Basics of Hematology and Clinical Blood Diagnosis

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PREFACE

Hematology is the study of blood and blood disorders. Hematologists and hematopathologists are highly trained healthcare providers who specialize in diseases of the blood and blood components. These include blood and bone marrow cells. There are many types of blood disorders, which can involve problems with red blood cells, white blood cells, platelets, blood vessels, bone marrow, lymph nodes, or the proteins involved in bleeding and clotting. This handbook is divided into the clinical approach and disease-specific areas. The clinical approach section outlines various symptoms and signs in patients with blood disease to enable the reader to formulate a sensible differential diagnosis before embarking on investigation and treatment. This book offers a concise and logical approach to caring for patients with diseases of the blood.

The author would like to express his sincere thanks to his friends and family for their support in the completion of this book. He wants to thank Springer MedPress Corporation to recognise the intensity of the subject and help me publish this masterpiece which will educate well the newcomers. Also thanks the staff and the editorial team for their kind support and encouragement. This book has all the relevant details with the new concepts.

-Author

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Red Blood Cell and Iron Disorders

ANEMIA

Anemia is a decrease in the total amount of red blood cells (RBCs) or hemoglobin in the blood, or a lowered ability of the blood to carry oxygen. When anemia comes on slowly, the symptoms are often vague and may include feeling tired, weakness, shortness of breath or a poor ability to exercise. Anemia that comes on quickly often has greater symptoms, which may include confusion, feeling like one is going to pass out, loss of consciousness, or increased thirst. Anemia must be significant before a person becomes noticeably pale. Additional symptoms may occur depending on the underlying cause.

The three main types of anemia are due to blood loss, decreased red blood cell production, and increased red blood cell breakdown. Causes of blood loss include trauma and gastrointestinal bleeding, among others. Causes of decreased production include iron deficiency, a lack of vitamin B_{12}, thalassemia, and a number of neoplasms of the bone marrow. Causes of increased breakdown include a number of genetic conditions such as sickle cell anemia, infections like malaria, and certain autoimmune diseases. It can also be classified based on the size of red blood cells and amount of hemoglobin in each cell. If the cells are small, it is microcytic anemia. If they are large, it is macrocytic anemia while if they are normal sized, it is normocytic anemia. Diagnosis in men is based on a hemoglobin of less than 130 to 140 g/L (13 to 14 g/dL), while in women, it must be less than 120 to 130 g/L (12 to 13 g/dL). Further testing is then required to determine the cause.

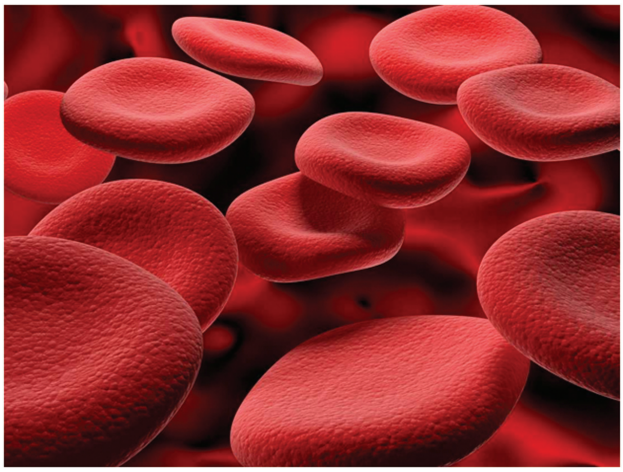


Fig. Anemia

Certain groups of individuals, such as pregnant women, benefit from the use of iron pills for prevention. Dietary supplementation, without determining the specific cause, is not recommended. The use of blood transfusions is typically based on a person's signs and symptoms. In those without symptoms, they are not recommended

unless hemoglobin levels are less than 60 to 80 g/L (6 to 8 g/dL). These recommendations may also apply to some people with acute bleeding. Erythropoiesis-stimulating medications are only recommended in those with severe anemia. Anemia is the most common blood disorder, affecting about a third of the global population. Iron-deficiency anemia affects nearly 1 billion people. In 2013, anemia due to iron deficiency resulted in about 183,000 deaths – down from 213,000 deaths in 1990. It is more common in women than men, during pregnancy, and in children and the elderly. Anemia increases costs of medical care and lowers a person's productivity through a decreased ability to work.

SIGNS AND SYMPTOMS

Anemia goes undetected in many people and symptoms can be minor. The symptoms can be related to an underlying cause or the anemia itself. Most commonly, people with anemia report feelings of weakness or fatigue, and sometimes poor concentration. They may also report 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 pre-existing heart disease is present), intermittent claudication of the legs, and symptoms of heart failure. On examination, the signs exhibited may include pallor (pale skin, lining mucosa, conjunctiva 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 thalassemia major) or leg ulcers. In severe anemia, there may be signs of a hyperdynamic circulation: tachycardia (a fast heart rate), bounding pulse, flow murmurs, and cardiac ventricular hypertrophy (enlargement). There may be signs of heart failure. Pica, the consumption of non-food items such as ice, but also paper, wax, or grass, and even hair or dirt, may be a symptom of iron deficiency, although it occurs often in those who have normal levels of hemoglobin. Chronic anemia may result in behavioural disturbances in children as a direct result of impaired neurological development in infants, and reduced academic performance in children of school age. Restless legs syndrome is more common in those with iron-deficiency anemia.

CAUSES

The 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 cause anemia eventually. Indeed, the most common cause of anemia is blood loss, but this usually does not 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 affects 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}. Lack of dietary B_{12} causes non-pernicious megaloblastic anemia.

  - Anemia of folic acid deficiency, as with vitamin B_{12}, causes megaloblastic anemia
  - Anemia of prematurity, by diminished erythropoietin response to declining hematocrit levels, combined with blood loss from laboratory testing, generally occurs in premature infants at two to six weeks of age.
  - Iron deficiency anemia, resulting in deficient heme synthesis
  - Thalassemias, causing deficient globin synthesis
  - Congenital dyserythropoietic anemias, causing ineffective erythropoiesis
  - 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 lactate dehydrogenase levels.

- Intrinsic (intracorpuscular) abnormalities 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 is another defect in membrane skeleton proteins.
  - Abetalipoproteinemia, causing defects in membrane lipids
  - Enzyme deficiencies
    a. Pyruvate kinase and hexokinase deficiencies, causing defect glycolysis
    b. Glucose-6-phosphate dehydrogenase deficiency and glutathione synthetase deficiency, causing increased oxidative stress
  - Hemoglobinopathies
    a. Sickle cell anemia
    b. Hemoglobinopathies causing unstable hemoglobins
  - Paroxysmal nocturnal hemoglobinuria
- Extrinsic (extracorpuscular) abnormalities
  - Antibody-mediated
    a. Warm autoimmune hemolytic anemia is 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.
    b. Cold agglutinin hemolytic anemia is primarily mediated by IgM. It can be idiopathic or result from an underlying condition.
    c. Rh disease, one of the causes of hemolytic disease of the newborn
    d. Transfusion reaction to blood transfusions
  - Mechanical trauma to red blood cells
    a. Microangiopathic hemolytic anemias, including thrombotic thrombocytopenic purpura and disseminated intravascular coagulation

b. Infections, including malaria
c. Heart surgery
d. Haemodialysis.

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 either acute bleeds (*e.g.,* variceal lesions, peptic ulcers) or chronic blood loss (*e.g.,* angiodysplasia)
- Gynecologic disturbances, also generally causing chronic blood loss
- From menstruation, mostly among young women or older women who have fibroids
- Infection by intestinal nematodes feeding on blood, such as hookworms and the whipworm *Trichuris trichiura*.

The roots of the words *anemia* and *ischemia* both refer to the basic idea of "lack of blood", but anemia and is chemiaare not the same thing in modern medical terminology. The word *anemia* used alone implies widespread *effects* from blood that either is too scarce (*e.g.,* blood loss) or is dysfunctional in its oxygen-supplying ability (due to whatever type of hemoglobin or erythrocyte problem). In contrast, the word *ischemia* refers solely to the lack of blood (poor perfusion). Thus ischemia in a body part can cause localized anemic effects within those tissues.

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.
- From the 6th week of pregnancy hormonal changes cause an increase in the mother's blood volume due to an increase in plasma.

Intestinal Inflammation

Certain gastrointestinal disorders can cause anemia. The mechanisms involved are multifactorial and not limited to malabsorption but mainly related to chronic intestinal inflammation, which causes dysregulation of hepcidin that leads to decreased access of iron to the circulation.

- *Helicobacter pylori* infection.
- Gluten-related disorders: untreated celiac disease and non-celiac gluten sensitivity. Anemia can be the only manifestation of celiac disease, in absence of gastrointestinal or any other symptoms.
- Inflammatory bowel disease.

DIAGNOSIS

Definitions

There are a number of definitions of anemia; reviews provide comparison and contrast of them. A strict but broad definition is an absolute decrease in red blood cell mass, however, a broader definition is a lowered ability of the blood to carry oxygen. An operational definition is a decrease in whole-blood hemoglobin concentration of more than 2 standard deviations below the mean of an age- and sex-matched reference range. It is difficult to directly measure RBC mass, so the hematocrit (amount of RBCs) or the hemoglobin (Hb) in the blood are often

used instead to indirectly estimate the value. Hemotocrit; however, is concentration dependent and is therefore not completely accurate. For example, during pregnancy a woman's RBC mass is normal but because of an increase in blood volume the hemoglobin and hematocrit are diluted and thus decreased. Another example would be bleeding where the RBC mass would decrease but the concentrations of hemoglobin and hematocrit initially remains normal until fluids shift from other areas of the body to the intravascular space. The anemia is also classified by severity into mild (110 g/L to normal), moderate (80 g/L to 110 g/L), and severe anemia (less than 80 g/L) in adult males and adult non pregnant females. Different values are used in pregnancy and children.

Testing

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 it 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, such as: ESR, ferritin, serum iron, transferrin, RBC folate level, serum vitamin B_{12}, hemoglobin electrophoresis, renal function tests (*e.g.,* serum creatinine) although the tests will depend on the clinical hypothesis that is being investigated. When the diagnosis remains difficult, a bone marrow examination allows direct examination of the precursors to red cells, although is rarely used as is painful, invasive and is hence reserved for cases where severe pathology needs to be determined or excluded.

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. Limitations of MCV include cases where the underlying cause is due to a combination of factors – such as iron deficiency (a cause of microcytosis) and vitamin B_{12} deficiency (a cause of macrocytosis) where the net result can be normocytic cells.

Production vs. Destruction or Loss

The "kinetic" approach to anemia yields arguably 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 or loss.

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 (microcytosis is not always present)
  - Anemia of chronic disease (more commonly presenting as normocytic anemia)
- Globin synthesis defect
  - Alpha, and beta-thalassemia
  - HbE syndrome
  - HbC syndrome
  - 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 due to insufficient dietary intake or absorption of iron to meet the body's needs. Infants, toddlers, and pregnant women have higher than average needs. Increased iron intake is also needed to offset blood losses due to digestive tract issues, frequent blood donations, or heavy menstrual periods. 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, 12% of all women of childbearing age have iron deficiency, compared with only 2% of adult men. The incidence is as high as 20% among African American and Mexican American women. Studies have shown iron deficiency without anemia causes poor school performance and lower IQ in teenage girls, although this may be due to socioeconomic factors. Iron deficiency is the most prevalent deficiency state on a worldwide basis. It is sometimes the cause of abnormal fissuring of the angular (corner) sections of the lips (angular stomatitis).

- In the United States, the most common cause of iron deficiency is bleeding or blood loss, usually from the gastrointestinal tract. Fecal occult blood testing, upper endoscopy and lower endoscopy should be performed to identify bleeding lesions. In older men and women, the chances are higher that bleeding from the gastrointestinal tract could be due to colon polyps or colorectal cancer.
- Worldwide, the most common cause of iron deficiency anemia is parasitic infestation (hookworms, amebiasis, schistosomiasis and whipworms).

The Mentzer index (mean cell volume divided by the RBC count) predicts whether microcytic anemia may be due to iron deficiency or thallasemia, although it requires confirmation.

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 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, which 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.
- Drugs such as methotrexate, zidovudine, and other substances may inhibit DNA replication such as heavy metals.

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 results in restricted cell division of the progenitor cells. The megaloblastic anemias often present with neutrophil hypersegmentation (six to 10 lobes). The non-megaloblastic macrocytic anemias have different etiologies (*i.e.,* 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 isolate the curative substance chemically 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 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

A dimorphic appearance on a peripheral blood smear occurs when there are two simultaneous populations of red blood cells, typically of different size and hemoglobin content (this last feature affecting the colour of the red blood cell on a stained peripheral blood smear). For example, a person recently transfused for iron deficiency would have small, pale, iron deficient red blood cells (RBCs) and the donor RBCs of normal size and colour. Similarly, a person transfused for severe folate or vitamin B_{12} deficiency would have two cell populations, but, in this case, the patient's RBCs would be larger and paler than the donor's RBCs. A person with sideroblastic anemia (a defect in heme synthesis, commonly caused by alcoholism, but also drugs/toxins, nutritional deficiencies, a few acquired and rare congenital diseases) can have a dimorphic smear from the sideroblastic anemia alone. Evidence for multiple causes appears with an elevated RBC distribution width (RDW), indicating a wider-than-normal range of red cell sizes, also seen in common nutritional anemia.

Heinz Body Anemia

Heinz bodies form in the cytoplasm of RBCs and appear as small dark dots under the microscope. In animals, Heinz body anemia has many causes. It may be drug-induced, for example in cats and dogs by acetaminophen (paracetamol), or may be caused by eating various plants or other substances:

- In cats and dogs after eating either raw or cooked plants from the genus *Alium*, for example, onions or garlic.
- In dogs after ingestion of zinc, for example, after eating U.S. pennies minted after 1982.
- In horses which eat dry or wilted red maple leaves.

Hyperanemia

Hyperanemia is a severe form of anemia, in which the hematocrit is below 10%.

Refractory anemia

Refractory anemia, an anemia which does not respond to treatment, is often seen secondary to myelodysplastic syndromes. Iron deficiency anemia may also be refractory as a clinical manifestation of gastrointestinal problems which disrupt iron absorption or cause occult bleeding.

TREATMENTS

Treatments for anemia depend on cause and severity. Vitamin supplements given orally (folic acid or vitamin B_{12}) or intramuscularly (vitamin B_{12}) will replace specific deficiencies.

Oral Iron

Nutritional iron deficiency is common in developing nations. An estimated two-thirds of children and of women of childbearing age in most developing nations are estimated to suffer from iron deficiency; one-third of them have the more severe form of the disorder, anemia. Iron deficiency from nutritional causes is rare in men and postmenopausal 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, stomach upset or darkening of the feces are commonly experienced. 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. In anemias of chronic disease, associated with chemotherapy, or associated with renal disease, some clinicians prescribe recombinant erythropoietin or epoetin alfa, to stimulate RBC production, although since there is also concurrent iron deficiency and inflammation present, parenteral iron is advised to be taken concurrently.

Injectable Iron

In cases where oral iron has either proven ineffective, would be too slow (for example, pre-operatively) or where absorption is impeded (for example in cases of inflammation), parenteral iron can be used. The body can absorb up to 6 mg iron daily from the gastrointestinal tract. In many cases the patient has a deficit of over 1,000 mg of iron which would require several months to replace. This can be given concurrently with erythropoietin to ensure sufficient iron for increased rates of erythropoiesis.

Blood Transfusions

Blood transfusions in those without symptoms is not recommended until the hemoglobin is below 60 to 80 g/L (6 to 8 g/dL). In those with coronary artery disease who are not actively bleeding transfusions are only recommended when the hemoglobin is below 70 to 80g/L (7 to 8 g/dL). Transfusing earlier does not improve survival. Transfusions otherwise should only be undertaken in cases of cardiovascular instability.

Erythropoiesis-stimulating Agent

The motive for the administration of an erythropoiesis-stimulating agent (ESA) is to maintain hemoglobin at the lowest level that both minimizes transfusions and meets the individual person's needs. They should not be used for mild or moderate anemia. They are not recommended in people with chronic kidney disease unless hemoglobin levels are less than 10 g/dL or they have symptoms of anemia. Their use should be along with parenteral iron.

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 given blood transfusions 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 HBO method. A 2005 review of the use of HBO in severe anemia found all publications reported positive results.

EPIDEMIOLOGY

A moderate degree of iron-deficiency anemia affected approximately 610 million people worldwide or 8.8% of the population. It is slightly more common in females (9.9%) than males (7.8%). Mild iron deficiency anemia affects another 375 million.

SICKLE-CELL DISEASE

Sickle-cell disease (SCD) is a group of blood disorders typically inherited from a person's parents. The most common type is known as sickle-cell anaemia (SCA). It results in an abnormality in the oxygen-carrying protein

haemoglobin (haemoglobin S) found in red blood cells. This leads to a rigid, sickle-like shape under certain circumstances. Problems in sickle cell disease typically begin around 5 to 6 months of age. A number of health problems may develop, such as attacks of pain ("sickle-cell crisis"), anemia, swelling in the hands and feet, bacterial infections, and stroke. Long term pain may develop as people get older. The average life expectancy in the developed world is 40 to 60 years.

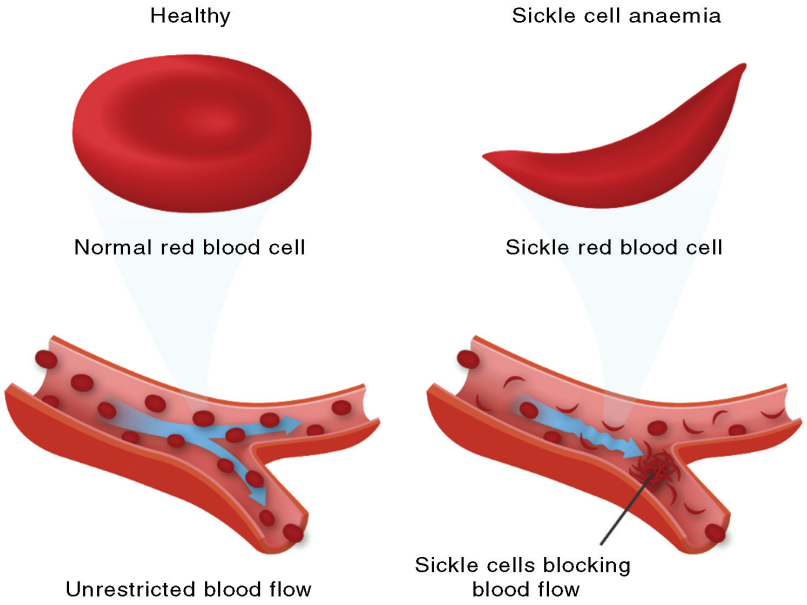


Fig. sickle cell anaemia.

Sickle-cell disease occurs when a person inherits two abnormal copies of the haemoglobin gene, one from each parent. This gene occurs in chromosome 11. Several subtypes exist, depending on the exact mutation in each haemoglobin gene. An attack can be set off by temperature changes, stress, dehydration, and high altitude. A person with a single abnormal copy does not usually have symptoms and is said to have sickle-cell trait. Such people are also referred to as carriers. Diagnosis is by a blood test and some countries test all babies at birth for the disease. Diagnosis is also possible during pregnancy. The care of people with sickle-cell disease may include infection prevention with vaccination and antibiotics, high fluid intake, folic acid supplementation, and pain medication. Other measures may include blood transfusion, and the medication hydroxycarbamide (hydroxyurea). A small percentage of people can be cured by a transplant of bone marrow cells. As of 2015, about 4.4 million people have sickle-cell disease while an additional 43 million have sickle-cell trait. About 80% of sickle-cell disease cases are believed to occur in sub-Saharan Africa. It also occurs relatively frequently in parts of India, the Arabian peninsula, and among people of African origin living in other parts of the world. In 2015, it resulted in about 114,800 deaths. The condition was first described in the medical literature by the American physician James B. Herrick in 1910. In 1949 the genetic transmission was determined by E. A. Beet and J. V. Neel. In 1954 the protective effect against malaria of sickle-cell trait was described.

SIGNS AND SYMPTOMS

Signs of sickle cell disease usually begin in early childhood. The severity of symptoms can vary from person to person. Sickle-cell disease may lead to various acute and chronic complications, several of which have a high mortality rate.

Sickle-cell Crisis

The terms "sickle-cell crisis" or "sickling crisis" may be used to describe several independent acute conditions occurring in patients with SCD. SCD results in anaemia and crises that could be of many types

including the vaso-occlusive crisis, aplastic crisis, sequestration crisis, haemolytic crisis, and others. Most episodes of sickle-cell crises last between five and seven days. "Although infection, dehydration, and acidosis (all of which favour sickling) can act as triggers, in most instances, no predisposing cause is identified."

Vaso-occlusive Crisis

The vaso-occlusive crisis is caused by sickle-shaped red blood cells that obstruct capillaries and restrict blood flow to an organ resulting in ischaemia, pain, necrosis, and often organ damage. The frequency, severity, and duration of these crises vary considerably. Painful crises are treated with hydration, analgesics, and blood transfusion; pain management requires opioid administration at regular intervals until the crisis has settled. For milder crises, a subgroup of patients manage on non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac or naproxen. For more severe crises, most patients require inpatient management for intravenous opioids; patient-controlled analgesia devices are commonly used in this setting. Vaso-occlusive crisis involving organs such as the penis or lungs are considered an emergency and treated with red-blood cell transfusions. Incentive spirometry, a technique to encourage deep breathing to minimise the development of atelectasis, is recommended.

Splenic Sequestration Crisis

Because of its narrow vessels and function in clearing defective red blood cells, the spleen is frequently affected. It is usually infarcted before the end of childhood in individuals suffering from sickle-cell anaemia. This spleen damage increases the risk of infection from encapsulated organisms; preventive antibiotics and vaccinations are recommended for those lacking proper spleen function.

Splenic sequestration crises are acute, painful enlargements of the spleen, caused by intrasplenic trapping of red cells and resulting in a precipitous fall in haemoglobin levels with the potential for hypovolemic shock. Sequestration crises are considered an emergency. If not treated, patients may die within 1–2 hours due to circulatory failure. Management is supportive, sometimes with blood transfusion. These crises are transient, they continue for 3–4 hours and may last for one day.

Acute Chest Syndrome

Acute chest syndrome (ACS) is defined by at least two of the following signs or symptoms: chest pain, fever, pulmonary infiltrate or focal abnormality, respiratory symptoms, or hypoxemia. It is the second-most common complication and it accounts for about 25% of deaths in patients with SCD, majority of cases present with vaso-occlusive crises then they develop ACS. Nevertheless, about 80% of patients have vaso-occlusive crises during ACS.

