50 kg (110 lb) and have a platelet count of at least 150 x 10^9/L (150,000 platelets per mm^3).

One unit has about 3×10^{11} platelets. Therefore, it takes 2 liters of blood having a platelet count of 150,000/mm^3 to produce one unit of platelets. Some regular donors have higher platelet counts (over 300,000/mm^3); for those donors, it only takes about one liter of their blood to produce a unit. Since the machine used to perform the procedure uses suction to pull blood out of your body, some people that can give whole blood may have veins too small to use for platelet donation. Your blood center can evaluate you prior to donation.

Blood accounts for about 8% of body weight, giving a 50 kg (110 lb) donor about four liters of blood. No more than 50% of platelets are ever extracted in one sitting, and they can be replenished by the body in about three days.

Most newer apheresis machines can separate a dose of platelets in about 60 to 120 minutes depending on the donor's health condition.

Platelet donation

After a short physical examination, the donor is taken into the donation room and sits in a chair next to the machine. The technician cleans one or both arms with iodine, or other disinfectant, and inserts the catheter into a vein in the arm. With some procedures both arms are used, one to draw blood and the other to return it. The process takes about one to two hours while blood is pulled into the machine, mixed with an anticoagulant such as sodium citrate spun around, and returned to the donor. "Double needle" procedures using both arms tend to be shorter since the blood is drawn and returned through different catheters, with "single needle" procedures a set volume is drawn and processed in the first part of the cycle and returned in the second part. The donor's blood undertakes 3-4 cycles of draw and return.

Side effects of the donation of platelets generally fall into three categories: blood pressure changes, problems with vein access, and effects of the returned anticoagulant. Blood pressure changes can sometimes cause nausea, fatigue, and dizziness. Venous access problems can cause bruising, referred to as a hematoma. While donating, the lips may begin to tingle or there may be a metallic taste; a supply of calcium antacid tablets is usually kept close by because the anticoagulant works by binding to the calcium in the blood. Since calcium is used in the operation of the nervous system, nerve-ending-dense

areas (such as the lips) are susceptible to the tingling. The donation process can also cause more serious problems such as fainting, and nerve irritation. These problems are extremely rare, but apheresis donors are typically not allowed to sleep during the long donation process so that they can be monitored.

Aside from the procedure, donating platelets is different from donating blood in a few ways.

Firstly, the donor must not take aspirin or other anti-platelet medications for anywhere from 36 to 72 hours prior to donation. (Guidelines vary by blood center.) The reason for this is that aspirin can prevent platelets from adhering. Some blood centers also prohibit the taking of any NSAID (non-steroidal-anti-inflammatory-drug) for 36 hours prior. Other medications such as clopidogrel (Plavix) may also affect platelet function and may affect donor eligibility.

Secondly, one is generally allowed to donate platelets anywhere from every 3–28 days. This is a stark contrast to whole-blood donation, which has an eight-week (or longer) waiting period between donations. Along those lines, since platelet donation does temporarily remove whole-blood from the body, it may become necessary to wait eight weeks after a whole blood donation to donate platelets. In the US, a donor is only allowed to donate 24 times each year and may not lose more red blood cells or plasma in a year than they would from the maximum allowable number of whole blood donations.

Thirdly, additional tests may be required before becoming a donor for the first time. These tests are used to establish a platelet count, and also possibly to determine the donor's compatibility with particular recipients through an HLA (Human Leukocyte Antigen) test. Multiparous women may be excluded from becoming donors due to heightened TRALI risk. The tests usually involve nothing more involved than the drawing of several tubes of blood.

Haemonetics

The Haemonetics machine draws a large amount of blood in each cycle, usually 5-7 cycles per donation (approx. 10 min per cycle). Up to two platelet units can be obtained during one donation (this is done with donors with a high count), and a unit of plasma can also be donated, at the center's discretion.

Trima Accel (CaridianBCT)

The Trima Accel Automated Blood Collection System can collect up to three units within two hours. This unit also draws more suction than the Haemonetics and lacks an automated arm cuff. This means it requires a large vein to support unless a portable blood pressure cuff is available.

The Trima Accel Automated Blood Collection System has incorporated a leukocyte reduction "cone", called LRS chamber, as part of the disposable kit. Use of this device

routinely produces platelet concentrates with white blood cell counts of less than 1×10^6 per product.

However, the Trima Accel draws and returns blood in very small amounts compared to the Haemonetics, resulting in more than 100 cycles/unit (draw 40 sec, return 15 sec). This generally results in a lower pressure drop during the cycle since less blood is out of the body at any one time.

"Trima Accel" can also perform the collection of platelets, plasma and red blood cells simultaneously.

COBE (CaridianBCT) Spectra

This older unit is still in use in some blood centers. While it can perform a single-needle donation, the most common method with this machine is to draw with one needle, and return with the other, continuously drawing the blood through a centrifuge (instead of using cycles). For obvious reasons, the single needle Trima and Haemonetics machines are more popular, while the COBE Spectra is being phased out.

Vein scarring

Repeated platelet donations at short intervals will cause the venipuncture site to scar. While cosmetically it is virtually invisible, the scarring also occurs on the vein itself, making it harder to insert a needle on future occasions. Anecdotal reports have said that rubbing Vitamin E oil (or the insides of a Vitamin E capsule) on the venipuncture site may reduce scarring.

It may be necessary for the donor to warn anybody who needs to draw blood from a scarred site that the vein may be somewhat tougher than normal. Failure to do so may result in the tech thinking they have missed the vein, not realizing that the vein simply may take a little more pressure to stick.