Aplastic Crisis

Aplastic crises are acute worsenings of the patient's baseline anaemia, producing pale appearance, fast heart rate, and fatigue. This crisis is normally triggered by parvovirus B19, which directly affects production of red blood cells by invading the red cell precursors and multiplying in and destroying them. Parvovirus infection almost completely prevents red blood cell production for two to three days. In normal individuals, this is of little consequence, but the shortened red cell life of SCD patients results in an abrupt, life-threatening situation. Reticulocyte counts drop dramatically during the disease (causing reticulocytopenia), and the rapid turnover of red cells leads to the drop in haemoglobin. This crisis takes 4 days to one week to disappear. Most patients can be managed supportively; some need blood transfusion.

Haemolytic Crisis

Haemolytic crises are acute accelerated drops in haemoglobin level. The red blood cells break down at a faster rate. This is particularly common in patients with coexistent G6PD deficiency. Management is supportive, sometimes with blood transfusions.

Other

One of the earliest clinical manifestations is dactylitis, presenting as early as six months of age, and may occur in children with sickle-cell trait. The crisis can last up to a month. Another recognised type of sickle crisis, acute chest syndrome, is characterised by fever, chest pain, difficulty breathing, and pulmonary infiltrate on a chest X-ray. Given that pneumonia and sickling in the lung can both produce these symptoms, the patient is treated for both conditions. It can be triggered by painful crisis, respiratory infection, bone-marrow embolisation, or possibly by atelectasis, opiate administration, or surgery. Hematopoietic ulcers may also occur.

GENETICS

Normally, humans have haemoglobin A, which consists of two alpha and two beta chains, haemoglobin A2, which consists of two alpha and two delta chains, and haemoglobin F, consisting of two alpha and two gamma chains in their bodies. Out of these three types, haemoglobin F dominates until about 6 weeks of age. Afterwards, haemoglobin A dominates throughout life. In people diagnosed with sickle cell disease, at least one of the β-globin subunits in haemoglobin A is replaced with what's known as haemoglobin S. In sickle cell anaemia, a common form of sickle cell disease, haemoglobin S replaces both β-globin subunits in the haemoglobin.

Sickle-cell conditions have an autosomal recessive pattern of inheritance from parents. The types of haemoglobin a person makes in the red blood cells depend on what haemoglobin genes are inherited from her or his parents. If one parent has sickle-cell anaemia and the other has sickle-cell trait, then the child has a 50% chance of having sickle-cell disease and a 50% chance of having sickle-cell trait. When both parents have sickle-cell trait, a child has a 25% chance of sickle-cell disease, 25% do not carry any sickle-cell alleles, and 50% have the heterozygous condition.

Sickle-cell gene mutation probably arose spontaneously in different geographic areas, as suggested by restriction endonuclease analysis. These variants are known as Cameroon, Senegal, Benin, Bantu, and Saudi-Asian. Their clinical importance is because some are associated with higher HbF levels, *e.g.,* Senegal and Saudi-Asian variants, and tend to have milder disease. The gene defect is a known mutation of a single nucleotide (GAG codon changing to GTG) of the β-globin gene, which results in glutamic acid (E/Glu) being substituted by valine (V/Val) at position 6.

Haemoglobin S with this mutation is referred to as HbS, as opposed to the normal adult HbA. This is normally a benign mutation, causing no apparent effects on the secondary, tertiary, or quaternary structures of haemoglobin in conditions of normal oxygen concentration. What it does allow for, under conditions of low oxygen concentration, is the polymerization of the HbS itself. The deoxy form of haemoglobin exposes a hydrophobic patch on the protein between the E and F helices (Phe 85, Leu 88). The hydrophobic side chain of the valine residue at position 6 of the beta chain in haemoglobin is able to associate with the hydrophobic patch, causing HbS molecules to aggregate and form fibrous precipitates.

In people heterozygous for HbS (carriers of sickling haemoglobin), the polymerisation problems are minor, because the normal allele is able to produce half of the haemoglobin. In people homozygous for HbS, the presence of long-chain polymers of HbS distort the shape of the red blood cell from a smooth doughnut-like shape

to ragged and full of spikes, making it fragile and susceptible to breaking within capillaries. Carriers have symptoms only if they are deprived of oxygen (for example, while climbing a mountain) or while severely dehydrated. The allele responsible for sickle-cell anaemia can be found on the short arm of chromosome 11, more specifically 11p15.5. A person who receives the defective gene from both father and mother develops the disease; a person who receives one defective and one healthy allele remains healthy, but can pass on the disease and is known as a carrier or heterozygote. Heterozygotes are still able to contract malaria, but their symptoms are generally less severe.

Due to the adaptive advantage of the heterozygote, the disease is still prevalent, especially among people with recent ancestry in malaria-stricken areas, such as Africa, the Mediterranean, India, and the Middle East. Malaria was historically endemic to southern Europe, but it was declared eradicated in the mid-20th century, with the exception of rare sporadic cases. The malaria parasite has a complex lifecycle and spends part of it in red blood cells. In a carrier, the presence of the malaria parasite causes the red blood cells with defective haemoglobin to rupture prematurely, making the *Plasmodium* parasite unable to reproduce. Further, the polymerization of Hb affects the ability of the parasite to digest Hb in the first place. Therefore, in areas where malaria is a problem, people's chances of survival actually increase if they carry sickle-cell trait (selection for the heterozygote).

In the United States, with no endemic malaria, the prevalence of sickle-cell anaemia among African Americans is lower (about 0.25%) than in West Africa (about 4.0%) and is falling. Without endemic malaria, the sickle-cell mutation is purely disadvantageous and tends to decline in the affected population by natural selection, and now artificially through prenatal genetic screening. However, the African American community descends from a significant admixture of several African and non-African ethnic groups and also represents the descendants of survivors of slavery and the slave trade. Thus, a lower degree of endogamy and, particularly, abnormally high health-selective pressure through slavery may be the most plausible explanations for the lower prevalence of sickle-cell anaemia (and, possibly, other genetic diseases) among African Americans compared to West Africans. Another factor that limits the spread of sickle-cell genes in North America is the absence of cultural proclivities to polygamy, which allows affected males to continue to seek unaffected children with multiple partners.

PATHOPHYSIOLOGY

The loss of red blood cell elasticity is central to the pathophysiology of sickle-cell disease. Normal red blood cells are quite elastic, which allows the cells to deform to pass through capillaries. In sickle-cell disease, low oxygen tension promotes red blood cell sickling and repeated episodes of sickling damage the cell membrane and decrease the cell's elasticity. These cells fail to return to normal shape when normal oxygen tension is restored. As a consequence, these rigid blood cells are unable to deform as they pass through narrow capillaries, leading to vessel occlusion and ischaemia. The actual anaemia of the illness is caused by haemolysis, the destruction of the red cells, because of their shape. Although the bone marrow attempts to compensate by creating new red cells, it does not match the rate of destruction. Healthy red blood cells typically function for 90–120 days, but sickled cells only last 10–20 days.

DIAGNOSIS

In HbS, the complete blood count reveals haemoglobin levels in the range of 6–8 g/dl with a high reticulocyte count (as the bone marrow compensates for the destruction of sickled cells by producing more red blood cells). In other forms of sickle-cell disease, Hb levels tend to be higher. A blood film may show features of hyposplenism (target cells and Howell-Jolly bodies). Sickling of the red blood cells, on a blood film, can be induced by the addition of sodium metabisulfite. The presence of sickle haemoglobin can also be demonstrated with the "sickle solubility test". A mixture of haemoglobin S (Hb S) in a reducing solution (such as sodium dithionite) gives a turbid appearance, whereas normal Hb gives a clear solution. Abnormal haemoglobin forms can be detected on

haemoglobin electrophoresis, a form of gel electrophoresis on which the various types of haemoglobin move at varying speeds. Sickle-cell haemoglobin (HgbS) and haemoglobin C with sickling (HgbSC)—the two most common forms—can be identified from there. The diagnosis can be confirmed with high-performance liquid chromatography. Genetic testing is rarely performed, as other investigations are highly specific for HbS and HbC. An acute sickle-cell crisis is often precipitated by infection. Therefore, a urinalysis to detect an occult urinary tract infection, and chest X-ray to look for occult pneumonia should be routinely performed. People who are known carriers of the disease often undergo genetic counseling before they have a child. A test to see if an unborn child has the disease takes either a blood sample from the fetus or a sample of amniotic fluid. Since taking a blood sample from a fetus has greater risks, the latter test is usually used. Neo-natal screening provides not only a method of early detection for individuals with sickle-cell disease, but also allows for identification of the groups of people that carry the sickle cell trait.

MANAGEMENT

Treatment involves a number of measures. L-glutamine use was supported by the FDA starting at the age of 5 as it decreases complications.

Folic Acid and Penicillin

From birth to five years of age, penicillin daily, due to the immature immune system that makes them more prone to early childhood illnesses is recommended. Dietary supplementation of folic acid had been previously recommended by the WHO. A 2016 Cochrane review of its use found "the effect of supplementation on anaemia and any symptoms of anaemia remains unclear" due to a lack of medical evidence.

Malaria Prevention

The protective effect of sickle-cell trait does not apply to people with sickle cell disease; in fact, they are more vulnerable to malaria, since the most common cause of painful crises in malarial countries is infection with malaria. It has therefore been recommended that people with sickle-cell disease living in malarial countries should receive anti-malarial chemoprophylaxis for life.

Vaso-occlusive Crisis

Most people with sickle-cell disease have intensely painful episodes called vaso-occlusive crises. However, the frequency, severity, and duration of these crises vary tremendously. Painful crises are treated symptomatically with pain medications; pain management requires opioid administration at regular intervals until the crisis has settled. For milder crises, a subgroup of patients manage on NSAIDs (such as diclofenac or naproxen). For more severe crises, most patients require inpatient management for intravenous opioids; patient-controlled analgesia (PCA) devices are commonly used in this setting. Diphenhydramine is also an effective agent that doctors frequently prescribe to help control itching associated with the use of opioids.

Acute Chest Crisis

Management is similar to vaso-occlusive crisis, with the addition of antibiotics (usually a quinolone or macrolide, since cell wall-deficient ["atypical"] bacteria are thought to contribute to the syndrome), oxygen supplementation for hypoxia, and close observation. Should the pulmonary infiltrate worsen or the oxygen requirements increase, simple blood transfusion or exchange transfusion is indicated. The latter involves the exchange of a significant portion of the person's red cell mass for normal red cells, which decreases the percent of haemoglobin S in the

patient's blood. The patient with suspected acute chest syndrome should be admitted to the hospital with worsening A-a gradient an indication for ICU admission.

Hydroxyurea

The first approved drug for the causative treatment of sickle-cell anaemia, hydroxyurea, was shown to decrease the number and severity of attacks in a study in 1995 and shown to possibly increase survival time in a study in 2003. This is achieved, in part, by reactivating fetal haemoglobin production in place of the haemoglobin S that causes sickle-cell anaemia. Hydroxyurea had previously been used as a chemotherapy agent, and there is some concern that long-term use may be harmful, but this risk has been shown to be either absent or very small and it is likely that the benefits outweigh the risks.

Blood Transfusion

Blood transfusions are often used in the management of sickle-cell disease in acute cases and to prevent complications by decreasing the number of red blood cells (RBC) that can sickle by adding normal red blood cells. In children preventative red blood cell (RBC) transfusion therapy has been shown to reduce the risk of first stroke or silent stroke when transcranial Doppler (TCD) ultrasonography shows abnormal cerebral blood flow. In those who have sustained a prior stroke event it also reduces the risk of recurrent stroke and additional silent strokes.

Bone Marrow Transplant

Bone marrow transplants have proven effective in children. Bone marrow transplants are the only known cure for SCD. However, bone marrow transplants are difficult to obtain because of the specific HLA typing necessary. Ideally, a close relative (allogeneic) would donate the bone marrow necessary for transplantation.

Avascular Necrosis

When treating avascular necrosis of the bone in people with sickle cell disease, the aim of treatment is to reduce or stop the pain and maintain joint mobility. Current treatment options are to rest the joint, physical therapy, pain relief medicine, joint replacement surgery, or bone grafting. High quality randomized controlled trials are needed to assess the most effective treatment option and determine if a combination of physical therapy and surgery are more effective than physical therapy alone.

Psychological Therapies

Psychological therapies such as patient education, cognitive therapy, behavioural therapy and psychodynamic psychotherapy, that aim to complement current medical treatments, require further research to determine their effectiveness.

PROGNOSIS

About 90% of people survive to age 20, and close to 50% survive beyond the fifth decade. In 2001, according to one study performed in Jamaica, the estimated mean survival for people with sickle-cell was 53 years old for men and 58 years old for women with homozygous SCD. The specific life expectancy in much of the developing world is unknown.

Complications

Sickle-cell anaemia can lead to various complications, including:

- Increased risk of severe bacterial infections due to loss of functioning spleen tissue (and comparable to the risk of infections after having the spleen removed surgically). These infections are typically caused by encapsulated organisms such as *Streptococcus pneumoniae* and *Haemophilus influenzae*. Daily penicillin prophylaxis is the most commonly used treatment during childhood, with some haematologists continuing treatment indefinitely. Patients benefit today from routine vaccination for *S. pneumoniae*.
- Stroke, which can result from a progressive narrowing of blood vessels, prevents oxygen from reaching the brain. Cerebral infarction occurs in children and cerebral haemorrhage in adults.
- Silent stroke causes no immediate symptoms, but is associated with damage to the brain. Silent stroke is probably five times as common as symptomatic stroke. About 10–15% of children with SCD suffer strokes, with silent strokes predominating in the younger patients.
- Cholelithiasis (gallstones) and cholecystitis may result from excessive bilirubin production and precipitation due to prolonged haemolysis.
- Avascular necrosis (aseptic bone necrosis) of the hip and other major joints may occur as a result of ischaemia.
- Decreased immune reactions due to hyposplenism (malfunctioning of the spleen)
- Priapism and infarction of the penis
- Osteomyelitis (bacterial bone infection), the most common cause of osteomyelitis in SCD is *Salmonella* (especially the atypical serotypes *Salmonella typhimurium, Salmonella enteritidis, Salmonella choleraesuis* and *Salmonella paratyphi* B), followed by *Staphylococcus aureus* and Gram-negative enteric bacilli perhaps because intravascular sickling of the bowel leads to patchy ischaemic infarction.
- Acute papillary necrosis in the kidneys
- Leg ulcers
- In eyes, background retinopathy, proliferative retinopathy, vitreous haemorrhages, and retinal detachments can result in blindness. Regular annual eye checks are recommended.
- During pregnancy, intrauterine growth retardation, spontaneous abortion, and pre-eclampsia
- Chronic pain: Even in the absence of acute vaso-occlusive pain, many patients have unreported chronic pain.
- Pulmonary hypertension (increased pressure on the pulmonary artery) can lead to strain on the right ventricle and a risk of heart failure; typical symptoms are shortness of breath, decreased exercise tolerance, and episodes of syncope. 21% of children and 30% of adults have evidence of pulmonary hypertension when tested; this is associated with reduced walking distance and increased mortality.
- Chronic kidney failure due to sickle-cell nephropathy manifests itself with hypertension, protein loss in the urine, loss of red blood cells in urine and worsened anaemia. If it progresses to end-stage renal failure, it carries a poor prognosis.

EPIDEMIOLOGY

The highest frequency of sickle cell disease is found in tropical regions, particularly sub-Saharan Africa, tribal regions of India and the Middle-East. Migration of substantial populations from these high prevalence areas to low prevalence countries in Europe has dramatically increased in recent decades and in some European countries sickle-cell disease has now overtaken more familiar genetic conditions such as haemophilia and cystic fibrosis. In 2015, it resulted in about 114,800 deaths. Sickle-cell disease occurs more commonly among people whose ancestors lived in tropical and sub-tropical sub-Saharan regions where malaria is or was common. Where malaria is common, carrying a single sickle-cell allele (trait) confers a heterozygote advantage: humans with one of the two alleles of sickle-cell disease show less severe symptoms when infected with malaria. This

condition is inherited in an autosomal recessive pattern, which means both copies of the gene in each cell have mutations. The parents each carry one copy of the mutated gene, but they typically do not show signs and symptoms of the condition.

Africa

Three-quarters of sickle-cell cases occur in Africa. A recent WHO report estimated that around 2% of newborns in Nigeria were affected by sickle cell anaemia, giving a total of 150,000 affected children born every year in Nigeria alone. The carrier frequency ranges between 10% and 40% across equatorial Africa, decreasing to 1–2% on the north African coast and <1% in South Africa. There have been studies in Africa that show a significant decrease in infant mortality rate, ages 2–16 months, because of the sickle-cell trait. This happened in predominant areas of malarial cases.

United States

The number of people with the disease in the United States is approximately 1 in 5,000, mostly affecting Americans of sub-Saharan African descent, according to the National Institutes of Health. In the United States, about one out of 500 African-American children and one in every 36,000 Hispanic-American children have sickle-cell anaemia.

It is estimated that sickle-cell disease affects 90,000 Americans. Most infants with SCD born in the United States are now identified by routine neo-natal screening. As of 2016 all 50 states include screening for sickle cell disease as part of their newborn screen. Patient advocates for sickle-cell disease have complained that it gets less government and private research funding than similar rare diseases like cystic fibrosis, with researcher Elliott Vichinsky saying this shows racial discrimination or the role of wealth in health care advocacy.

France

As a result of population growth in African-Caribbean regions of overseas France and immigration from North and sub-Saharan Africa to mainland France, sickle-cell disease has become a major health problem in France. SCD has become the most common genetic disease in the country, with an overall birth prevalence of 1/2,415 in Metropolitan France, ahead of phenylketonuria (1/10,862), congenital hypothyroidism (1/3,132), congenital adrenal hyperplasia (1/19,008) and cystic fibrosis (1/5,014) for the same reference period. Since 2000, neo-natal screening of SCD has been performed at national level for all newborns defined as being "at risk" for SCD based on ethnic origin (defined as those born to parents originating from sub-Saharan Africa, North Africa, the Mediterranean area (South Italy, Greece and Turkey), the Arabic peninsula, the French overseas islands and the Indian subcontinent).

United Kingdom

In the United Kingdom (UK) it is thought that between 12,000 and 15,000 people have sickle cell disease with an estimate of 250,000 carriers of the condition in England alone. As the number of carriers is only estimated, all newborn babies in the UK receive a routine blood test to screen for the condition. Due to many adults in high-risk groups not knowing if they are carriers, pregnant women and both partners in a couple are offered screening so they can get counselling if they have the sickle cell trait. In addition blood donors from those in high-risk groups are also screened to confirm whether they are carriers and whether their blood filters properly. Donors who are found to be carriers are then informed and their blood, while often used for those of the same ethnic group, is not used for those with sickle cell disease who require a blood transfusion.

Middle East

In Saudi Arabia, about 4.2% of the population carry the sickle-cell trait and 0.26% have sickle-cell disease. The highest prevalence is in the Eastern province where approximately 17% of the population carry the gene and 1.2% have sickle-cell disease. In 2005 in Saudi Arabia a mandatory pre-marital test including HB electrophoresis was launched and aimed to decrease the incidence of SCD and thalassemia. In Bahrain a study published in 1998 that covered about 56,000 people in hospitals in Bahrain found that 2% of newborns have sickle cell disease, 18% of the surveyed people have the sickle cell trait, and 24% were carriers of the gene mutation causing the disease. The country began screening of all pregnant women in 1992 and newborns started being tested if the mother was a carrier. In 2004, a law was passed requiring couples planning to get married to undergo free premarital counseling. These programmes were accompanied by public education campaigns.

India and Nepal

Sickle-cell disease is common in ethnic groups of central India who share a genetic linkage with African communities, where the prevalence has ranged from 9.4 to 22.2% in endemic areas of Madhya Pradesh, Rajasthan and Chhattisgarh. It is also endemic among Tharu people of Nepal and India; however, they have a sevenfold lower incidence of malaria despite living in a malaria infested zone.

Caribbean Islands

In Jamaica, 10% of the population carries the sickle-cell gene, making it the most prevalent genetic disorder in the country.

HISTORY

The first modern report of sickle-cell disease may have been in 1846, where the autopsy of an executed runaway slave was discussed; the key findings was the absence of the spleen. There were also reports amongst African slaves in the United States exhibiting resistance to malaria but being prone to leg ulcers. The abnormal characteristics of the red blood cells, which later lent their name to the condition, was first described by Ernest E. Irons (1877–1959), intern to the Chicago cardiologist and professor of medicine James B. Herrick (1861–1954), in 1910. Irons saw "peculiar elongated and sickle-shaped" cells in the blood of a man named Walter Clement Noel, a 20-year-old first-year dental student from Grenada. Noel had been admitted to the Chicago Presbyterian Hospital in December 1904 suffering from anaemia. Noel was readmitted several times over the next three years for "muscular rheumatism" and "bilious attacks" but completed his studies and returned to the capital of Grenada (St. George's) to practice dentistry. He died of pneumonia in 1916 and is buried in the Catholic cemetery at Sauteurs in the north of Grenada. Shortly after the report by Herrick, another case appeared in the *Virginia Medical Semi-Monthly* with the same title, "Peculiar Elongated and Sickle-Shaped Red Blood Corpuscles in a Case of Severe Anemia." This article is based on a patient admitted to the University of Virginia Hospital on November 15, 1910. In the later description by Verne Mason in 1922, the name "sickle cell anemia" is first used. Childhood problems related to sickle cells disease were not reported until the 1930s, despite the fact that this cannot have been uncommon in African-American populations.

The Memphis physician Lemuel Diggs, a prolific researcher into sickle cell disease, first introduced the distinction between sickle cell disease and trait in 1933, although it took until 1949 until the genetic characteristics were elucidated by James V. Neel and E.A. Beet. 1949 was the year when Linus Pauling described the unusual chemical behaviour of haemoglobin S, and attributed this to an abnormality in the molecule itself. The actual

molecular change in HbS was described in the late 1950s BY Vernon Ingram. The late 1940s and early 1950s saw further understanding in the link between malaria and sickle cell disease. In 1954, the introduction of haemoglobin electrophoresis allowed the discovery of particular subtypes, such as HbSC disease. Large scale natural history studies and further intervention studies were introduced in the 1970s and 1980s, leading to widespread use of prophylaxis against pneumococcal infections amongst other interventions. Bill Cosby's Emmy-winning 1972 TV movie, *To All My Friends on Shore*, depicted the story of the parents of a child suffering from sickle-cell disease. The 1990s saw the development of hydroxycarbamide, and reports of cure through bone marrow transplantation appeared in 2007. Some old texts refer to it as drepanocytosis.

SOCIETY AND CULTURE

Effective September 15, 2017, the U.S. Social Security Administration issued a Policy Interpretation Ruling providing background information on sickle cell disease and a description of how Social Security evaluates the disease during its adjudication process for disability claims.

RESEARCH

Umbilical Cord Blood Transplant

While umbilical cord blood transplant can potentially cure the condition, a suitable donor is available in only 10% of people. About 7% of people also die as a result of the procedure and graft versus host disease may occur.

Gene Therapy

In 2001 it was reported that sickle-cell disease had been successfully treated in mice using gene therapy. The researchers used a viral vector to make the mice—which have essentially the same defect that causes human sickle cell disease—express production of fetal haemoglobin (HbF), which an individual normally ceases to produce shortly after birth. In humans, using hydroxyurea to stimulate the production of HbF has been known to temporarily alleviate sickle cell disease symptoms. The researchers demonstrated that this gene therapy method is a more permanent way to increase therapeutic HbF production. Phase 1 clinical trials of gene therapy for sickle cell disease in humans were started in 2014. The clinical trials will assess the safety and initial evidence for efficacy of an autologous transplant of lentiviral vector-modified bone marrow for adults with severe sickle cell disease. As of 2016, however, no randomized controlled trials have been reported. A case report for the first person treated was published in March 2017.

THALASSEMIA

Thalassemias are inherited blood disorders characterized by abnormal hemoglobin production. Symptoms depend on the type and can vary from none to severe. Often there is mild to severe anemia (low red blood cells). Anemia can result in feeling tired and pale skin. There may also be bone problems, an enlarged spleen, yellowish skin, dark urine, and among children slow growth. Thalassemias are genetic disorders inherited from a person's parents. There are two main types, alpha thalassemia and beta thalassemia. The severity of alpha and beta thalassemia depends on how many of the four genes for alpha globin or two genes for beta globin are missing. Diagnosis is typically by blood tests including a complete blood count, special hemoglobin tests, and genetic tests. Diagnosis may occur before birth through prenatal testing. Treatment depends on the type and severity. Treatment for those with more severe disease often includes regular blood transfusions, iron chelation, and

folic acid. Iron chelation may be done with deferoxamine or deferasirox. Occasionally, a bone marrow transplant may be an option. Complications may include iron overload from the transfusions with resulting heart or liver disease, infections, and osteoporosis. If the spleen becomes overly enlarged, surgical removal may be required.

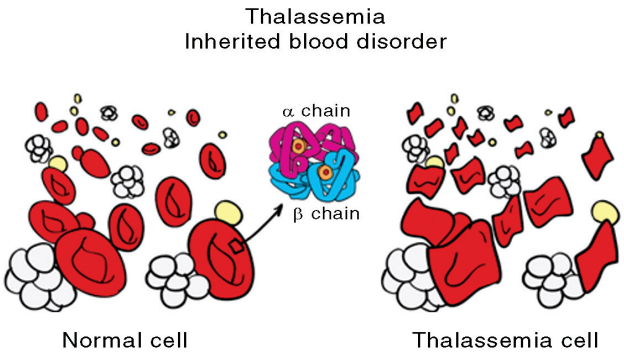


Fig. Thalassemia.

As of 2013, thalassemia occurs in about 280 million people, with about 439,000 having severe disease. It is most common among people of Italian, Greek, Middle Eastern, South Asian, and African descent. Males and females have similar rates of disease. It resulted in 16,800 deaths in 2015, down from 36,000 deaths in 1990. Those who have minor degrees of thalassemia, similar to those with sickle-cell trait, have some protection against malaria, explaining why they are more common in regions of the world where malaria exists.

SIGNS AND SYMPTOMS

- Iron overload: People with thalassemia can get an overload of iron in their bodies, either from the disease itself or from frequent blood transfusions. Too much iron can result in damage to the heart, liver, and endocrine system, which includes glands that produce hormones that regulate processes throughout the body. The damage is characterized by excessive deposits of iron. Without adequate iron chelation therapy, almost all patients with beta-thalassemia accumulate potentially fatal iron levels.
- Infection: People with thalassemia have an increased risk of infection. This is especially true if the spleen has been removed.
- Bone deformities: Thalassemia can make the bone marrow expand, which causes bones to widen. This can result in abnormal bone structure, especially in the face and skull. Bone marrow expansion also makes bones thin and brittle, increasing the risk of broken bones.
- Enlarged spleen: The spleen aids in fighting infection and filters unwanted material, such as old or damaged blood cells. Thalassemia is often accompanied by the destruction of a large number of red blood cells and the task of removing these cells causes the spleen to enlarge. Splenomegaly can make anemia worse, and it can reduce the life of transfused red blood cells. Severe enlargement of the spleen may necessitate its removal.
- Slowed growth rates: anemia can cause a child's growth to slow. Puberty also may be delayed in children with thalassemia.
- Heart problems: Diseases, such as congestive heart failure and abnormal heart rhythms, may be associated with severe thalassemia.

CAUSE

Both α- and β-thalassemias are often inherited in an autosomal recessive manner. Cases of dominantly inherited α- and β-thalassemias have been reported, the first of which was in an Irish family with two deletions of 4 and 11 bp in exon 3 interrupted by an insertion of 5 bp in the β-globin gene. For the autosomal recessive

forms of the disease, both parents must be carriers for a child to be affected. If both parents carry a hemoglobinopathy trait, the risk is 25% for each pregnancy for an affected child. Estimates suggest that approximately 1.5% of the global population (80 - 90 million people) are β-thalassemia carriers. However, exact data on carrier rates in many populations are lacking, particularly in developing areas of the world known or expected to be heavily affected. Because of the prevalence of the disease in countries with little knowledge of thalassemia, access to proper treatment and diagnosis can be difficult. While there are some diagnostic and treatment facilities in developing countries, in most cases these are not provided by government services, and are available only to patients that can afford them. In general, poorer populations only have access to limited diagnostic facilities together with blood transfusions. In some developing countries, there are virtually no facilities for diagnosis or management of thalassemia.

Evolution

Having a single genetic variant for thalassemia may protect against malaria and thus be an advantage. People diagnosed with heterozygous (carrier) β-thalassemia have some protection against coronary heart disease.

PATHOPHYSIOLOGY

Normally, the majority of adult hemoglobin (HbA) is composed of four protein chains, two α and two β globin chains arranged into a heterotetramer. In thalassemia, patients have defects in either the α or β globin chain, causing production of abnormal red blood cells (In sickle-cell disease, the mutation is specific to β globin). The thalassemias are classified according to which chain of the hemoglobin molecule is affected. In α-thalassemias, production of the α globin chain is affected, while in β-thalassemia, production of the β globin chain is affected. The β globin chains are encoded by a single gene on chromosome 11; α globin chains are encoded by two closely linked genes on chromosome 16. Thus, in a normal person with two copies of each chromosome, two loci encode the β chain, and four loci encode the α chain. Deletion of one of the α loci has a high prevalence in people of African or Asian descent, making them more likely to develop α-thalassemia. β-Thalassemias are not only common in Africans, but also in Greeks and Italians.

Alpha-thalassemias

The α-thalassemias involve the genes *HBA1* and *HBA2*, inherited in a Mendelian recessive fashion. Two gene loci and so four alleles exist. It is also connected to the deletion of the 16p chromosome. α Thalassemias result in decreased alpha-globin production, therefore fewer alpha-globin chains are produced, resulting in an excess of β chains in adults and excess γ chains in newborns. The excess β chains form unstable tetramers (called hemoglobin H or HbH of 4 beta chains), which have abnormal oxygen dissociation curves.

Beta-thalassemia

Beta thalassemias are due to mutations in the *HBB gene* on chromosome 11, also inherited in an autosomal, recessive fashion. The severity of the disease depends on the nature of the mutation and on the presence of mutations in one or both alleles. Mutated alleles are called β when partial function is conserved (either the protein has a reduced function, or it functions normally but is produced in reduced quantity) or β, when no functioning protein is produced.

The situation of both alleles determines the clinical picture:

- β thalassemia major (Mediterranean anemia or Cooley anemia) is caused by a β/β genotype. No functional β chains are produced, and thus no hemoglobin A can be assembled. This is the most severe form of β-thalassemia;
- β thalassemia intermedia is caused by a β/β or β/β genotype. In this form, some hemoglobin A is produced;

- β thalassemia minor is caused by a β/β or β/β genotype. Only one of the two β globin alleles contains a mutation, so β chain production is not terribly compromised and patients may be relatively asymptomatic.

Delta-thalassemia

As well as alpha and beta chains present in hemoglobin, about 3% of adult hemoglobin is made of alpha and delta chains. Just as with beta thalassemia, mutations that affect the ability of this gene to produce delta chains can occur.

Combination Hemoglobinopathies

Thalassemia can coexist with other hemoglobinopathies. The most common of these are:

- Hemoglobin E/thalassemia: common in Cambodia, Thailand, and parts of India, it is clinically similar to β thalassemia major or thalassemia intermedia.
- Hemoglobin S/thalassemia: common in African and Mediterranean populations, is clinically similar to sickle-cell anemia, with the additional feature of splenomegaly.
- Hemoglobin C/thalassemia: common in Mediterranean and African populations, hemoglobin C/β thalassemia causes a moderately severe hemolytic anemia with splenomegaly; hemoglobin C/β thalassemia produces a milder disease.
- Hemoglobin D/thalassemia: common in the northwestern parts of India and Pakistan (Punjab region).

DIAGNOSIS

Thalassemia can be diagnosed via a complete blood count, hemoglobin electrophoresis, and DNA testing.

PREVENTION

The American College of Obstetricians and Gynecologists recommends all people thinking of becoming pregnant be tested to see if they have thalassemia. Genetic counseling and genetic testing are recommended for families who carry a thalassemia trait. A screening policy exists in Cyprus to reduce the rate of thalassemia, which, since the program's implementation in the 1970s (which also includes prenatal screening and abortion), has reduced the number of children born with the disease from one of every 158 births to almost zero. In Iran as a premarital screening, the man's red cell indices are checked first, if he has microcytosis (mean cell hemoglobin < 27 pg or mean red cell volume < 80 fl), the woman is tested.

When both are microcytic, their hemoglobin A2 concentrations are measured. If both have a concentration above 3.5% (diagnostic of thalassemia trait) they are referred to the local designated health post for genetic counseling. Large scale awareness campaigns are being organized in India both by government and non-government organizations in favour of voluntary premarital screening to detect carriers of thalassemia and marriage between both carriers are strongly discouraged.

MANAGEMENT

Mild thalassemia: people with thalassemia traits do not require medical or follow-up care after the initial diagnosis is made. People with â-thalassemia trait should be warned that their condition can be misdiagnosed as the more common iron deficiency anemia. They should avoid routine use of iron supplements; iron deficiency can develop, though, during pregnancy or from chronic bleeding. Counseling is indicated in all persons with genetic disorders, especially when the family is at risk of a severe form of disease that may be prevented.

Blood Transfusions

People with severe thalassemia require medical treatment. A blood transfusion regimen was the first measure effective in prolonging life.

Medications

Multiple blood transfusions can result in iron overload. The iron overload related to thalassemia may be treated by chelation therapy with the medications deferoxamine, deferiprone, or deferasirox. These treatments have resulted in improving life expectancy in those with thalassemia major. Deferoxamine is only effective via daily injections which makes its long-term use more difficult. It has the benefit of being inexpensive and decent long-term safety. Adverse effects are primary skin reactions around the injection site and hearing loss. Deferasirox has the benefit of being an oral medication. Common side effects include: nausea, vomiting and diarrhea. It however is not effective in everyone and is probably not suitable in those with significant cardiac issues related to iron overload. The cost is also significant. Deferiprone is a medication that is given by mouth. Nausea, vomiting, and diarrhea are relatively common with its use. It is available in both Europe and the United States. It appears to be the most effective agent when the heart is significantly involved. There is no evidence from randomized controlled trial to support zinc supplementation in thalassemia.

Bone Marrow Transplant

Bone marrow transplantation may offer the possibility of a cure in young people who have an HLA-matched donor. Success rates have been in the 80–90% range. Mortality from the procedure is about 3%. There are no randomized controlled trials which have tested the safety and efficacy of non-identical donor bone marrow transplantation in persons with â- thalassemia who are dependent on blood transfusion. If the person does not have an HLA-matched compatible donor, another method called bone marrow transplantation (BMT) from haploidentical mother to child (mismatched donor) may be used. In a study of 31 people, the thalassemia-free survival rate 70%, rejection 23%, and mortality 7%. The best results are with very young people.

EPIDEMIOLOGY

The beta form of thalassemia is particularly prevalent among Mediterranean peoples, and this geographical association is responsible for its naming. Thalassemia resulted in 25,000 deaths in 2013 down from 36,000 deaths in 1990. In Europe, the highest concentrations of the disease are found in Greece, coastal regions in Turkey (particularly the Aegean Region such as Izmir, Balikesir, Aydin, Mugla, and Mediterranean Region such as Antalya, Adana, Mersin), in parts of Italy, particularly southern Italy and the lower Po valley. The major Mediterranean islands (except the Balearics) such as Sicily, Sardinia, Malta, Corsica, Cyprus, and Crete are heavily affected in particular.

Other Mediterranean people, as well as those in the vicinity of the Mediterranean, also have high rates of thalassemia, including people from West Asia and North Africa. Far from the Mediterranean, South Asians are also affected, with the world's highest concentration of carriers (30% of the population) being in the Maldives. Nowadays, it is found in populations living in Africa, the Americas, and in Tharu people in the Terai region of Nepal and India. It is believed to account for much lower malaria sicknesses and deaths, accounting for the historic ability of Tharus to survive in areas with heavy malaria infestation, where others could not. Thalassemias are particularly associated with people of Mediterranean origin, Arabs (especially Palestinians and people of Palestinian descent), and Asians. The Maldives has the highest incidence of thalassemia in the world with a carrier rate of 18% of the population. The estimated prevalence is 16% in people from Cyprus, 1% in Thailand,

and 3–8% in populations from Bangladesh, China, India, Malaysia and Pakistan. Thalassemias also occur in descendants of people from Mediterranean countries (*e.g.,* Greece, Italy, Spain, and others), in Latin America.

SOCIETY AND CULTURE

In 2008, in Spain, a baby was selectively implanted to be a cure for his brother's thalassemia. The child was born from an embryo screened to be free of the disease before implantation with *in vitro* fertilization. The baby's supply of immunologically compatible cord blood was saved for transplantation to his brother. The transplantation was considered successful. In 2009, a group of doctors and specialists in Chennai and Coimbatore registered the successful treatment of thalassemia in a child using an unaffected sibling's umbilical cord blood.

HEMOLYTIC DISEASE OF THE NEWBORN

Hemolytic disease of the newborn, also known as hemolytic disease of the fetus and newborn, HDN, HDFN, or erythroblastosis fetalis, is an alloimmune condition that develops in a peripartum fetus, when the IgG molecules (one of the five main types of antibodies) produced by the mother pass through the placenta. Among these antibodies are some which attack antigens on the red blood cells in the fetal circulation, breaking down and destroying the cells (hemolysis).

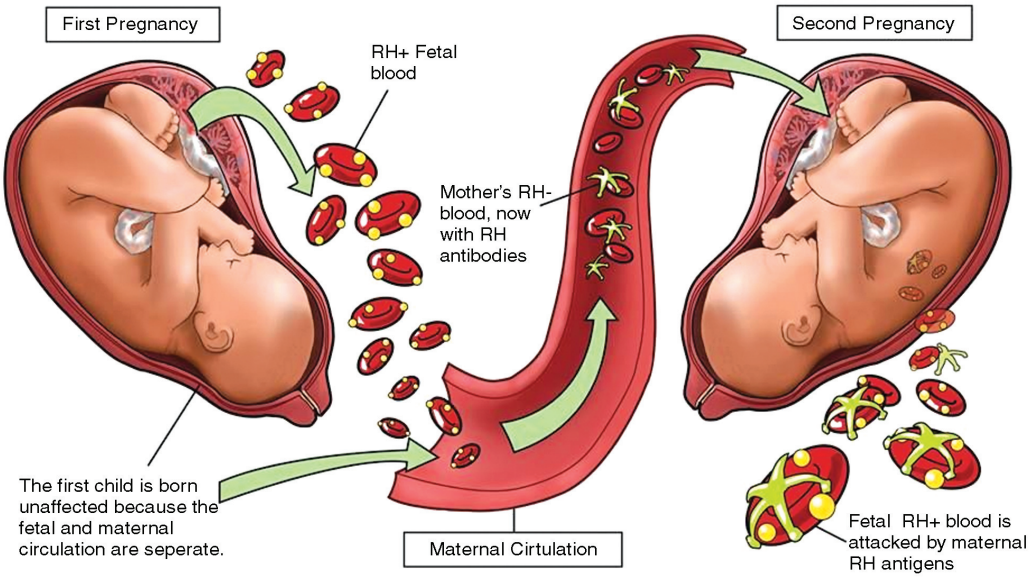


Fig. Hemolytic disease of the newborn.

The fetus can develop reticulocytosis and anemia. This fetal disease ranges from mild to very severe, and fetal death from heart failure (hydrops fetalis) can occur. When the disease is moderate or severe, many erythroblasts (immature red blood cells) are present in the fetal blood, and so these forms of the disease can be called *erythroblastosis fetalis* (or *erythroblastosis foetalis*). HDFN represents a breach of immune privilege for the fetus or some other form of impairment of the immune tolerance of pregnancy. Various types of HDFN are classified by which alloantigen provokes the response. In order of incidence, the types include ABO, anti-RhD, anti-RhE, anti-Rhc, anti-Rhe, anti-RhC, multiantigen combinations, and anti-Kell.

SIGNS AND SYMPTOMS

Signs of hemolytic disease of the newborn include a positive direct Coombs test (also called direct agglutination test), elevated cord bilirubin levels, and hemolytic anemia. It is possible for a newborn with this disease to have neutropenia and neo-natal alloimmune thrombocytopenia as well. Hemolysis leads to elevated bilirubin levels.

After delivery bilirubin is no longer cleared (via the placenta) from the neonate's blood and the symptoms of jaundice (yellowish skin and yellow discoloration of the whites of the eyes, or icterus) increase within 24 hours after birth. Like other forms of severe neonatal jaundice, there is the possibility of the neonate developing acute or chronic kernicterus, however the risk of kernicterus in HDN is higher because of the rapid and massive destruction of blood cells. It is important to note that isoimmunization is a risk factor for neurotoxicity and lowers the level at which kernicterus can occur. Untreated profound anemia can cause high-output heart failure, with pallor, enlarged liver and/or spleen, generalized swelling, and respiratory distress. HDN can be the cause of hydrops fetalis, an often-severe form of prenatal heart failure that causes fetal edema.

Complications

Complications of HDN could include kernicterus, hepatosplenomegaly, inspissated (thickened or dried) bile syndrome and/or greenish staining of the teeth, hemolytic anemia and damage to the liver due to excess bilirubin. Similar conditions include acquired hemolytic anemia, congenital toxoplasma, congenital syphilis infection, congenital obstruction of the bile duct, and cytomegalovirus (CMV) infection.

- High at birth or rapidly rising bilirubin
- Prolonged hyperbilirubinemia
- Bilirubin Induced Neurological Dysfunction
- Cerebral Palsy
- Kernicterus
- Neutropenia
- Thrombocytopenia
- Hemolytic anemia - Must NOT be treated with iron
- Late onset anemia - Must NOT be treated with iron. Can persist up to 12 weeks after birth.

PATHOPHYSIOLOGY

Antibodies are produced when the body is exposed to an antigen foreign to the make-up of the body. If a mother is exposed to a foreign antigen and produces IgG (as opposed to IgM which does not cross the placenta), the IgG will target the antigen, if present in the fetus, and may affect it *in utero* and persist after delivery. The three most common models in which a woman becomes sensitized towards (*i.e.,* produces IgG antibodies against) a particular antigen are hemorrhage, blood transfusion, and ABO incompatibility.

Fetal-maternal hemorrhage, which is the movement of fetal blood cells across the placenta, can occur during abortion, ectopic pregnancy, childbirth, ruptures in the placenta during pregnancy (often caused by trauma), or medical procedures carried out during pregnancy that breach the uterine wall. In subsequent pregnancies, if there is a similar incompatibility in the fetus, these antibodies are then able to cross the placenta into the fetal bloodstream to attach to the red blood cells and cause their destruction (hemolysis). This is a major cause of HDN, because 75% of pregnancies result in some contact between fetal and maternal blood, and 15-50% of pregnancies have hemorrhages with the potential for immune sensitization. The amount of fetal blood needed to cause maternal sensitization depends on the individual's immune system and ranges from 0.1 mL to 30 mL.

csion. ABO blood group system and the D antigen of the Rhesus (Rh) blood group system typing are routine prior to transfusion. Suggestions have been made that women of child bearing age or young girls should not be given a transfusion with Rhc-positive blood or $Kell_1$-positive blood to avoid possible sensitization, but this would strain the resources of blood transfusion services, and it is currently considered uneconomical to screen for these blood groups. HDFN can also be caused by antibodies to a variety of other blood group system antigens, but Kell and Rh are the most frequently encountered.

The third sensitization model can occur in women of blood type O. The immune response to A and B antigens, that are widespread in the environment, usually leads to the production of IgM or IgG anti-A and anti-B antibodies early in life. Women of blood type O are more prone than women of types A and B to making IgG anti-A and anti-B antibodies, and these IgG antibodies are able to cross the placenta. For unknown reasons, the incidence of maternal antibodies against type A and B antigens of the IgG type that could potentially cause hemolytic disease of the newborn is greater than the observed incidence of "ABO disease." About 15% of pregnancies involve a type O mother and a type A or type B child; only 3% of these pregnancies result in hemolytic disease due to A/B/O incompatibility. In contrast to antibodies to A and B antigens, Rhesus antibodies are generally not produced from exposure to environmental antigens. In cases where there is ABO incompatibility and Rh incompatibility, the risk of alloimmunization is decreased because fetal red blood cells are removed from maternal circulation due to anti-ABO antibodies before they can trigger an anti-Rh response.

ANTIBODY SPECIFIC INFORMATION

- Anti-D is the only preventable form of HDN. Since the 1968 introduction of Rho-D immunoglobulin, (Rhogam), which prevents the production of maternal Rho-D antibodies, the incidence of anti-D HDN has decreased dramatically.
- Anti-C and anti-c can both show a negative DAT but still have a severely affected infant. An indirect Coombs must also be run.
- Anti-M also recommends antigen testing to rule out the presence of HDN as the direct coombs can come back negative in a severely affected infant.
- Anti-Kell can cause severe anemia regardless of titer. Anti-Kell suppresses the bone marrow, by inhibiting the erythroid progenitor cells.
- Kidd antigens are also present on the endothelial cells of the kidneys
- One study done by Moran et al., found that titers are not reliable for anti-E. Their most severe case of hemolytic disease of the newborn occurred with titers 1:2. Moran states that it would be unwise routinely to dismiss anti-E as being of little clinical consequence.

DIAGNOSIS

The diagnosis of HDN is based on history and laboratory findings: Blood tests done on the newborn baby.

- Biochemistry tests for jaundice
- Peripheral blood morphology shows increased reticulocytes. Erythroblasts (also known as nucleated red blood cells) occur in moderate and severe disease.
- Positive direct Coombs test (might be negative after fetal interuterine blood transfusion)

Blood tests done on the mother

- Positive indirect Coombs test

Blood tests done on the father

- Erythrocyte antigen status.

Types (Classified by Serology)

Types of HDN are classified by the type of antigens involved. The main types are ABO HDN, Rhesus HDN, Kell HDN, and other antibodies. ABO hemolytic disease of the newborn can range from mild to severe, but generally it is a mild disease. It can be caused by anti-A and anti-B antibodies. Rhesus D hemolytic disease of the newborn (often called Rh disease) is the most common form of severe HDN. Rhesus c hemolytic disease of the newborn can range from a mild to severe disease - is the third most common form of severe HDN. Rhesus e and

rhesus C hemolytic disease of the newborn are rare. Combinations of antibodies, for example, anti-Rhc and anti-RhE occurring together can be especially severe. Anti-Kell hemolytic disease of the newborn is most commonly caused by anti-K_1 antibodies, the second most common form of severe HDN. Over half of the cases of anti-K_1 related HDN are caused by multiple blood transfusions. Antibodies to the other Kell antigens are rare.

PREVENTION

In cases of Rho(D) incompatibility, Rho(D) immunoglobulin is given to prevent sensitization. However, there is no comparable immunotherapy available for other blood group incompatibilities.

Early Pregnancy

- IVIG - IVIG stands for Intravenous Immunoglobulin. It is used in cases of previous loss, high maternal titers, known aggressive antibodies, and in cases where religion prevents blood transfusion. IVIG can be more effective than IUT alone. Fetal mortality was reduced by 36% in the IVIG and IUT group than in the IUT alone group. IVIG and plasmapheresis together can reduce or eliminate the need for an IUT.
- Plasmapheresis - Plasmapheresis aims to decrease the maternal titer by direct plasma replacement and physical removal of antibody. Plasmapheresis and IVIG together can even be used on women with previously hydropic fetuses and fetal losses.

Mid- to Late- pregnancy

- IUT - Intrauterine Transfusion (IUT) is done either by intraperitoneal transfusion (IPT) or intravenous transfusion (IVT). IVT is preferred over IPT. IUTs are only done until 35 weeks. After that, the risk of an IUT is greater than the risk from post birth transfusion.
- Steroids - Steroids are sometimes given to the mother before IUTs and early delivery to mature the fetal lungs.
- Phenobarbital - Phenobarbital is sometimes given to the mother to help mature the fetal liver and reduce hyperbilirubinemia.
- Early Delivery - Delivery can occur anytime after the age of viability. Emergency delivery due to failed IUT is possible, along with induction of labour at 35–38 weeks.

Rhesus-negative mothers who are pregnant with a rhesus-positive infant are offered Rho(D) immune globulin (RhIG, or RhoGam) at 28 weeks during pregnancy, at 34 weeks, and within 48 hours after delivery to prevent sensitization to the D antigen. It works by binding any fetal red blood cells with the D antigen before the mother is able to produce an immune response and form anti-D IgG. A drawback to pre-partum administration of RhIG is that it causes a positive antibody screen when the mother is tested, which can be difficult to distinguish from natural immunological responses that result in antibody production. Without Rho(D) immunoglobulin, the risk of isoimmunization is approximately 17%; with proper administration the risk is reduced to less than 0.1-0.2%.

AFTER BIRTH TESTING

- Coombs - after birth baby will have a direct Coombs test run to confirm the antibodies attached to the infant's red blood cells. This test is run on the infant's cord blood.

In some cases, the direct Coombs will be negative but severe, even fatal HDN can occur. An indirect Coombs needs to be run in cases of anti-C, anti-c, and anti-M. Infants with Anti-M are also recommended to receive antigen testing to rule out the presence of HDN.

- Hgb - the infant's hemoglobin should be tested from cord blood.
- Reticulocyte count - Reticulocytes are elevated when the infant is producing more red blood cells in response to anemia. A rise in the retic count can mean that an infant may not need additional transfusions. Low retic is observed in infants treated with IUT and in those with HDN from anti-Kell.
- Neutrophils - as neutropenia is one of the complications of HDN, the neutrophil count should be checked.
- Thrombocytes - as thrombocytopenia is one of the complications of HDN, the thrombocyte count should be checked.
- Bilirubin should be tested from cord blood.
- Ferritin - because most infants affected by HDN have iron overload, a ferritin must be run before giving the infant any additional iron.
- Newborn Screening Tests - Transfusion with donor blood during pregnancy or shortly after birth can affect the results of the Newborn Screening Tests. It is recommended to wait and retest 10–12 months after last transfusion. In some cases, DNA testing from saliva can be used to rule out certain conditions.

TREATMENT

After birth, treatment depends on the severity of the condition, but could include temperature stabilization and monitoring, phototherapy, transfusion with compatible packed red blood, exchange transfusion with a blood type compatible with both the infant and the mother, sodium bicarbonate for correction of acidosis and/or assisted ventilation.

- Phototherapy - Exposure to ultraviolet light (phototherapy) is recommended when the cord bilirubin is 3 or higher. Some doctors use it at lower levels while awaiting lab results. This converts conjugated bilirubin to an unconjugated form that is easier for the infant to clear.
- IVIG - IVIG has been used to successfully treat many cases of HDN. It has been used not only on anti-D, but on anti-E as well. IVIG can be used to reduce the need for exchange transfusion and to shorten the length of phototherapy. The AAP recommends "In isoimmune hemolytic disease, administration of intravenous γ-globulin (0.5-1 g/kg over 2 hours) is recommended if the TSB is rising despite intensive phototherapy or the TSB level is within 2 to 3 mg/dL (34-51 μmol/L) of the exchange level. If necessary, this dose can be repeated in 12 hours (evidence quality B: benefits exceed harms). Intravenous γ-globulin has been shown to reduce the need for exchange transfusions in Rh and ABO hemolytic disease."
- Exchange transfusion - Exchange transfusion is used when bilirubin reaches either the high or medium risk lines on the non-ogram provided by the American Academy of Pediatrics. Cord bilirubin >4 is also indicative of the need for exchange transfusion.

TRANSFUSION REACTIONS

Once a woman has antibodies, she is at high risk for a future transfusion reaction if she is in need of a blood transfusion. For this reason, she must carry a medical alert card at all times and inform all doctors and emergency personnel of her antibody status. The absence of antibodies however does not preclude a woman from having a transfusion reaction: "Acute hemolytic transfusion reactions may be either immune-mediated or non-immune-mediated. Immune-mediated hemolytic transfusion reactions caused by immunoglobulin M (IgM) anti-A, anti-B, or anti-A, B typically result in severe, potentially fatal complement-mediated intravascular hemolysis. Immune-mediated hemolytic reactions caused by IgG, Rh, Kell, Duffy, or other non-ABO antibodies typically result in extravascular sequestration, shortened survival of transfused red cells, and relatively mild clinical reactions.

Acute hemolytic transfusion reactions due to immune hemolysis may occur in patients who have no antibodies detectable by routine laboratory procedures."

EPIDEMIOLOGY

In 2003, the incidence of Rh(D) sensitization in the United States was 6.8 per 1000 live births; 0.27% of women with an Rh incompatible fetus experience alloimmunization.

OTHER ANIMALS

Hemolytic disease of the newborn is most commonly seen in kittens (where it is known as "fading kitten syndrome") and foals. It has also been reported in puppies.

HEMOLYTIC ANEMIA

Hemolytic anemia or haemolytic anaemia is a form of anemia due to hemolysis, the abnormal breakdown of red blood cells (RBCs), either in the blood vessels (intravascular hemolysis) or elsewhere in the human body (extravascular, but usually in the spleen). It has numerous possible consequences, ranging from relatively harmless to life-threatening. The general classification of hemolytic anemia is either inherited or acquired. Treatment depends on the cause and nature of the breakdown. Symptoms of hemolytic anemia are similar to other forms of anemia (fatigue and shortness of breath), but in addition, the breakdown of red cells leads to jaundice and increases the risk of particular long-term complications, such as gallstones and pulmonary hypertension.

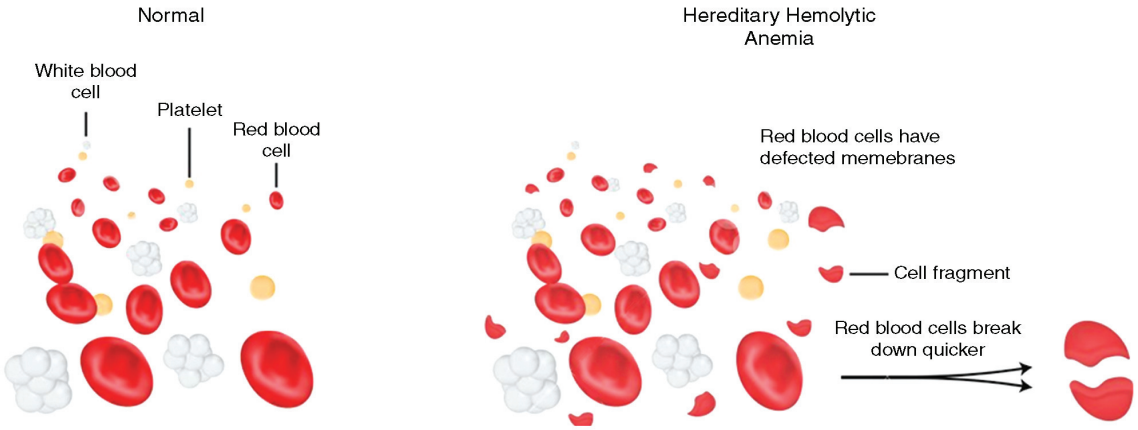


Fig. Hereditary Hemolytic Anemia.

SIGNS AND SYMPTOMS

In general, signs of anemia (pallor, fatigue, shortness of breath, and potential for heart failure) are present. In small children, failure to thrive may occur in any form of anemia. Certain aspects of the medical history can suggest a cause for hemolysis, such as drugs, consumption of fava beans due to Favism, the presence of prosthetic heart valve, or other medical illness. Chronic hemolysis leads to an increased excretion of bilirubin into the biliary tract, which in turn may lead to gallstones. The continuous release of free hemoglobin has been linked with the development of pulmonary hypertension (increased pressure over the pulmonary artery); this, in turn, leads to episodes of syncope (fainting), chest pain, and progressive breathlessness. Pulmonary hypertension eventually causes right ventricular heart failure, the symptoms of which are peripheral edema (fluid accumulation in the skin of the legs) and ascites (fluid accumulation in the abdominal cavity).

CAUSES

They may be classified according to the means of hemolysis, being either intrinsic in cases where the cause is related to the red blood cell (RBC) itself, or extrinsic in cases where factors external to the RBC dominate. Intrinsic effects may include problems with RBC proteins or oxidative stress handling, whereas external factors include immune attack and microvascular angiopathies (RBCs are mechanically damaged in circulation).

Intrinsic Causes

Hereditary (inherited) hemolytic anemia can be due to:

- Defects of red blood cell membrane production (as in hereditary spherocytosis and hereditary elliptocytosis)
- Defects in hemoglobin production (as in thalassemia, sickle-cell disease and congenital dyserythropoietic anemia)
- Defective red cell metabolism (as in glucose-6-phosphate dehydrogenase deficiency and pyruvate kinase deficiency)
- Paroxysmal nocturnal hemoglobinuria (PNH), sometimes referred to as Marchiafava-Micheli syndrome, is a rare, acquired, potentially life-threatening disease of the blood characterized by complement-induced intravascular hemolytic anemia.

Extrinsic Causes

Acquired hemolytic anemia may be caused by immune-mediated causes, drugs and other miscellaneous causes.

- Immune-mediated causes could include transient factors as in *Mycoplasma pneumoniae* infection (cold agglutinin disease) or permanent factors as in autoimmune diseases like autoimmune hemolytic anemia (itself more common in diseases such as systemic lupus erythematosus, rheumatoid arthritis, Hodgkin's lymphoma, and chronic lymphocytic leukemia).
- Spur cell hemolytic anemia
- Any of the causes of hypersplenism (increased activity of the spleen), such as portal hypertension.
- Acquired hemolytic anemia is also encountered in burns and as a result of certain infections (*e.g.,* malaria).
- Lead poisoning resulting from the environment causes non-immune hemolytic anemia.
- Similarly, poisoning by arsine or stibine also causes hemolytic anemia.
- Runners can suffer hemolytic anemia due to "footstrike hemolysis", owing to the destruction of red blood cells in feet at foot impact.
- Low-grade hemolytic anemia occurs in 70% of prosthetic heart valve recipients, and severe hemolytic anemia occurs in 3%.

MECHANISM

Hemolytic anemia involves the following:

1. Abnormal and accelerated destruction of red cells and, in some anemias, their precursors
2. Increased breakdown of hemoglobin, which may result in:
   - Increased bilirubin level (mainly indirect-reacting) with jaundice
   - Increased fecal and urinary urobilinogen
   - Hemoglobinemia, methemalbuminemia, hemoglobinuria and hemosiderinuria (where there is significant intravascular hemolysis).

3. Bone marrow compensatory reaction:
   - Erythroid hyperplasia with accelerated production of red cells, reflected by reticulocytosis, and slight macrocytosis in peripheral blood
   - Expansion of bone marrow in infants and children with severe chronic hemolysis - changes in bone configuration visible on X-ray
4. The balance between red cell destruction and marrow compensation determines the severity of anemias.

In a healthy person, a red blood cell survives 90 to 120 days in the circulation, so about 1% of human red blood cells break down each day. The spleen (part of the reticulo-endothelial system) is the main organ that removes old and damaged RBCs from the circulation. In healthy individuals, the breakdown and removal of RBCs from the circulation is matched by the production of new RBCs in the bone marrow. In conditions where the rate of RBC breakdown is increased, the body initially compensates by producing more RBCs; however, breakdown of RBCs can exceed the rate that the body can make RBCs, and so anemia can develop. Bilirubin, a breakdown product of hemoglobin, can accumulate in the blood, causing jaundice. In general, hemolytic anemia occurs as a modification of the RBC life cycle. That is, instead of being collected at the end of its useful life and disposed of normally, the RBC disintegrates in a manner allowing free iron-containing molecules to reach the blood. With their complete lack of mitochondria, RBCs rely on glycolysis for the materials needed to reduce oxidative damage. Any limitations of glycolysis can result in more susceptibility to oxidative damage and a short or abnormal lifecycle.

If the cell is unable to signal to the reticuloendothelial phagocytes by externalizing phosphatidylserine, it is likely to lyse through uncontrolled means. The distinguishing feature of intravascular hemolysis is the release of RBC contents into the blood stream. The metabolism and elimination of these products, largely iron-containing compounds capable of doing damage through Fenton reactions, is an important part of the condition. Several reference texts exist on the elimination pathways, for example. Free hemoglobin can bind to haptoglobin, and the complex is cleared from the circulation; thus, a decrease in haptoglobin can support a diagnosis of hemolytic anemia. Alternatively, hemoglobin may oxidize and release the heme group that is able to bind to either albumin or hemopexin. The heme is ultimately converted to bilirubin and removed in stool and urine. Hemoglobin may be cleared directly by the kidneys resulting in fast clearance of free hemoglobin but causing the continued loss of hemosiderin loaded renal tubular cells for many days. Additional effects of free hemoglobin seem to be due to specific reactions with NO.

DIAGNOSIS

The diagnosis of hemolytic anemia can be suspected on the basis of a constellation of symptoms and is largely based on the presence of anemia, an increased proportion of immature red cells (reticulocytes) and a decrease in the level of haptoglobin, a protein that binds free hemoglobin. Examination of a peripheral blood smear and some other laboratory studies can contribute to the diagnosis. Symptoms of hemolytic anemia include those that can occur in all anemias as well as the specific consequences of hemolysis. All anemias can cause fatigue, shortness of breath, decreased ability to exercise when severe. Symptoms specifically related to hemolysis include jaundice and dark colored urine due to the presence of hemoglobin (hemaglobinuria). When restricted to the morning hemaglobinuria may suggest paroxysmal nocturnal haemoglobinuria. Direct examination of blood under a microscope in a peripheral blood smear may demonstrate red blood cell fragments called schistocytes, red blood cells that look like spheres (spherocytes), and/or red blood cells missing small pieces (bite cells). An increased number of newly made red blood cells (reticulocytes) may also be a sign of bone marrow compensation for anemia. Laboratory studies commonly used to investigate hemolytic anemia include blood tests for breakdown products of red blood cells, bilirubin and lactate dehydrogenase, a test for the free hemoglobin binding protein haptoglobin, and the direct Coombs test to evaluate antibody binding to red blood cells suggesting autoimmune hemolytic anemia.

TREATMENT

Definitive therapy depends on the cause:

- Symptomatic treatment can be given by blood transfusion, if there is marked anemia. A positive Coombs test is a relative contraindication to transfuse the patient. In cold hemolytic anemia there is advantage in transfuse warmed blood
- In severe immune-related hemolytic anemia, steroid therapy is sometimes necessary.
- In steroid resistant cases, consideration can be given to rituximab or addition of an immunosuppressant (azathioprine, cyclophosphamide)
- Association of methylprednisolone and intravenous immunoglobulin can control hemolysis in acute severe cases
- Sometimes splenectomy can be helpful where extravascular hemolysis, or hereditary spherocytosis, is predominant (*i.e.,* most of the red blood cells are being removed by the spleen).

ANIMALS

Hemolytic anemia affects non-human species as well as humans. It has been found, in a number of animal species, to result from specific triggers. Some notable cases include hemolytic anemia found in black rhinos kept in captivity, with the disease, in one instance, affecting 20% of captive rhinos at a specific facility. The disease is also found in wild rhinos. Dogs and cats differ slightly from humans in some details of their RBC composition and have altered susceptibility to damage, notably, increased susceptibility to oxidative damage from consumption of onion. Garlic is less toxic to dogs than onion.

SPHEROCYTOSIS

Spherocytosis is the presence in the blood of spherocytes, *i.e.,* erythrocytes (red blood cells) that are sphere-shaped rather than bi-concave disk shaped as normal. Spherocytes are found in all hemolytic anemias to some degree. Hereditary spherocytosis and autoimmune hemolytic anemia are characterized by having *only* spherocytes.

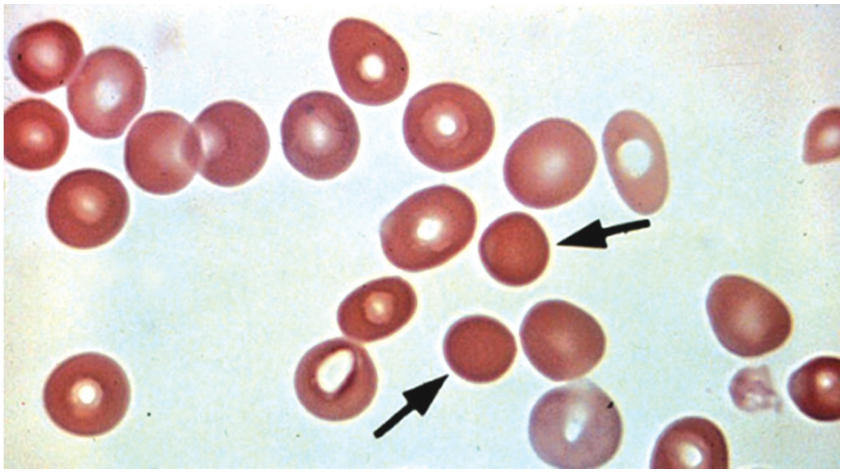


Fig. Hereditary Spherocytosis.

CAUSES

Spherocytes are most commonly found in immunologically-mediated hemolytic anemias and in hereditary spherocytosis, but the former would have a positive direct Coombs test and the latter would not. The misshapen but

otherwise healthy red blood cells are mistaken by the spleen for old or damaged red blood cells and it thus constantly breaks them down, causing a cycle whereby the body destroys its own blood supply (auto-hemolysis). A complete blood count (CBC) may show increased reticulocytes, a sign of increased red blood cell production, and decreased hemoglobin and hematocrit. The term "non-hereditary spherocytosis" is occasionally used, albeit rarely.

Lists of causes:

- Warm autoimmune hemolytic anemia
- Cold autoimmune hemolytic anemia/paroxysmal cold hemoglobinuria
- Acute and delayed hemolytic transfusion reactions
- ABO hemolytic diseases of newborn/Rh hemolytic disease of newborn
- Hereditary spherocytosis
- Intravenous water infusion or drowning (fresh water)
- hypophosphatemia
- Bartonellosis
- Snake bite
- hyposplenism
- Rh-null phenotype.

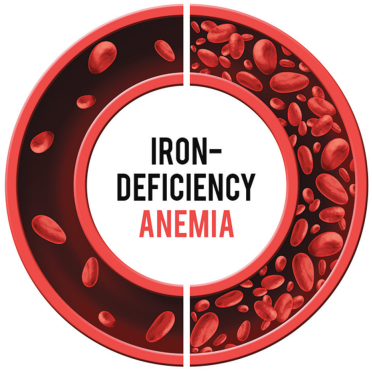
PHYSIOPATHOLOGY

Spherocytosis most often refers to hereditary spherocytosis. This is caused by a molecular defect in one or more of the proteins of the red blood cell cytoskeleton, including spectrin, ankyrin, Band 3, or Protein 4.2. Because the cell skeleton has a defect, the blood cell contracts to a sphere, which is its most surface tension efficient and least flexible configuration. Though the spherocytes have a smaller surface area through which oxygen and carbon dioxide can be exchanged, they in themselves perform adequately to maintain healthy oxygen supplies. However, they have a high osmotic fragility—when placed into water, they are more likely to burst than normal red blood cells. These cells are more prone to physical degradation.

IRON-DEFICIENCY ANEMIA

Iron-deficiency anemia is anemia caused by a lack of iron. Anemia is defined as a decrease in the number of red blood cells or the amount of hemoglobin in the blood. When onset is slow, symptoms are often vague, including feeling tired, weakness, shortness of breath, or poor ability to exercise. Anemia that comes on quickly often has greater symptoms, including: confusion, feeling like one is going to pass out, and increased thirst. There needs to be significant anemia before a person becomes noticeably pale. Problems with growth and development may occur in children. There may be additional symptoms depending on the underlying cause.

Iron-deficiency anemia is usually caused by blood loss, insufficient dietary intake, or poor absorption of iron from food. Sources of blood loss can include heavy periods, childbirth, uterine fibroids, stomach ulcers, colon cancer, and urinary tract bleeding. A poor ability to absorb iron may occur as a result of Crohn's disease or a gastric bypass. In the developing world, parasitic worms, malaria, and HIV/AIDS increase the risk. Diagnosis is generally confirmed by blood tests. Prevention is by eating a diet high in iron or iron supplementation in those at risk. Treatment depends on the underlying cause and may include dietary changes, medications, or surgery. Iron supplements and vitamin C may be recommended. Severe cases may be treated with blood transfusions or iron injections. Iron-deficiency anemia affected about 1.48 billion people in 2015. A lack of dietary iron is estimated to cause approximately half of all anemia cases globally. Women and young children are most commonly affected. In 2015 anemia due to iron deficiency resulted in about 54,000 deaths – down from 213,000 deaths in 1990.



SIGNS AND SYMPTOMS

Iron-deficiency anemia is characterized by the sign of pallor (reduced oxyhemoglobin in skin or mucous membranes), and the symptoms of fatigue, lightheadedness, and weakness. None of these symptoms (or any of the others below) are sensitive or specific. Pallor of mucous membranes (primarily the conjunctiva) in children suggests anemia with the best correlation to the disease, but in a large study was found to be only 28% sensitive and 87% specific (with high predictive value) in distinguishing children with anemia [hemoglobin (Hb) <11.0 g/dl] and 49% sensitive and 79% specific in distinguishing severe anemia (Hb < 7.0 g/dl). Thus, this sign is reasonably predictive when present, but not helpful when absent, as only one-third to one-half of children who are anemic (depending on severity) will show pallor. Because iron-deficiency anemia tends to develop slowly, adaptation occurs to the systemic effects that anemia causes, and the disease often goes unrecognized for some time. In severe cases, dyspnea can occur. Pica may also develop; pagophagia has been suggested to be "the most specific for iron deficiency."

Other possible symptoms and signs of iron-deficiency anemia include:

- Irritability
- Angina
- Palpitations
- Breathlessness
- Tingling, numbness, or burning sensations
- Glossitis (inflammation or infection of the tongue)
- Angular cheilitis (inflammatory lesions at the mouth's corners)
- Koilonychia (spoon-shaped nails) or nails that are brittle
- Poor appetite
- Dysphagia due to formation of esophageal webs (Plummer-Vinson syndrome)
- Restless legs syndrome.

Child Development

Iron-deficiency anemia is associated with poor neurological development, including decreased learning ability and altered motor functions. Causation has not been established, but there is a possible long-term impact from these neurological issues.

CAUSE

A diagnosis of iron-deficiency anemia requires further investigation into its cause. It can be caused by increased iron demand/loss or decreased iron intake. For instance, chronic gastrointestinal blood loss can be

considered, which could be linked to a possible malignancy. In babies and adolescents, rapid growth may outpace dietary intake of iron and result in deficiency in the absence of disease or a grossly abnormal diet. In women of childbearing age, heavy menstrual periods can also cause iron-deficiency anemia.

Parasitic Disease

The leading cause of iron-deficiency anemia worldwide is a parasitic disease known as a helminthiasis caused by infestation with parasitic worms (helminths); specifically, hookworms, which include *Ancylostoma duodenale*, *Ancylostoma ceylanicum*, and *Necator americanus,* are most commonly responsible for causing iron-deficiency anemia. The World Health Organization estimates that "approximately two billion people are infected with soil-transmitted helminths worldwide." Parasitic worms cause both inflammation and chronic blood loss by binding to a human's small-intestinal mucosa, and through their means of feeding and degradation, they can ultimately cause iron-deficiency anemia.

Blood Loss

Blood contains iron within red blood cells, so blood loss leads to a loss of iron. There are several common causes of blood loss. Women with menorrhagia (heavy menstrual periods) are at risk of iron-deficiency anemia because they are at higher-than-normal risk of losing a larger amount blood during menstruation than is replaced in their diet. Slow, chronic blood loss within the body — such as from a peptic ulcer, angiodysplasia, a colon polyp or gastrointestinal cancer, or excessively heavy periods — can cause iron-deficiency anemia. Gastrointestinal bleeding can result from regular use of some groups of medication, such as NSAIDs (*e.g.,* aspirin), as well as anticoagulants such as clopidogrel and warfarin; however, these are required in some patients, especially those with states causing thrombophilia.

Diet

The body normally gets the iron it requires from foods. If a person consumes too little iron, or iron that is poorly absorbed (non-heme iron), they can become iron deficient over time. Examples of iron-rich foods include meat, eggs, leafy green vegetables and iron-fortified foods. For proper growth and development, infants and children need iron from their diet. A high intake of cow's milk is associated with an increased risk of iron-deficiency anemia. Other risk factors for iron-deficiency anemia include low meat intake and low intake of iron-fortified products.

Iron Malabsorption

Iron from food is absorbed into the bloodstream in the small intestine, primarily in the duodenum. Iron malabsorption is a less common cause of iron-deficiency anemia, but many gastrointestinal disorders can reduce the body's ability to absorb iron. There are different mechanisms that may be present. In celiac disease, abnormal changes in the structure of the duodenum can decrease iron absorption. Abnormalities or surgical removal of the stomach can also lead to malabsorption by altering the acidic environment needed for iron to be converted into its absorbable form. If there is insufficient production of hydrochloric acid in the stomach, hypochlorhydria/achlorhydria can occur (often due to chronic H. pylori infections or long-term proton pump inhibitor therapy), inhibiting the conversion of ferric iron to the absorbable ferrous iron.

Pregnancy

Without iron supplementation, iron-deficiency anemia occurs in many pregnant women because their iron stores need to serve their own increased blood volume, as well as be a source of hemoglobin for the growing

fetus and for placental development. Other less common causes are intravascular hemolysis and hemoglobinuria. Iron deficiency in pregnancy appears to cause long-term and irreversible cognitive deficits in the baby.

MECHANISM

Anemia can result from significant iron deficiency. When the body has sufficient iron to meet its needs (functional iron), the remainder is stored for later use in cells, mostly in the bone marrow and liver. These stores are called ferritin complexes and are part of the human (and other animals) iron metabolism systems. Iron is a mineral that is important in the formation of red blood cells in the body, particularly as a critical component of hemoglobin. After being absorbed in the small intestine, iron travels through blood, bound to transferrin, and eventually ends up in the bone marrow, where it is involved in red blood cell formation. When red blood cells are degraded, the iron is recycled by the body and stored. When the amount of iron needed by the body exceeds the amount of iron that is readily available, the body can use iron stores (ferritin) for a period of time, and red blood cell formation continues normally. However, as these stores continue to be used, iron is eventually depleted to the point that red blood cell formation is abnormal. Ultimately, anemia ensues, which by definition is a hemoglobin lab value below normal limits.

DIAGNOSIS

Conventionally, a definitive diagnosis requires a demonstration of depleted body iron stores obtained by bone marrow aspiration, with the marrow stained for iron. However, with the availability of reliable blood tests that can be more readily collected for iron-deficiency anemia diagnosis, a bone marrow aspiration is usually not obtained. Furthermore, a study published April 2009 questions the value of stainable bone marrow iron following parenteral iron therapy.

History

The diagnosis of iron-deficiency anemia will be suggested by history that includes common causes of the condition, such as a menstruating woman or the presence of occult blood (*i.e.,* hidden blood) in the stool. A travel history to areas in which hookworms and whipworms are endemic may be helpful in guiding certain stool tests for parasites or their eggs. Although symptoms can play a role in identifying iron-deficiency anemia, these are often non-specific symptoms, especially in mild cases, which may limit their contribution to determining the diagnosis.

Blood tests

Anemia is often discovered by routine blood tests, which generally include a complete blood count (CBC). A sufficiently low hemoglobin (Hb) by definition makes the diagnosis of anemia, and a low hematocrit value is also characteristic of anemia. Further studies will be undertaken to determine the anemia's cause. If the anemia is due to iron deficiency, one of the first abnormal values to be noted on a CBC, as the body's iron stores begin to be depleted, will be a high red blood cell distribution width (RDW), reflecting an increased variability in the size of red blood cells (RBCs). A low mean corpuscular volume (MCV) also appears during the course of body iron depletion. It indicates a high number of abnormally small red blood cells. A low MCV, a low mean corpuscular hemoglobin or mean corpuscular hemoglobin concentration (MCH), and the corresponding appearance of RBCs on visual examination of a peripheral blood smear narrows the problem to a microcytic anemia (literally, a "small red blood cell" anemia). The blood smear of a person with iron-deficiency anemia shows many hypochromic (pale, relatively colorless) and small RBCs, and may also show poikilocytosis (variation in shape)

and anisocytosis (variation in size). With more severe iron-deficiency anemia, the peripheral blood smear may show hypochromic, pencil-shaped cells and, occasionally, small numbers of nucleated red blood cells. The platelet count may be slightly above the high limit of normal in iron-deficiency anemia (termed a mild thrombocytosis), but severe cases can present with thrombocytopenia (low platelet count). Iron-deficiency anemia is confirmed by tests that include serum ferritin, serum iron level, serum transferrin, and total iron binding capacity (TIBC). A low serum ferritin is most commonly found.

However, serum ferritin can be elevated by any type of chronic inflammation and thus is not consistently decreased in iron-deficiency anemia. Serum iron levels may be measured, but serum iron concentration is not as reliable as the measurement of both serum iron and serum iron-binding protein levels (TIBC). The ratio of serum iron to TIBC (called iron saturation or transferrin saturation index or percent) is a value with defined parameters that can help to confirm the diagnosis of iron-deficiency anemia; however, other conditions must also be considered, including other types of anemia. Another finding that can be used is the level of free erythrocyte protoporphyrin (FEP). During haemoglobin synthesis, trace amounts of zinc will be incorporated into protoporphyrin in the place of iron which is lacking.

We can separate the protoporphyrin from its zinc moeity and measure it, known as the FEP, providing an indirect measurement of the zinc-protoporphyrin complex. The level of FEP is expressed in either ìg/dl of whole blood or µg/dl of RBC. An iron insufficiency in the bone marrow can be detected very early by a rise in FEP. Further testing may be necessary to differentiate iron-deficiency anemia from other disorders, such as thalassemia minor. It is very important not to treat people with thalassemia with an iron supplement, as this can lead to hemochromatosis. A hemoglobin electrophoresis provides useful evidence for distinguishing these two conditions, along with iron studies.

Screening

It is unclear if screening pregnant women for iron-deficiency anemia during pregnancy improves outcomes in the United States. The same holds true for screening children who are “6 to 24 months” old.

TREATMENT

When treating iron-deficiency anemia, considerations of the proper treatment methods are done in light of the “cause and severity” of the condition. If the iron-deficiency anemia is a downstream effect of blood loss or another underlying cause, treatment is geared towards addressing the underlying cause when possible. In severe acute cases, treatment measures are taken for immediate management in the interim, such as blood transfusions or even intravenous iron. Iron-deficiency anemia treatment for less severe cases includes dietary changes to incorporate iron-rich foods into regular oral intake. Foods rich in ascorbic acid (vitamin C) can also be beneficial, since ascorbic acid enhances iron absorption. Other oral options are iron supplements in the form of pills or drops for children.

As iron-deficiency anemia becomes more severe, or if the anemia does not respond to oral treatments, other measures may become necessary. In addition to the previously mentioned indication for intravenous iron or blood transfusions, intravenous iron may also be used when oral intake is not tolerated, as well as for other indications. Specifically, for those on dialysis, parenteral iron is commonly used. Individuals on dialysis who are taking forms of erythropoietin or some “erythropoiesis-stimulating agent” are given parenteral iron, which helps the body respond to the erythropoietin agents and produce red blood cells. The various forms of treatment are not without possible adverse effects. Iron supplementation by mouth commonly causes negative gastrointestinal effects, including constipation. Intravenous iron can induce an allergic response that can be as serious as anaphylaxis, although different formulations have decreased the likelihood of this adverse effect.

EPIDEMIOLOGY

A moderate degree of iron-deficiency anemia affects approximately 610 million people worldwide or 8.8% of the population. It is slightly more common in females (9.9%) than males (7.8%). Mild iron deficiency anemia affects another 375 million. The prevalence of iron deficiency as a cause of anemia varies among countries; in the groups in which anemia is most common, including young children and a subset of non-pregnant women, iron deficiency accounts for a fraction of anemia cases in these groups ("25% and 37%, respectively"). Iron deficiency is a more common cause of anemia in other groups, including pregnant women. Within the United States, iron-deficiency anemia affects about 2% of adult males, 10.5% of Caucasian women, and 20% of African-American and Mexican-American women.

IRON OVERLOAD

Iron overload (variously known as haemochromatosis, hemochromatosis, hemochromocytosis, Celtic curse, Irish illness, British gene, Scottish sickness and bronzing diabetes) indicates accumulation of iron in the body from any cause. The most important causes are hereditary haemochromatosis (HHC), a genetic disorder, and transfusional iron overload, which can result from repeated blood transfusions.

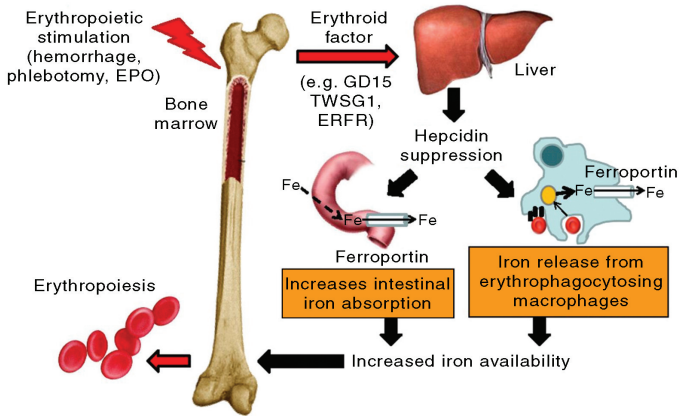


Fig. Impact of iron overload.

SIGNS AND SYMPTOMS

Organs most commonly affected by haemochromatosis are the liver, heart, and endocrine glands.

Haemochromatosis may present with the following clinical syndromes:

- Chronic liver disease and cirrhosis of the liver
- Heart involvement: heart failure, irregular heart rhythm
- Hormonal issues: diabetes and hypogonadism (insufficiency of the sex hormone producing glands) which leads to low sex drive and/or loss of fertility in men and loss of menstrual cycle in women.

Diabetes in people with iron overload occurs as a result of selective iron deposition in islet beta cells in the pancreas leading to functional failure and cell death. Arthritis, from calcium pyrophosphate deposition in joints leading to joint pains. The most commonly affected joints are those of the hands, particularly the knuckles of the second and third fingers. Bronzing of the skin. This deep tan colour, in concert with insulin insufficiency due to pancreatic damage, is the source of a nickname for this condition: "bronze diabetes".

CAUSES AND FORMS

The causes can be distinguished between primary cases (hereditary or genetically determined) and less frequent secondary cases (acquired during life). People of Celtic (Irish, Scottish, Welsh, Cornish, Breton *etc.*),

English, and Scandinavian origin have a particularly high incidence of whom about 10% are carriers of the C282Y mutation on the HFE gene associated with HLA-A3 and 1% have the condition.

Primary Haemochromatosis

Although it was known most of the 20th century that most cases of haemochromatosis were inherited, they were incorrectly assumed to depend on a single gene. The overwhelming majority depend on mutations of the HFE gene discovered in 1996, but since then others have been discovered and sometimes are grouped together as "non-classical hereditary haemochromatosis", "non-HFE related hereditary haemochromatosis", or "non-HFE haemochromatosis".

Description	OMIM	Mutation
Haemochromatosis type 1: "classical" haemochromatosis	235200	HFE
Haemochromatosis type 2A: juvenile haemochromatosis	602390	Haemojuvelin ("HJV", also known as RGMc and HFE2)
Haemochromatosis type 2B: juvenile haemochromatosis	606464	hepcidin antimicrobial peptide (*HAMP*) or HFE2B
Haemochromatosis type 3	604250	transferrin receptor-2 (TFR2 or HFE3)
Haemochromatosis type 4/ African iron overload	604653	ferroportin (SLC11A3/SLC40A1)
Neonatal haemochromatosis	231100	(unknown)
Acaeruloplasminaemia (very rare)	604290	caeruloplasmin
Congenital atransferrinaemia (very rare)	209300	transferrin
GRACILE syndrome (very rare)	603358	BCS1L

Most types of hereditary haemochromatosis have autosomal recessive inheritance, while type 4 has autosomal dominant inheritance.

Secondary Haemochromatosis

- Severe chronic haemolysis of any cause, including intravascular haemolysis and ineffective erythropoiesis (haemolysis within the bone marrow)
- Multiple frequent blood transfusions (either whole blood or just red blood cells), which are usually needed either by individuals with hereditary anaemias (such as beta-thalassaemia major, sickle cell anaemia, and Diamond–Blackfan anaemia) or by older patients with severe acquired anaemias such as in myelodysplastic syndromes
- Excess parenteral iron supplements, such as what can acutely happen in iron poisoning
- Excess dietary iron
- Some disorders do not normally cause haemochromatosis on their own, but may do so in the presence of other predisposing factors. These include cirrhosis (especially related to alcohol abuse), steatohepatitis of any cause, porphyria cutanea tarda, prolonged haemodialysis, and post-portacaval shunting.

DIAGNOSIS

There are several methods available for diagnosing and monitoring iron loading. Blood tests are usually the first test if there is a clinical suspicion of iron overload. Serum ferritin testing is a low-cost, readily available, and minimally invasive method for assessing body iron stores. However, the major problem with using it as an indicator of iron overload is that it can be elevated in a range of other medical conditions unrelated to iron levels including infection, inflammation, fever, liver disease, kidney disease, and cancer. Also, total iron binding capacity may be low, but can also be normal. Serum ferritin: In males and postmenopausal females, a serum ferritin value of over 300 ng/mL (670 pmol/L) indicates iron overload. In premenopausal females, a serum

ferritin value of over 150 or 200 ng/mL (330 or 440 pmol/L) indicates iron overload. If the person is capable of showing the symptoms, they may need to be tested more than once throughout their lives as a precautionary, most commonly in women after menopause. Transferrin saturation is a more specific test. DNA/screening: the standard of practice in diagnosis of haemochromatosis places emphasis on genetic testing. Positive HFE analysis confirms the clinical diagnosis of haemochromatosis in asymptomatic individuals with blood tests showing increased iron stores, or for predictive testing of individuals with a family history of haemochromatosis. The alleles evaluated by HFE gene analysis are evident in ~80% of patients with haemochromatosis; a negative report for HFE gene does not rule out haemochromatosis. First degree relatives of those with primary haemochromatosis should be screened to determine if they are a carrier or if they could develop the disease. This can allow preventive measures to be taken. Screening the general population is not recommended.

Liver biopsy is the removal of small sample in order to be studied and can determine the cause of inflammation or cirrhosis. In someone with negative HFE gene testing, elevated iron status for no other obvious reason, and family history of liver disease, additional evaluation of liver iron concentration is indicated. In this case, diagnosis of haemochromatosis is based on biochemical analysis and histologic examination of a liver biopsy. Assessment of the hepatic iron index (HII) is considered the "gold standard" for diagnosis of haemochromatosis. Magnetic resonance imaging (MRI) is used as a non-invasive way to accurately estimate iron deposition levels in the liver as well as heart, joints, and pituitary gland.

TREATMENT

Phlebotomy/venesection: routine treatment consists of regularly scheduled phlebotomies (bloodletting or erythrocytapheresis). When first diagnosed, the phlebotomies may be performed every week or fortnight, until iron levels can be brought to within normal range. Once the serum ferritin and transferrin saturation are within the normal range, treatments may be scheduled every two to three months depending upon the rate of reabsorption of iron. A phlebotomy session typically draws between 450 and 500 mL of blood. Diet low in iron is generally recommended, but has little effect compared to venesection. The human diet contains iron in two forms - heme iron and non-heme iron. Heme iron is the most easily absorbed form of iron. People with iron overload may be advised to avoid food that are high in heme iron. Highest in heme iron is red meat such as beef, venison, lamb, buffalo, and fish such as bluefin tuna. A strict low iron diet is usually not necessary. Non-heme iron is not as easily absorbed in the human system and is found in plant-based foods like grains, beans, vegetables, fruits, nuts, and seeds.

Medication: For those unable to tolerate routine blood draws, there are chelating agents available for use. The drug deferoxamine binds with iron in the bloodstream and enhances its elimination in urine and faeces. Typical treatment for chronic iron overload requires subcutaneous injection over a period of 8–12 hours daily. Two newer iron chelating drugs that are licensed for use in patients receiving regular blood transfusions to treat thalassaemia (and, thus, who develop iron overload as a result) are deferasirox and deferiprone.

PROGNOSIS

In general, provided there has been no liver damage, patients should expect a normal life expectancy if adequately treated by venesection. If the serum ferritin is greater than 1000 ug/L at diagnosis there is a risk of liver damage and cirrhosis which may eventually shorten their life. The presence of cirrhosis increases the risk of hepatocellular carcinoma.

EPIDEMIOLOGY

It is most common in certain European populations (such as the Irish and Norwegians) and occurs in 0.6% of the population. Men with the disease are 24 times more likely to experience symptoms than affected women.

HISTORY

Stone Age

Two factors are thought to have had large influence on the mutation of genes related to Iron overload during the Stone Age: diet and the environment. Starting during the Mesolithic Era, communities of people lived in an environment that was fairly sunny, warm and had the dry climates of the Middle East. Most of the humans who lived at the time were foragers and their diets consisted mostly of hunting game, gathering and even fishing when and if the opportunity arose. With the archaeologists studying dental plaque and the assumptions of what would have been available to the people due to their environment, leads to the theories of Mesolithic foragers eating substances such as tubers, nuts, plantains, grass and much of the food would have been was very rich in iron. Over hundreds of years the body was very well adapted to the high level of iron content in the consumption. Moving forward in time and studying the Neolithic Era, which was during the end of the Stone Age, we see serious advancements in both the environment and diet of the travelling people.

During the European Neolithic era, some communities of foragers started to migrate north. The lifestyle and environment started to change to a decrease in temperatures and a change in the landscaping in which the foragers now needed to adapt to. As the people began to develop and advance their use of tools and learn new ways of producing food, hunting and gathering was no longer the main source, but farming also slowly started to develop. The change that the travellers encountered would have lead to serious stress on the body and a decrease in iron rich consumption. This transition is a key factor in which researchers can start to see the link between the travellers diets, environment and the mutations of genes, especially those that regulated the iron absorption within the body. The iron, that makes of 70% of our red blood cell composition, is a critical micronutrient for effective thermoregulation in the body. When the body encounters a deficiency of its micronutrients, in this case iron, it will lead to a drop in the core temperature.

When the travellers encountered the much more chilly and damp environments of Europe, the supplementary iron from there food was a necessity to help keep their temperatures regulated- however, without the iron supplements from the food the human body would have undergone serious stress to make up for the lost iron and would have started to store iron at higher rates that normal. This theory hypothesizes that the pressures cause by the migration would be the initiation to the gene mutation that allowed the body to absorb and store higher amounts of iron. There is no way to know the exact time at which the gene mutated or in which location these people were in when it happened. However, when the time frame at which the migrators would have started to move north and the increasing probability of the people's bodies adapting, lead to the assumption that the travellers would have moved and helped to develop the Celtic Empire during the Bronzing Age. This theory would also offer an explanation of why the name 'Celtic Curse' became a well known alternative/nickname for hemochromatosis.

Viking Hypothesis

Many studies and surveys are being conducted in order to determine the frequencies of the disease in countries and also to figure out how the mutation migrated around the globe. The theory that this disease initially evolved from travellers migrating north helped to give an understanding of how it could have initially evolved. Through the surveys and counting of affected, there was a very particular distribution pattern of the disease in which there are large clusters and frequencies of the gene mutations being found along the coastline of Europe.

This is the pattern that has been noticed is what helped lead to the development of the "Viking Hypothesis". The locations of clusters and mapped patterns of this mutation have a very close correlation to the migration of Vikings and locations of Viking settlements in Europe that occurred around the time of c.700 AD to c.1100 AD.

The Vikings originally came from the three countries of Scandinavia (Norway, Sweden and Denmark) and when on land, they had multiple Kingdoms and their way of life mostly evolved around farming and trade. When the Northmen took to the sea they were given the name 'Vikings' which was developed from the Old Norse language and meant 'pirates'. The Viking ships made their way along the coastline of Europe in search for trade, riches, land and the migration of their people. The genetic studies to date along with the extremely high frequency patterns in some European countries lead to the suggestion that the mutation could have been easily spread Vikings and later by the Normans. And with this, indicating a genetic link between hereditary hemochromatosis and Viking ancestry.

Modern Times

In 1865, Armand Trousseau (a French internist) was one of the first to describe many of the symptoms of a diabetic patient with cirrhosis of the liver and had bronzed skin colour. The term hemochromatosis was first used by German pathologist named Friedrich Daniel von Recklinghausen in 1890 when he introduced an accumulation of iron in body tissue. In 1935 J.H. Sheldon, a British physician, described the pathophysiology mechanism linked to iron metabolism for the first time. In 1996 Felder and colleagues identified the hemochromatosis gene, HFE gene. Felder found that the HFE gene has two main mutations, C282Y and H63D, which were the main cause of hereditary hemochromatosis. The next year the CDC and the National Human Genome Research Institute sponsored an examination of hemochromatosis following the discovery of the HFE gene which helped lead to the population screenings and estimates that are still being used today.

TERMINOLOGY

Historically, the term *haemochromatosis* (spelled *hemochromatosis* in American English) was initially used to refer to what is now more specifically called haemochromatosis type 1 (or HFE-related hereditary haemochromatosis). Currently, haemochromatosis (without further specification) is mostly defined as iron overload with a hereditary or primary cause, or originating from a metabolic disorder. However, the term is currently also used more broadly to refer to any form of iron overload, thus requiring specification of the cause, for example, *hereditary haemochromatosis*.

Hereditary haemochromatosis is an autosomal recessive disorder with estimated prevalence in the population of 1 in 200 among patients with European ancestry, with lower incidence in other ethnic groups. The gene responsible for hereditary haemochromatosis (known as HFE gene) is located on chromosome 6; the majority of hereditary haemochromatosis patients have mutations in this HFE gene.

Hereditary haemochromatosis is characterized by an accelerated rate of intestinal iron absorption and progressive iron deposition in various tissues. This typically begins to be expressed in the third to fifth decades of life, but may occur in children. The most common presentation is hepatic (liver) cirrhosis in combination with hypopituitarism, cardiomyopathy, diabetes, arthritis, or hyperpigmentation. Because of the severe sequelae of this disorder if left untreated, and recognizing that treatment is relatively simple, early diagnosis before symptoms or signs appear is important. In general, the term *haemosiderosis* is used to indicate the pathological effect of iron accumulation in any given organ, which mainly occurs in the form of the iron-storage complex haemosiderin. Sometimes, the simpler term siderosis is used instead.

Other definitions distinguishing haemochromatosis or haemosiderosis that are occasionally used include:

- Haemosiderosis is haemochromatosis caused by excessive blood transfusions, that is, haemosiderosis is a form of secondary haemochromatosis.
- Haemosiderosis is haemosiderin deposition within cells, while haemochromatosis is haemosiderin within cells and interstitium.

- Haemosiderosis is iron overload that does not cause tissue damage, while haemochromatosis does.
- Haemosiderosis is arbitrarily differentiated from haemochromatosis by the reversible nature of the iron accumulation in the reticuloendothelial system.

SIDEROBLASTIC ANEMIA

Sideroblastic anemia or sideroachrestic anemia is a form of anemia in which the bone marrow produces ringed sideroblasts rather than healthy red blood cells (erythrocytes). In sideroblastic anemia, the body has iron available but cannot incorporate it into hemoglobin, which red blood cells need in order to transport oxygen efficiently. The disorder may be caused either by a genetic disorder or indirectly as part of myelodysplastic syndrome, which can develop into hematological malignancies (especially acute myeloid leukemia).

Sideroblasts (*sidero-* + *-blast*) are atypical, abnormal nucleated erythroblasts (precursors to mature red blood cells) with granules of iron accumulated in the mitochondria surrounding the nucleus. Normally, sideroblasts are present in the bone marrow, and enter the circulation after maturing into a normal erythrocyte. The presence of sideroblasts *per se* does not define Sideroblastic anemia. Only the finding of ring (or ringed) sideroblasts characterizes Sideroblastic anemia.

Ring sideroblasts are named so because iron-laden mitochondria form a ring around the nucleus. It is a subtype of basophilic granules of the erythrocyte, but which can only be seen in bone marrow. To count a cell as a ring sideroblast, the ring must encircle a third or more of the nucleus and contain five or more iron granules, according to the 2008 WHO classification of the tumors of the hematopoietic and lymphoid tissues.

The WHO International Working Group on Morphology of MDS (IWGM-MDS) defined three types of sideroblasts:

1. Type 1 sideroblasts: fewer than 5 siderotic granules in the cytoplasm
2. Type 2 sideroblasts: 5 or more siderotic granules, but not in a perinuclear distribution
3. Type 3 or ring sideroblasts: 5 or more granules in a perinuclear position, surrounding the nucleus or encompassing at least one third of the nuclear circumference.

Type 1 and type 2 are found in Non-sideroblastic anemias. Type 3 is found only in Sideroblastic anemia.

CLASSIFICATION

Sideroblastic anemia is typically divided into subtypes based on its cause.

- Hereditary or congenital sideroblastic anemia may be X-linked or autosomal.

OMIM	Name	Gene
300751	X-linked sideroblastic anemia (XLSA)	ALAS2
301310	sideroblastic anemia with spinocerebellar ataxia (ASAT)	ABCB7
205950	pyridoxine-refractory autosomal recessive sideroblastic anemia	SLC25A38
206000	pyridoxine-responsive sideroblastic anemia	(vitamin B_6 deficiency; pyridoxal phosphate required for heme synthesis)

GLRX5 has also been implicated.

- Acquired, or secondary, sideroblastic anemia develops after birth and is divided according to its cause.

SYMPTOMS

Symptoms of sideroblastic anemia include skin paleness, fatigue, dizziness, and enlarged spleen and liver. Heart disease, liver damage, and kidney failure can result from iron buildup in these organs.

CAUSES

Causes of sideroblastic anemia can be categorized into three groups: congenital sideroblastic anemia, acquired clonal sideroblastic anemia, and acquired reversible sideroblastic anemia. All cases involve dysfunctional heme synthesis or processing. This leads to granular deposition of iron in the mitochondria that form a ring around the nucleus of the developing red blood cell. Congenital forms often present with normocytic or microcytic anemia while acquired forms of sideroblastic anemia are often normocytic or macrocytic.

- Congenital sideroblastic anemia:
  - X-linked sideroblastic anemia: This is the most common congenital cause of sideroblastic anemia and involves a defect in ALAS2, which is involved in the first step of heme synthesis. Although X-linked, approximately one third of patients are women due to skewed X-inactivation (lyonizations).
  - Autosomal recessive sideroblastic anemia involves mutations in the SLC25A38 gene. The function of this protein is not fully understood, but it is involved in mitochondrial transport of glycine. Glycine is a substrate for ALAS2 and necessary for heme synthesis. The autosomal recessive form is typically severe in presentation.
  - Genetic syndromes: Rarely, sideroblastic anemia may be part of a congenital syndrome and present with associated findings, such as ataxia, myopathy, and pancreatic insufficiency.
- Acquired clonal sideroblastic anemia:
  - Clonal sideroblastic anemias fall under the broader category of myelodysplastic syndromes (MDS). Three forms exist and include refractory anemia with ringed sideroblasts (RARS), refractory anemia with ringed sideroblasts and thrombocytosis (RARS-T), and refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS). These anemias are associated with increased risk for leukemic evolution.
- Acquired reversible sideroblastic anemia:
  - Causes include excessive alcohol use (the most common cause of sideroblastic anemia), pyridoxine deficiency (vitamin B_6 is the cofactor in the first step of heme synthesis), lead poisoning and copper deficiency. Excess zinc can indirectly cause sideroblastic anemia by decreasing absorption and increasing excretion of copper. Antimicrobials that may lead to sideroblastic anemia include isoniazid (which interferes with pyridoxine metabolism), chloramphenicol (which, by inhibiting the synthesis of mitochondrial membrane protein, impairs mitochondrial respiration), cycloserine, and linezolid.

DIAGNOSIS

Ringed sideroblasts are seen in the bone marrow. On the peripheral blood smear can be found erythrocytes with basophilic stippling (cytoplasmic granules of RNA precipitates) and Pappenheimer bodies (cytoplasmic granules of iron). The anemia is moderate to severe and dimorphic. Microscopic viewing of the red blood cells will reveal marked unequal cell size and abnormal cell shape. Basophilic stippling is marked and target cells are common. The mean cell volume is commonly decreased (*i.e.,* a microcytic anemia), but it may also be normal or even high. The RDW is increased with the red blood cell histogram shifted to the left. Leukocytes and platelets are normal. Bone marrow shows erythroid hyperplasia with a maturation arrest. In excess of 40% of the developing erythrocytes are ringed sideroblasts. Serum iron, percentage saturation and ferritin are increased. The total iron-binding capacity of the cells is normal to decreased. Stainable marrow hemosiderin is increased.

Laboratory Findings

- Serum Iron: High

- Increased ferritin levels
- Normal to decreased total iron-binding capacity
- High transferrin saturation
- Hematocrit of about 20-30%
- The mean corpuscular volume or MCV is usually normal or low for congenital causes of sideroblastic anemia but normal or high for acquired forms.
- With lead poisoning
- Specific test: Prussian blue stain of RBC in marrow shows ringed sideroblasts. Prussian blue staining involves a non-enzymatic reaction of ferrous iron with ferrocyanide forming ferric-ferrocyanide, which is blue in colour. A counterstain may be used to provide better visualization.

TREATMENT

Occasionally, the anemia is so severe that support with transfusion is required. These patients usually do not respond to erythropoietin therapy. Some cases have been reported that the anemia is reversed or heme level is improved through use of moderate to high doses of pyrodoxine (vitamin B_6). In severe cases of SBA, bone marrow transplant is also an option with limited information about the success rate. Some cases are listed on MedLine and various other medical sites. In the case of isoniazid-induced sideroblastic anemia, the addition of B_6 is sufficient to correct the anemia. Desferrioxamine, a chelating agent, is used to treat iron overload from transfusions. Therapeutic phlebotomy can be used to manage iron overload.

COURSE AND PROGNOSIS

Sideroblastic anemias are often described as responsive or non-responsive in terms of increased hemoglobin levels to pharmacological doses of vitamin B_6.

1. Congenital: 80% are responsive, though the anemia does not completely resolve.
2. Acquired clonal: 40% are responsive, but the response may be minimal.
3. Acquired reversible: 60% are responsive, but course depends on treatment of the underlying cause.

Severe refractory sideroblastic anemias requiring regular transfusions and/or that undergo leukemic transformation (5-10%) significantly reduce life expectancy.

CONGENITAL DYSERYTHROPOIETIC ANEMIA

Congenital dyserythropoietic anemia (CDA) is a rare blood disorder, similar to the thalassemias. CDA is one of many types of anemia, characterized by ineffective erythropoiesis, and resulting from a decrease in the number of red blood cells (RBCs) in the body and a less than normal quantity of hemoglobin in the blood.

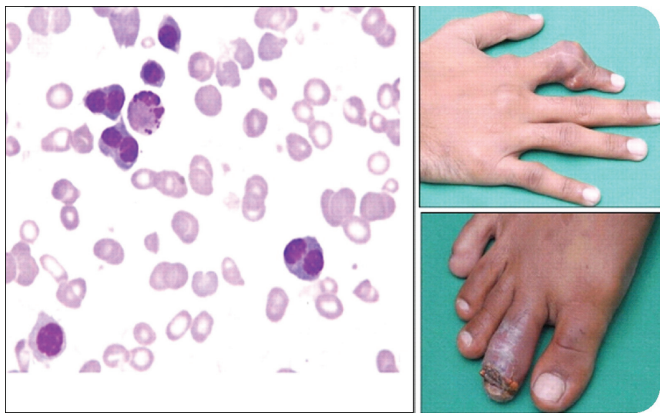


Fig. Congenital dyserythropoietic anemia.

SIGNS/SYMPTOMS

The symptoms and signs of congenital dyserythropoietic anemia are consistent with:

- Tiredness (fatigue)
- Weakness
- Pale skin.

GENETICS

CDA may be transmitted by both parents autosomal recessively or dominantly and has four different subtypes, CDA Type I, CDA Type II, CDA Type III, and CDA Type IV. CDA type II (CDA II) is the most frequent type of congenital dyserythropoietic anemias. More than 300 cases have been described, but with the exception of a report by the International CDA II Registry, these reports include only small numbers of cases and no data on the lifetime evolution of the disease.

Type	OMIM	Gene	Locus
CDAN1	224120	*CDAN1 (gene)*	15q15
CDAN2	224100	*SEC23B*	20p11.2
CDAN3	105600	*KIF23*	15q21
CDAN4	613673	*KLF1*	19p13.13-p13.12

DIAGNOSIS

The diagnosis of congenital dyserythropoietic anemia can be done via sequence analysis of the entire coding region, types I, II, III and IV (is a relatively new form of CDA that had been found, just 4 cases have been reported) according to the genetic testing registry.

Types:

- *Congenital dyserythropoietic anemia type I*-is defined by moderate to severe macrocytic anemia (commonly in neonates as intrauterine growth retardation).
- *Congenital dyserythropoietic anemia type II*-is defined by moderate anemia, splenomegaly, and hepatomegaly.
- *Congenital dyserythropoietic anemia type III*- is defined by mild anemia and retinal degeneration.
- *Congenital dyserythropoietic anemia type IV*- is defined by having severe anemia at birth (type V and VI are recognized).

TREATMENT

Treatment of individuals with CDA usually consist of frequent blood transfusions, but this can vary depending on the type that the individual has. Patients report going every 2–3 weeks for blood transfusions. In addition, they must undertake chelation therapy to survive; either deferoxamine, deferasirox, or deferiprone to eliminate the excess iron that accumulates. Removal of the spleen and gallbladder are common. Hemoglobin levels can run anywhere between 8.0 g/dl and 11.0 g/dl in untransfused patients, the amount of blood received by the patient is not as important as their baseline pre-transfusion hemoglobin level.

This is true for ferritin levels and iron levels in the organs as well, it is important for patients to go regularly for transfusions in order to maximize good health, normal ferritin levels run anywhere between 24 and 336 ng/ml, hematologists generally do not begin chelation therapy until ferritin levels reach at least 1000 ng/ml. It is more important to check iron levels in the organs through MRI scans, however, than to simply get regular blood tests to check ferritin levels, which only show a trend, and do not reflect actual organ iron content.

Gene Therapy

Gene therapy, as well as, bone marrow transplant are also possible treatments for the disorder, but each have their own risks at this point in time. Bone marrow transplantation is the more used method between the two, whereas researchers are still trying to definitively establish the results of gene therapy treatment. It generally requires a 10/10 HLA matched donor, however, who is usually a sibling. As most patients do not have this, they must rely on gene therapy research to potentially provide them with an alternative. CDA at both clinical and genetic aspects are part of a heterogeneous group of genetic conditions. Gene therapy is still experimental and has largely only been tested in animal models until now. This type of therapy has promise, however, as it allows for the autologous transplantation of the patient's own healthy stem cells rather than requiring an outside donor, thereby bypassing any potential for graft vs. host disease (GVHD). In the United States, the FDA approved clinical trials on Beta thalassemia patients in 2012. The first study, which took place in July 2012, recruited human subjects with thalassemia major,

MEGALOBLASTIC ANEMIA

Megaloblastic anemia (or megaloblastic anaemia) is an anemia (of macrocytic classification) that results from inhibition of DNA synthesis during red blood cell production. When DNA synthesis is impaired, the cell cycle cannot progress from the G2 growth stage to the mitosis (M) stage. This leads to continuing cell growth without division, which presents as macrocytosis. Megaloblastic anemia has a rather slow onset, especially when compared to that of other anemias.

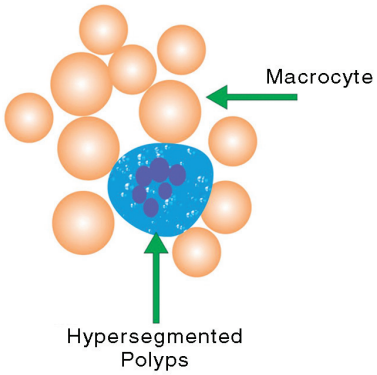


Fig. Megaloblastic anemia.

The defect in red cell DNA synthesis is most often due to hypovitaminosis, specifically a deficiency of vitamin B_{12} and/or folic acid. Vitamin B_{12} deficiency alone will not cause the syndrome in the presence of sufficient folate, as the mechanism is loss of B_{12} dependent folate recycling, followed by folate-deficiency loss of nucleic acid synthesis (specifically thymine), leading to defects in DNA synthesis. Folic acid supplementation in the absence of vitamin B_{12} prevents this type of anemia (although other vitamin B_{12}-specific pathologies may be present). Loss of micronutrients may also be a cause. Copper deficiency resulting from an excess of zinc from unusually high oral consumption of zinc-containing denture-fixation creams has been found to be a cause. Megaloblastic anemia not due to hypovitaminosis may be caused by antimetabolites that poison DNA production directly, such as some chemotherapeutic or antimicrobial agents (for example azathioprine or trimethoprim). The pathological state of megaloblastosis is characterized by many large immature and dysfunctional red blood cells (megaloblasts) in the bone marrow and also by hypersegmented neutrophils (those exhibiting five or more nuclear lobes ("segments"), with up to four lobes being normal). These hypersegmented neutrophils can be detected in the peripheral blood (using a diagnostic smear of a blood sample).

CAUSES

- Vitamin B_{12} deficiency leading to folate deficiency:
  - Achlorhydria-induced malabsorption
  - Deficient intake
  - Deficient intrinsic factor, a molecule produced by cells in the stomach that is required for B_{12} absorption (pernicious anemia or gastrectomy)
  - Coeliac disease
  - Biological competition for vitamin B_{12} by diverticulosis, fistula, intestinal anastomosis, or infection by the marine parasite *Diphyllobothrium latum (fish tapeworm)*
  - Selective vitamin B_{12} malabsorption (congenital—juvenile megaloblastic anemia 1—and drug-induced)
  - Chronic pancreatitis
  - Ileal resection and bypass
  - Nitrous oxide anesthesia (usually requires repeated instances).
- Folate deficiency:
  - Alcoholism
  - Deficient intake
  - Increased needs: pregnancy, infant, rapid cellular proliferation, and cirrhosis
  - Malabsorption (congenital and drug-induced)
  - Intestinal and jejunal resection
  - (indirect) Deficient thiamine and factors (*e.g.,* enzymes) responsible for folate metabolism.
- Combined Deficiency: vitamin B_{12} and folate.
- Inherited Pyrimidine Synthesis Disorders: Orotic aciduria
- Inherited DNA Synthesis Disorders
- Toxins and Drugs:
  - Folic acid antagonists (methotrexate)
  - Purine synthesis antagonists (6-mercaptopurine)
  - Pyrimidine antagonists (cytarabine)
  - Phenytoin
  - Nitrous Oxide
- Erythroleukemia
- Inborn genetic mutations of the Methionine synthase gene.

HEMATOLOGICAL FINDINGS

The blood film can point towards vitamin deficiency:

- Decreased red blood cell (RBC) count and hemoglobin levels
- Increased mean corpuscular volume (MCV, >100 fL) and mean corpuscular hemoglobin (MCH)
- Normal mean corpuscular hemoglobin concentration (MCHC, 32–36 g/dL)
- The reticulocyte count is decreased due to destruction of fragile and abnormal megaloblastic erythroid precursor.
- The platelet count may be reduced.
- Neutrophil granulocytes may show multisegmented nuclei ("senile neutrophil"). This is thought to be due to decreased production and a compensatory prolonged lifespan for circulating neutrophils, which increase numbers of nuclear segments with age.

- Anisocytosis (increased variation in RBC size) and poikilocytosis (abnormally shaped RBCs).
- Macrocytes (larger than normal RBCs) are present.
- Ovalocytes (oval-shaped RBCs) are present.
- Howell-Jolly bodies (chromosomal remnant) also present.

Blood chemistries will also show:

- An increased lactic acid dehydrogenase (LDH) level. The isozyme is LDH-2 which is typical of the serum and hematopoetic cells.
- Increased homocysteine and methylmalonic acid in Vitamin B_{12} deficiency
- Increased homocysteine in folate deficiency.

Normal levels of both methylmalonic acid and total homocysteine rule out clinically significant cobalamin deficiency with virtual certainty. Bone marrow (not normally checked in a patient suspected of megaloblastic anemia) shows megaloblastic hyperplasia.

DIAGNOSIS

The gold standard for the diagnosis of Vitamin B_{12} deficiency is a low blood level of Vitamin B_{12}. A low level of blood Vitamin B_{12} is a finding that normally can and should be treated by injections, supplementation, or dietary or lifestyle advice, but it is not a diagnosis. Hypovitaminosis B_{12} can result from a number of mechanisms, including those listed above. For determination of cause, further patient history, testing, and empirical therapy may be clinically indicated. A measurement of methylmalonic acid (methylmalonate) can provide an indirect method for partially differentiating Vitamin B_{12} and folate deficiencies. The level of methylmalonic acid is not elevated in folic acid deficiency. Direct measurement of blood cobalamin remains the gold standard because the test for elevated methylmalonic acid is not specific enough. Vitamin B_{12} is one necessary prosthetic group to the enzyme methylmalonyl-coenzyme A mutase. Vitamin B_{12} deficiency is but one among the conditions that can lead to dysfunction of this enzyme and a buildup of its substrate, methylmalonic acid, the elevated level of which can be detected in the urine and blood. Due to the lack of available radioactive Vitamin B_{12}, the Schilling test is now largely a historical artifact. The Schilling test was performed in the past to help determine the nature of the vitamin B_{12} deficiency. An advantage of the Schilling test was that it often included Vitamin B_{12} with intrinsic factor.

VITAMIN B_{12} DEFICIENCY ANEMIA

Vitamin B_{12} deficiency anemia, of which pernicious anemia is a type, is a disease in which not enough red blood cells are produced due to a deficiency of vitamin B_{12}. The most common initial symptom is feeling tired. Other symptoms may include shortness of breath, pale skin, chest pain, numbness in the hands and feet, poor balance, a smooth red tongue, poor reflexes, depression and confusion. Without treatment some of these problems may become permanent. Although pernicious anemia technically refers to cases resulting from not enough intrinsic factor, it is often used to describe all cases of anemia due to not enough vitamin B_{12}. Lack of intrinsic factor is most commonly due to an autoimmune attack on the cells that create it in the stomach. It can also occur following the surgical removal of part of the stomach or from an inherited disorder. Other causes of low vitamin B_{12} include not enough dietary intake (such as in a vegan diet), celiac disease, or tapeworm infection. When suspected, diagnosis is made by blood and, occasionally, bone marrow tests. Blood tests may show fewer but larger red blood cells, low numbers of young red blood cells, low levels of vitamin B_{12}, and antibodies to intrinsic factor.

Pernicious anemia, due to lack of intrinsic factor, is not preventable. Vitamin B_{12} deficiency due to other causes may be prevented with a balanced diet or with supplements. Pernicious anemia can be easily treated with either injections or pills of vitamin B_{12}. If the symptoms are severe, injections are typically recommended initially. For those who have trouble swallowing pills, a nasal spray is available. Often, treatment is lifelong.

Pernicious anemia due to autoimmune problems occurs in about one per 1000 people. Among those over the age of 60, about 2% have the condition. It more commonly affects people of northern European descent. Women are more commonly affected than men. With proper treatment, most people live normal lives. Due to a higher risk of stomach cancer, those with pernicious anemia should be checked regularly for this. The first clear description was by Thomas Addison in 1849. The term "pernicious" means "insidiously harmful"; the term was coined and apt prior to the availability of effective treatment.

SIGNS AND SYMPTOMS

The symptoms of pernicious anemia come on slowly. Untreated, it can lead to neurological complications, and in serious cases, death. Many of the signs and symptoms are due to anemia itself, when anemia is present. Symptoms may consist of the triad of tingling or other skin sensations (paresthesia), tongue soreness (glossitis), and fatigue and general weakness. It presents with a number of further common symptoms, including depressive mood, low-grade fevers, diarrhea, dyspepsia, weight loss, neuropathic pain, jaundice, sores at the corner of the mouth (angular cheilitis), a look of exhaustion with pale and dehydrated or cracked lips and dark circles around the eyes, as well as brittle nails, and thinning and early greying of the hair. Because PA may affect the nervous system, symptoms may also include difficulty in proprioception, memory changes, mild cognitive impairment (including difficulty concentrating and sluggish responses, colloquially referred to as brain fog), and even psychoses, impaired urination, loss of sensation in the feet, unsteady gait, difficulty in walking, muscle weakness and clumsiness.

Anemia may also lead to tachycardia (rapid heartbeat), cardiac murmurs, a yellow waxy pallor, altered blood pressure (low or high), and a shortness of breath (known as "the sighs"). The deficiency also may present with thyroid disorders. In severe cases, the anemia may cause evidence of congestive heart failure. A complication of severe chronic PA is subacute combined degeneration of spinal cord, which leads to distal sensory loss (posterior column), absent ankle reflex, increased knee reflex response, and extensor plantar response. Other than anemia, hematological symptoms may include cytopenias, intramedullary hemolysis, and pseudothrombotic microangiopathy. Pernicious anemia can contribute to a delay in physical growth in children, and may also be a cause for delay in puberty for adolescents.

CAUSES

Vitamin B_{12} cannot be produced by the human body, and must be obtained from the diet. When foods containing B_{12} are eaten, the vitamin is usually bound to protein and is released by proteases released by the pancreas in the small bowel. Following its release, most B_{12} is absorbed by the body in the small bowel (ileum) after binding to a protein known as intrinsic factor. Intrinsic factor is produced by parietal cells of the gastric mucosa (stomach lining) and the intrinsic factor-B_{12} complex is absorbed by cubilin receptors on the ileum epithelial cells. PA is characterised by B_{12} deficiency caused by the absence of intrinsic factor.

PA may be considered as an end stage of immune gastritis, a disease characterised by stomach atrophy and the presence of antibodies to parietal cells and intrinsic factor. A specific form of chronic gastritis, type A gastritis or atrophic body gastritis, is highly associated with PA. This autoimmune disorder is localised to the body of the stomach, where parietal cells are located. Antibodies to intrinsic factor and parietal cells cause the destruction of the oxyntic gastric mucosa, in which the parietal cells are located, leading to the subsequent loss of intrinsic factor synthesis. Without intrinsic factor, the ileum can no longer absorb the B_{12}.

Although the exact role of *Helicobacter pylori* infection in PA remains controversial, evidence indicates *H. pylori* is involved in the pathogenesis of the disease. A long-standing *H. pylori* infection may cause gastric autoimmunity by a mechanism known as molecular mimicry. Antibodies produced by the immune system can be cross-reactive and may bind to both *H. pylori* antigens and those found in the gastric mucosa. The antibodies are produced by activated B cells that recognise both pathogen and self-derived peptides. The autoantigens

believed to cause the autoreactivity are the alpha and beta subunits of the H/K-ATPase. Less commonly, *H. pylori* and Zollinger-Ellison syndrome may also cause a form of non-autoimmune gastritis that can lead to pernicious anemia.

Impaired B_{12} absorption can also occur following gastric removal (gastrectomy) or gastric bypass surgery. In these surgeries, either the parts of the stomach that produce gastric secretions are removed or they are bypassed. This means intrinsic factor, as well as other factors required for B_{12} absorption, are not available. However, B_{12} deficiency after gastric surgery does not usually become a clinical issue. This is probably because the body stores many years' worth of B_{12} in the liver and gastric surgery patients are adequately supplemented with the vitamin. Although no specific PA susceptibility genes have been identified, a genetic factor likely is involved in the disease. Pernicious anemia is often found in conjunction with other autoimmune disorders, suggesting common autoimmune susceptibility genes may be a causative factor. In spite of that, previous family studies and case reports focusing on PA have suggested that there is a tendency of genetic heritance of PA in particular, and close relatives of the PA patients seem to have higher incidence of PA and associated PA conditions. Moreover, it was further indicated that the formation of antibodies to gastric cells was autosomal dominant gene determined, and the presence of antibodies to the gastric cells might not be necessarily related to the occurrence of atrophic gastritis related to PA.

PATHOPHYSIOLOGY

Although the healthy body stores three to five years' worth of B_{12} in the liver, the usually undetected autoimmune activity in one's gut over a prolonged period of time leads to B_{12} depletion and the resulting anemia. B_{12} is required by enzymes for two reactions: the conversion of methylmalonyl CoA to succinyl CoA, and the conversion of homocysteine to methionine. In the latter reaction, the methyl group of 5-methyltetrahydrofolate is transferred to homocysteine to produce tetrahydrofolate and methionine. This reaction is catalyzed by the enzyme methionine synthase with B_{12} as an essential cofactor. During B_{12} deficiency, this reaction cannot proceed, which leads to the accumulation of 5-methyltetrahydrofolate. This accumulation depletes the other types of folate required for purine and thymidylate synthesis, which are required for the synthesis of DNA. Inhibition of DNA replication in red blood cells results in the formation of large, fragile megaloblastic erythrocytes. The neurological aspects of the disease are thought to arise from the accumulation of methylmalonyl CoA due to the requirement of B_{12} as a cofactor to the enzyme methylmalonyl CoA mutase.

DIAGNOSIS

PA may be suspected when a patient's blood smear shows large, fragile, immature erythrocytes, known as megaloblasts. A diagnosis of PA first requires demonstration of megaloblastic anemia by conducting a full blood count and blood smear, which evaluates the mean corpuscular volume (MCV), as well the mean corpuscular hemoglobin concentration (MCHC). PA is identified with a high MCV (macrocytic anemia) and a normal MCHC (normochromic anemia). Ovalocytes are also typically seen on the blood smear, and a pathognomonic feature of megaloblastic anemias (which include PA and others) is hypersegmented neutrophils.

Serum vitamin B_{12} levels are used to detect its deficiency, but they do not distinguish its causes. Vitamin B_{12} levels can be falsely high or low and data for sensitivity and specificity vary widely. Normal serum levels may be found in cases of deficiency where myeloproliferative disorders, liver disease, transcobalamin II deficiency, or intestinal bacterial overgrowth are present. Low levels of serum vitamin B_{12} may be caused by other factors than B_{12} deficiency, such as folate deficiency, pregnancy, oral contraceptive use, haptocorrin deficiency, and myeloma.

The presence of antibodies to gastric parietal cells and intrinsic factor is common in PA. Parietal cell antibodies are found in other autoimmune disorders and also in up to 10% of healthy individuals, making the test non-

specific. However, around 85% of PA patients have parietal cell antibodies, which means they are a sensitive marker for the disease. Intrinsic factor antibodies are much less sensitive than parietal cell antibodies, but they are much more specific. They are found in about half of PA patients and are very rarely found in other disorders. These antibody tests can distinguish between PA and food-B_{12} malabsorption. The combination of both tests of intrinsic factor antibodies and parietal cell antibodies may improve overall sensitivity and specificity of the diagnostic results. A buildup of certain metabolites occurs in B_{12} deficiency due to its role in cellular physiology. Methylmalonic acid (MMA) can be measured in both the blood and urine, whereas homocysteine is only measured in the blood. An increase in both MMA and homocysteine can distinguish between B_{12} deficiency and folate deficiency because only homocysteine increases in the latter.

Elevated gastrin levels can be found in around 80-90% of PA cases, but they may also be found in other forms of gastritis. Decreased pepsinogen I levels or a decreased pepsinogen I to pepsinogen II ratio may also be found, although these findings are less specific to PA and can be found in food-B_{12} malabsorption and other forms of gastritis. The diagnosis of atrophic gastritis type A should be confirmed by gastroscopy and stepwise biopsy. About 90% of individuals with PA have antibodies for parietal cells; however, only 50% of all individuals in the general population with these antibodies have pernicious anemia.

Forms of vitamin B_{12} deficiency other than PA must be considered in the differential diagnosis of megaloblastic anemia. For example, a B_{12}-deficient state which causes megaloblastic anemia and which may be mistaken for classical PA may be caused by infection with the tapeworm *Diphyllobothrium latum*, possibly due to the parasite's competition with host for vitamin B_{12}. The classic test for PA, the Schilling test, is no longer widely used, as more efficient methods are available. This historic test consisted, in its first step, of taking an oral dose of radiolabelled vitamin B_{12}, followed by quantitation of the vitamin in the patient's urine over a 24-hour period via measurement of the radioactivity. A second step of the test repeats the regimen and procedure of the first step, with the addition of oral intrinsic factor. A patient with PA presents lower than normal amounts of intrinsic factor; hence, addition of intrinsic factor in the second step results in an increase in vitamin B_{12} absorption (over the baseline established in the first). The Schilling test distinguished PA from other forms of B_{12} deficiency, specifically, from Imerslund-Grasbeck Syndrome (IGS), a vitamin B_{12}-deficiency caused by mutations in the cobalamin receptor.

TREATMENT

The treatment of PA varies by country and area. Opinions vary over the efficacy of administration (parenteral/oral), the amount and time interval of the doses, or the forms of vitamin B_{12} (*e.g.,* cyanocobalamin/hydroxocobalamin). More comprehensive studies are still needed in order to validate the feasibility of a particular therapeutic method for PA in clinical practices. A permanent cure for PA is lacking, although repletion of B_{12} should be expected to result in cessation of anemia-related symptoms, a halt in neurological deterioration, and in cases where neurological problems are not advanced, neurological recovery and a complete and permanent remission of all symptoms, so long as B_{12} is supplemented. Repletion of B_{12} can be accomplished in a variety of ways.

Intramuscular Injections

The standard treatment for PA has been intramuscular injections of cobalamin in the form of cyanocobalamin (CN-Cbl), hydroxocobalamin (OH-Cbl) or methylcobalamin.

Oral Doses

Treatment with high-dose vitamin B_{12} by mouth also appears effective.

PROGNOSIS

A person with well-treated PA can live a healthy life. Failure to diagnose and treat in time, however, may result in permanent neurological damage, excessive fatigue, depression, memory loss, and other complications. In severe cases, the neurological complications of pernicious anemia can lead to death - hence the name, "pernicious", meaning deadly. An association has been observed between pernicious anemia and certain types of gastric cancer, but a causal link has not been established.

EPIDEMIOLOGY

PA is estimated to affect 0.1% of the general population and 1.9% of those over 60, accounting for 20–50% of B_{12} deficiency in adults. A review of literature shows that the prevalence of PA is higher in Northern Europe, especially in Scandinavian countries, and among people of African descent, and that increased awareness of the disease and better diagnostic tools might play a role in apparently higher rates of incidence.

HISTORY

The symptoms are first described in 1822 by Dr James Scarth Combe in the *Transactions of the Medico-Chirurgical Society of Edinburgh*, under the title of *History of a Case of Anaemia*.

However, this was not investigated in more depth until 1849, by British physician Thomas Addison, from which it acquired the common name of Addison's anemia. In 1871, German physician Michael Anton Biermer (1827–1892) noticed the particular characteristic of the anemia in one of his patients; he later coined the term "progressive pernicious anemia". In 1907, Richard Clarke Cabot reported on a series of 1200 patients with PA; their average survival was between one and three years. William Bosworth Castle performed an experiment whereby he ingested raw hamburger meat and regurgitated it after an hour, and subsequently fed it to a group of 10 patients. Untreated raw hamburger meat was fed to the control group. The former group showed a disease response, whereas the latter group did not. This was not a sustainable practice, but it demonstrated the existence of an 'intrinsic factor' from gastric juice.

Pernicious anemia was a fatal disease before about the year 1920, when George Whipple suggested raw liver as a treatment. The first workable treatment for pernicious anemia began when Whipple made a discovery in the course of experiments in which he bled dogs to make them anemic, then fed them various foods to see which would make them recover most rapidly (he was looking for treatments for anemia from bleeding, not pernicious anemia). Whipple discovered ingesting large amounts of liver seemed to cure anemia from blood loss, and tried liver ingestion as a treatment for pernicious anemia, reporting improvement there, also, in a paper in 1920. George Minot and William Murphy then set about to partly isolate the curative property in liver, and in 1926 showed it was contained in raw liver juice (in the process also showing it was the iron in liver tissue, not the soluble factor in liver juice, which cured the anemia from bleeding in dogs); thus, the discovery of the liver juice factor as a treatment for pernicious anemia had been by coincidence. Frieda Robscheit-Robbins worked closely with Whipple, co-authoring 21 papers from 1925-30.

For the discovery of the cure of a previously fatal disease of unknown cause, Whipple, Minot, and Murphy shared the 1934 Nobel Prize in Medicine. After Minot and Murphy's verification of Whipple's results in 1926, pernicious anemia victims ate or drank at least one-half pound of raw liver, or drank raw liver juice, every day. This continued for several years, until a concentrate of liver juice became available. In 1928, chemist Edwin Cohn prepared a liver extract that was 50 to 100 times more potent than the natural food (liver). The extract could even be injected into muscle, which meant patients no longer needed to eat large amounts of liver or juice. This also reduced the cost of treatment considerably.

The active ingredient in liver remained unknown until 1948, when it was isolated by two chemists, Karl A. Folkers of the United States and Alexander R. Todd of Great Britain. The substance was a cobalamin, which the discoverers named vitamin B_{12}. The new vitamin in liver juice was eventually completely purified and characterized in the 1950s, and other methods of producing it from bacteria were developed. It could be injected into muscle with even less irritation, making it possible to treat PA with even more ease. Pernicious anemia was eventually treated with either injections or large oral doses of B_{12}, typically between 1 and 4 mg daily. One writer has hypothesized that Mary Todd Lincoln, the wife of American President Abraham Lincoln, had pernicious anemia for decades and died from it.

RESEARCH

SNAC Complex

Although oral megadoses and intramuscular injections are the most common methods of treatment currently available, several novel methods are being tested, with high promise for future incorporation into mainstream treatment methods. As injections are unfavourable vehicles for drug delivery, current research involves improving the passive diffusion across the ileum upon oral ingestion of cobalamin derivatives.

Researchers have recently taken advantage of the novel compound sodium N-[8-(2-hydroxybenzoyl)amino]caprylate (SNAC), which greatly enhances both bioavailability and metabolic stability. SNAC is able to form a non-covalent complex with cobalamin while preserving its chemical integrity. This complex is much more lipophilic than the water-soluble vitamin B_{12}, so is able to pass through cellular membranes with greater ease.

Recombinant Intrinsic Factor

Another method for increasing absorption through the ileum is to ingest a Cbl complex to which IF is already bound. The lack of intrinsic factor produced by the patient's body can be supplemented by using synthetic human IF produced from pea plant recombinants. However, in cases where IF-antibodies are the reason for malabsorption across the ileum, this treatment would be ineffective.

Sublingual/Intranasal Delivery

Sublingual treatments have also been postulated to be more effective than oral treatments alone. A 2003 study found, while this method is effective, a dose of 500 µg of cyanocobalamin given either orally or sublingually, is equally efficacious in restoring normal physiological concentrations of cobalamin. Intranasal methods have also been studied as a vehicle for the delivery of cobalamin. A 1997 study monitored the plasma cobalamin concentration of six patients with pernicious anemia over a period of 35 days while being treated with 1500 µg of intranasal hydroxocobalamin. One hour after administration, all patients showed on average an immediate eight-fold increase in plasma cobalamin concentration and a two-fold increase after 35 days with three 1500 µg treatments. However, further studies are needed to investigate the long-term effectiveness of this delivery method.

Exploratory Treatments

One exploratory, and potential alternative method for the treatment of pernicious anemia is the use of transdermal patches. In one such system, the patches are composed of cyanocobalamin, its stabilizers, and

epidermal penetration enhancers. The transdermal route allows the cobalamin derivative to passively diffuse through the stratum corneum, epidermis, and dermis, and ultimately entering the bloodstream; hence, the cobalamin avoids the hepatic first pass effect, and so offers the potential for improved bioavailability and efficacy. Slow release increases cobalamin half-life, offering the potential of decreases in required dosage required relative to oral delivery methods. In one such system, a drug-loaded polycaprolactone fibre that is prepared as a electrospun nanofibre can release hundreds of micrograms of cobabalmin per day.

2

White Blood Cell Disorders

INTRODUCTION

White blood cells (WBCs), also called leukocytes or leucocytes, are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. All white blood cells are produced and derived from multipotent cells in the bone marrow known as hematopoietic stem cells. Leukocytes are found throughout the body, including the blood and lymphatic system.

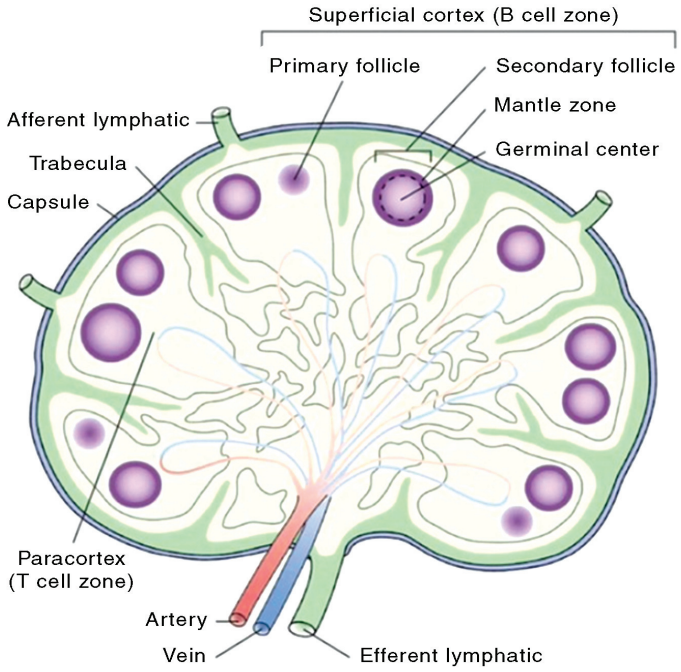


Fig. White blood cell disorders.

All white blood cells have nuclei, which distinguishes them from the other blood cells, the anucleated red blood cells (RBCs) and platelets. Types of white blood cells can be classified in standard ways. Two pairs of broadest categories classify them either by structure (granulocytes or agranulocytes) or by cell division lineage (myeloid cells or lymphoid cells). These broadest categories can be further divided into the five main types: neutrophils, eosinophils (acidophilus), basophils, lymphocytes, and monocytes. These types are distinguished by their physical and functional characteristics. Monocytes and neutrophils are phagocytic. Further subtypes can be classified; for example, among lymphocytes, there are B cells, T cells, and NK cells. The number of leukocytes in the blood is often an indicator of disease, and thus the WBC count is an important subset of the complete blood count. The normal white cell count is usually between 4 × 10/L and 1.1 × 10/L. In the US, this is usually expressed

as 4,000 to 11,000 white blood cells per microliter of blood. They make up approximately 1% of the total blood volume in a healthy adult, making them substantially less numerous than the RBCs at 40% to 45%. However, this 1% of the blood makes a large difference to health, because immunity depends on it. An increase in the number of leukocytes over the upper limits is called leukocytosis. It is normal when it is part of healthy immune responses, which happen frequently. It is occasionally abnormal, when it is neoplastic or autoimmune in origin. A decrease below the lower limit is called leukopenia. This indicates a weakened immune system.

TYPES

Type	Appearance (micrograph)	Appearance (illustration)	Approx. % in adults	Diameter (μm)	Main targets	Nucleus	Granules	Lifetime
Neutrophil			62%	10–12	• Bacteria • Fungi	Multilobed	Fine, faintly pink (H&E stain)	6 hours–few days (days in spleen and other tissue)
Eosinophil			2.3%	10–12	• Larger parasites • Modulate allergic inflammatory responses	Bi-lobed	Full of pink-orange (H&E stain)	8–12 days (circulate for 4–5 hours)
Basophil			0.4%	12–15	• Release histamine for inflammatory responses	Bi-lobed or tri-lobed	Large blue	A few hours to a few days
Lymphocyte			30%	Small lymphocytes 7–8 Large lymphocytes 12–15	• B cells: releases antibodies and assists activation of T cells • T cells: ○ CD4+ Th (T helper) cells: activate and regulate T and B cells ○ CD8+ cytotoxic T cells: virus-infected and tumor cells. ○ γδ T cells: bridge between innate and adaptive immune responses; phagocytosis ○ Regulatory (suppressor) T cells: Returns the functioning of the immune system to normal operation after infection; prevents autoimmunity • Natural killer cells: virus-infected and tumor cells.	Deeply staining, eccentric	NK-cells and cytotoxic (CD8+) T-cells	Years for memory cells, weeks for all else.
Monocyte			5.3%	15–30	Monocytes migrate from the bloodstream to other tissues and differentiate into tissue resident macrophages, Kupffer cells in the liver.	Kidney shaped	None	Hours to days

All white blood cells are nucleated, which distinguishes them from the anucleated red blood cells and platelets. Types of leukocytes can be classified in standard ways. Two pairs of broadest categories classify them either by structure (granulocytes or agranulocytes) or by cell lineage (myeloid cells or lymphoid cells). These broadest categories can be further divided into the five main types: neutrophils, eosinophils, basophils, lymphocytes, and monocytes. These types are distinguished by their physical and functional characteristics. Monocytes and neutrophils are phagocytic. Further subtypes can be classified. Granulocytes are distinguished from agranulocytes by their nucleus shape (lobed versus round, that is, polymorphonuclear versus mononuclear) and by their cytoplasm granules (present or absent, or more precisely, visible on light microscopy or not thus visible). The other dichotomy is by lineage: Myeloid cells (neutrophils, monocytes, eosinophils and basophils) are distinguished from lymphoid cells (lymphocytes) by hematopoietic lineage (cellular differentiation lineage). Lymphocytes can be further classified as T cells, B cells, and natural killer cells.

NEUTROPHIL

Neutrophils (also known as neutrocytes) are the most abundant type of granulocytes and the most abundant (40% to 70%) type of white blood cells in most mammals. They form an essential part of the innate immune system. Their functions vary in different animals. They are formed from stem cells in the bone marrow and differentiated into subpopulations of neutrophil-killers and neutrophil-cagers. They are short-lived and highly motile, or mobisle, as they can enter parts of tissue where other cells/molecules cannot. Neutrophils may be subdivided into segmented neutrophils and banded neutrophils (or bands). They form part of the polymorphonuclear cells family (PMNs) together with basophils and eosinophils.

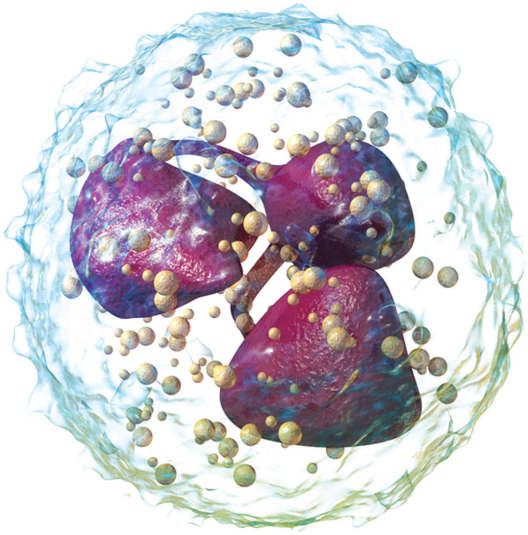


Fig. Neutrophil.

The name *neutrophil* derives from staining characteristics on hematoxylin and eosin (H&E) histological or cytological preparations. Whereas basophilic white blood cells stain dark blue and eosinophilic white blood cells stain bright red, neutrophils stain a neutral pink. Normally, neutrophils contain a nucleus divided into 2–5 lobes.

Neutrophils are a type of phagocyte and are normally found in the bloodstream. During the beginning (acute) phase of inflammation, particularly as a result of bacterial infection, environmental exposure, and some cancers, neutrophils are one of the first-responders of inflammatory cells to migrate towards the site of inflammation. They migrate through the blood vessels, then through interstitial tissue, following chemical signals such as Interleukin-8 (IL-8), C5a, fMLP, Leukotriene B4 and H_2O_2 in a process called chemotaxis. They are the predominant cells in

pus, accounting for its whitish/yellowish appearance. Neutrophils are recruited to the site of injury within minutes following trauma and are the hallmark of acute inflammation; however, due to some pathogens being indigestible, they can be unable to resolve certain infections without the assistance of other types of immune cells.

STRUCTURE

When adhered to a surface, neutrophil granulocytes have an average diameter of 12–15 micrometers (μm) in peripheral blood smears. In suspension, human neutrophils have an average diameter of 8.85 μm. With the eosinophil and the basophil, they form the class of *polymorphonuclear cells*, named for the nucleus' multilobulated shape (as compared to lymphocytes and monocytes, the other types of white cells). The nucleus has a characteristic lobed appearance, the separate lobes connected by chromatin. The nucleolus disappears as the neutrophil matures, which is something that happens in only a few other types of nucleated cells. In the cytoplasm, the Golgi apparatus is small, mitochondria and ribosomes are sparse, and the rough endoplasmic reticulum is absent. The cytoplasm also contains about 200 granules, of which a third are azurophilic. Neutrophils are sexually dimorphic.

Neutrophils from women exhibit a small additional X chromosome structure, known as a "neutrophil drumstick". Neutrophils will show increasing segmentation (many segments of the nucleus) as they mature. A normal neutrophil should have 3–5 segments. Hypersegmentation is not normal but occurs in some disorders, most notably vitamin B_{12} deficiency. This is noted in a manual review of the blood smear and is positive when most or all of the neutrophils have 5 or more segments. Neutrophils are the most abundant white blood cells in humans (approximately 10 are produced daily); they account for approximately 50–70% of all white blood cells (leukocytes). The stated normal range for human blood counts varies between laboratories, but a neutrophil count of 2.5–7.5 × 10/L is a standard normal range. People of African and Middle Eastern descent may have lower counts, which are still normal. A report may divide neutrophils into segmented neutrophils and bands.

When circulating in the bloodstream and inactivated, neutrophils are spherical. Once activated, they change shape and become more amorphous or amoeba-like and can extend pseudopods as they hunt for antigens. Neutrophils have a preference to engulf refined carbohydrates (from ingested glucose, fructose, sucrose, honey and orange juice) over bacteria. In 1973 Sanchez et al. found that the neutrophil phagocytic capacity to engulf bacteria is affected when simple sugars are digested, and that fasting strengthens the neutrophils' phagocytic capacity to engulf bacteria. However, the digestion of normal starches has no effect. It was concluded that the function, and not the number, of phagocytes in engulfing bacteria was altered by the ingestion of sugars. In 2007 researchers at the Whitehead Institute of Biomedical Research found that given a selection of sugars, neutrophils engulf some types of sugar preferentially.

DEVELOPMENT

Life Span

The average lifespan of inactivated human neutrophils in the circulation has been reported by different approaches to be between 5 and 90 hours. Upon activation, they marginate (position themselves adjacent to the blood vessel endothelium) and undergo selectin-dependent capture followed by integrin-dependent adhesion in most cases, after which they migrate into tissues, where they survive for 1–2 days. Neutrophils are much more numerous than the longer-lived monocyte/macrophage phagocytes. A pathogen (disease-causing microorganism or virus) is likely to first encounter a neutrophil. Some experts hypothesize that the short lifetime of neutrophils is an evolutionary adaptation. The short lifetime of neutrophils minimizes propagation of those pathogens that parasitize phagocytes because the

more time such parasites spend outside a host cell, the more likely they will be destroyed by some component of the body's defences. Also, because neutrophil antimicrobial products can also damage host tissues, their short life limits damage to the host during inflammation. Neutrophils will be removed after phagocytosis of pathogens by macrophages. PECAM-1 and phosphatidylserine on the cell surface are involved in this process.

FUNCTION

Chemotaxis

Neutrophils undergo a process called chemotaxis via amoeboid movement, which allows them to migrate towards sites of infection or inflammation. Cell surface receptors allow neutrophils to detect chemical gradients of molecules such as interleukin-8 (IL-8), interferon gamma (IFN-γ), C3a, C5a, and Leukotriene B4, which these cells use to direct the path of their migration. Neutrophils have a variety of specific receptors, including ones for complement, cytokines like interleukins and IFN-γ, chemokines, lectins, and other proteins. They also express receptors to detect and adhere to endothelium and Fc receptors for opsonin.

In leukocytes responding to a chemoattractant, the cellular polarity is regulated by activities of small Rho guanosine triphosphatases (Rho GTPases) and the phosphoinositide 3-kinases (PI3Ks). In neutrophils, lipid products of PI3Ks regulate activation of Rho GTPases and are required for cell motility. They accumulate asymmetrically to the plasma membrane at the leading edge of polarized cells. Spatially regulating Rho GTPases and organizing the leading edge of the cell, PI3Ks and their lipid products could play pivotal roles in establishing leukocyte polarity, as compass molecules that tell the cell where to crawl. It has been shown in mice that in certain conditions neutrophils have a specific type of migration behaviour referred to as neutrophil swarming during which they migrate in a highly coordinated manner and accumulate and cluster to sites of inflammation.

Anti-microbial Function

Being highly motile, neutrophils quickly congregate at a focus of infection, attracted by cytokines expressed by activated endothelium, mast cells, and macrophages. Neutrophils express and release cytokines, which in turn amplify inflammatory reactions by several other cell types. In addition to recruiting and activating other cells of the immune system, neutrophils play a key role in the front-line defence against invading pathogens. Neutrophils have three methods for directly attacking micro-organisms: phagocytosis (ingestion), degranulation (release of soluble anti-microbials), and generation of neutrophil extracellular traps (NETs).

Phagocytosis

Neutrophils are phagocytes, capable of ingesting microorganisms or particles. For targets to be recognized, they must be coated in opsonins—a process known as antibody opsonization. They can internalize and kill many microbes, each phagocytic event resulting in the formation of a phagosome into which reactive oxygen species and hydrolytic enzymes are secreted. The consumption of oxygen during the generation of reactive oxygen species has been termed the "respiratory burst", although unrelated to respiration or energy production.

The respiratory burst involves the activation of the enzyme NADPH oxidase, which produces large quantities of superoxide, a reactive oxygen species. Superoxide decays spontaneously or is broken down via enzymes known as superoxide dismutases (Cu/ZnSOD and MnSOD), to hydrogen peroxide, which is then converted to hypochlorous acid (HClO), by the green heme enzyme myeloperoxidase. It is thought that the bactericidal properties of HClO are enough to kill bacteria phagocytosed by the neutrophil, but this may instead be a step necessary for the activation of proteases.

Degranulation

Neutrophils also release an assortment of proteins in three types of granules by a process called degranulation. The contents of these granules have antimicrobial properties, and help combat infection.

Granule type	Protein
Azurophilic granules (or "primary granules")	Myeloperoxidase, bactericidal/permeability-increasing protein (BPI), defensins, and the serine proteases neutrophil elastase and cathepsin G
Specific granules (or "secondary granules")	Alkaline phosphatase, lysozyme, NADPH oxidase, collagenase, lactoferrin, histaminase, and cathelicidin
Tertiary granules	Cathepsin, gelatinase and collagenase.

Neutrophil Extracellular Traps

In 2004, Brinkmann and colleagues described a striking observation that activation of neutrophils causes the release of web-like structures of DNA; this represents a third mechanism for killing bacteria. These neutrophil extracellular traps (NETs) comprise a web of fibres composed of chromatin and serine proteases that trap and kill extracellular microbes. It is suggested that NETs provide a high local concentration of antimicrobial components and bind, disarm, and kill microbes independent of phagocytic uptake. In addition to their possible antimicrobial properties, NETs may serve as a physical barrier that prevents further spread of pathogens. Trapping of bacteria may be a particularly important role for NETs in sepsis, where NETs are formed within blood vessels. Recently, NETs have been shown to play a role in inflammatory diseases, as NETs could be detected in preeclampsia, a pregnancy-related inflammatory disorder in which neutrophils are known to be activated. In addition, NETs are known to exhibit pro-thrombotic effects both *in vitro* and *in vivo*.

CLINICAL SIGNIFICANCE

Low neutrophil counts are termed *neutropenia*. This can be congenital (developed at or before birth) or it can develop later, as in the case of aplastic anemia or some kinds of leukemia. It can also be a side-effect of medication, most prominently chemotherapy. Neutropenia makes an individual highly susceptible to infections. It can also be the result of colonization by intracellular neutrophilic parasites.

In alpha 1-antitrypsin deficiency, the important neutrophil enzyme elastase is not adequately inhibited by alpha 1-antitrypsin, leading to excessive tissue damage in the presence of inflammation – the most prominent one being pulmonary emphysema. In Familial Mediterranean fever (FMF), a mutation in the *pyrin* (or *marenostrin*) gene, which is expressed mainly in neutrophil granulocytes, leads to a constitutively active acute-phase response and causes attacks of fever, arthralgia, peritonitis, and – eventually – amyloidosis. Decreases in neutrophil function have been linked to hyperglycemia. Dysfunction in the neutrophil biochemical pathway myeloperoxidase as well as reduced degranulation are associated with hyperglycemia.

The Absolute neutrophil count (ANC) is also used in diagnosis and prognosis. ANC is the gold standard for determining severity of neutropenia, and thus neutropenic fever. Any ANC < 1500 cells/mm is considered neutropenia, but <500 cells/mm is considered severe. There is also new research tying ANC to myocardial infarction as an aid in early diagnosis.

NEUTROPHIL ANTIGENS

There are five (HNA 1-5) sets of neutrophil antigens recognized. The three HNA-1 antigens (a-c) are located on the low affinity Fc-γ receptor IIIb (FCGR3B :CD16b) The single known HNA-2a antigen is located on CD177. The HNA-3 antigen system has two antigens (3a and 3b) which are located on the seventh exon of the CLT2 gene (SLC44A2). The HNA-4 and HNA-5 antigen systems each have two known antigens (a and b) and

are located in the β2 integrin. HNA-4 is located on the αM chain (CD11b) and HNA-5 is located on the áL integrin unit (CD11a).

SUBPOPULATIONS

Two functionally unequal subpopulations of neutrophils were identified on the basis of different levels of their reactive oxygen metabolite generation, membrane permeability, activity of enzyme system, and ability to be inactivated. The cells of one subpopulation with high membrane permeability (neutrophil-killers) intensively generate reactive oxygen metabolites and are inactivated in consequence of interaction with the substrate, whereas cells of another subpopulation (neutrophil-cagers) produce reactive oxygen species less intensively, don't adhere to substrate and preserve their activity.

EOSINOPHIL

Eosinophils sometimes called eosinophiles or, less commonly, acidophils, are a variety of white blood cells and one of the immune system components responsible for combating multicellular parasites and certain infections in vertebrates. Along with mast cells and basophils, they also control mechanisms associated with allergy and asthma. They are granulocytes that develop during hematopoiesis in the bone marrow before migrating into blood, after which they are terminally differentiated and do not multiply. These cells are eosinophilic or "acid-loving" due to their large acidophilic cytoplasmic granules, which show their affinity for acids by their affinity to coal tar dyes: Normally transparent, it is this affinity that causes them to appear brick-red after staining with eosin, a red dye, using the Romanowsky method. The staining is concentrated in small granules within the cellular cytoplasm, which contain many chemical mediators, such as eosinophil peroxidase, ribonuclease (RNase), deoxyribonucleases (DNase), lipase, plasminogen, and major basic protein. These mediators are released by a process called degranulation following activation of the eosinophil, and are toxic to both parasite and host tissues.

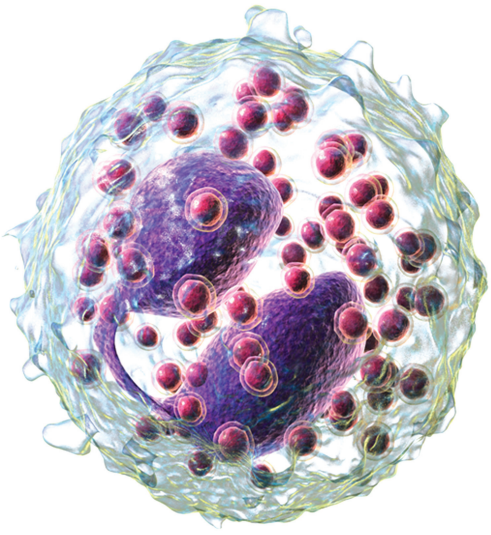


Fig. Eosinophil.

In normal individuals, eosinophils make up about 1–3% of white blood cells, and are about 12–17 micrometres in size with bilobed nuclei. While they are released into the bloodstream as neutrophils are, eosinophils reside in tissue They are found in the medulla and the junction between the cortex and medulla of the thymus, and, in the lower gastrointestinal tract, ovary, uterus, spleen, and lymph nodes, but not in the lung, skin, esophagus, or some other internal organs under normal conditions. The presence of eosinophils in these latter organs is associated with disease. For instance, patients with eosinophilic asthma have high levels of eosinophils that lead to

inflammation and tissue damage, making it more difficult for patients to breathe. Eosinophils persist in the circulation for 8–12 hours, and can survive in tissue for an additional 8–12 days in the absence of stimulation. Pioneering work in the 1980s elucidated that eosinophils were unique granulocytes, having the capacity to survive for extended periods of time after their maturation as demonstrated by ex-vivo culture experiments.

DEVELOPMENT

TH2 and ILC2 cells both express the transcription factor GATA-3 which promotes the production of TH2 cytokines, including the interleukins (ILs). IL-5 controls the development of eosinophils in the bone marrow, as they differentiate from myeloid precursor cells. Their lineage fate is determined by transcription factors, including GATA and C/EBP. Eosinophils produce and store many secondary granule proteins prior to their exit from the bone marrow. After maturation, eosinophils circulate in blood and migrate to inflammatory sites in tissues, or to sites of helminth infection in response to chemokines like CCL11 (eotaxin-1), CCL24 (eotaxin-2), CCL5 (RANTES), 5-hydroxyicosatetraenoic acid and 5-oxo-eicosatetraenoic acid, and certain leukotrienes like leukotriene B4 (LTB4) and MCP1/4. Interleukin-13, another TH2 cytokine, primes eosinophilic exit from the bone marrow by lining vessel walls with adhesion molecules such as VCAM-1 and ICAM-1. When eosinophils are activated, they undergo cytolysis, where the breaking of the cell releases eosinophilic granules found in extracellular DNA traps. High concentrations of these DNA traps are known to cause cellular damage, as the granules they contain are responsible for the ligand-induced secretion of eosinophilic toxins which cause structural damage. There is evidence to suggest that eosinophil granule protein expression is regulated by the non-coding RNA EGOT.

FUNCTION

Following activation, eosinophils effector functions include production of:

- Cationic granule proteins and their release by degranulation.
- Reactive oxygen species such as hypobromite, superoxide, and peroxide (hypobromous acid, which is preferentially produced by eosinophil peroxidase).
- Lipid mediators like the eicosanoids from the leukotriene (*e.g.,* LTC_4, LTD_4, LTE_4) and prostaglandin (*e.g.,* PGE_2) families.
- Enzymes, such as elastase.
- Growth factors such as TGF beta, VEGF, and PDGF.
- Cytokines such as IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-13, and TNF alpha.

There are also eosinophils that play a role in fighting viral infections, which is evident from the abundance of RNases they contain within their granules, and in fibrin removal during inflammation. Eosinophils, along with basophils and mast cells, are important mediators of allergic responses and asthma pathogenesis and are associated with disease severity. They also fight helminth (worm) colonization and may be slightly elevated in the presence of certain parasites. Eosinophils are also involved in many other biological processes, including postpubertal mammary gland development, oestrus cycling, allograft rejection and neoplasia. They have also been implicated in antigen presentation to T cells. Eosinophils are responsible for tissue damage and inflammation in many diseases, including asthma. High levels of interleukin-5 has been observed to up regulate the expression of adhesion molecules, which then facilitate the adhesion of eosinophils to endothelial cells, thereby causing inflammation and tissue damage. An accumulation of eosinophils in the nasal mucosa is considered a major diagnostic criterion for allergic rhinitis (nasal allergies).

GRANULE PROTEINS

Following activation by an immune stimulus, eosinophils degranulate to release an array of cytotoxic granule cationic proteins that are capable of inducing tissue damage and dysfunction.

These include:

- Major basic protein (MBP)
- Eosinophil cationic protein (ECP)
- Eosinophil peroxidase (EPX)
- Eosinophil-derived neurotoxin (EDN).

Major basic protein, eosinophil peroxidase, and eosinophil cationic protein are toxic to many tissues. Eosinophil cationic protein and eosinophil-derived neurotoxin are ribonucleases with antiviral activity. Major basic protein induces mast cell and basophil degranulation, and is implicated in peripheral nerve remodelling. Eosinophil cationic protein creates toxic pores in the membranes of target cells allowing potential entry of other cytotoxic molecules to the cell, can inhibit proliferation of T cells, suppress antibody production by B cells, induce degranulation by mast cells, and stimulate fibroblast cells to secrete mucus and glycosaminoglycan. Eosinophil peroxidase forms reactive oxygen species and reactive nitrogen intermediates that promote oxidative stress in the target, causing cell death by apoptosis and necrosis.

CLINICAL SIGNIFICANCE

Eosinophilia

An increase in eosinophils, *i.e.,* the presence of more than 500 eosinophils/microlitre of blood is called an eosinophilia, and is typically seen in people with a parasitic infestation of the intestines; autoimmune and collagen vascular disease (such as rheumatoid arthritis) and Systemic lupus erythematosus; malignant diseases such as eosinophilic leukemia, clonal hypereosinophilia, and Hodgkin's disease; lymphocyte-variant hypereosinophilia; extensive skin diseases (such as exfoliative dermatitis); Addison's disease and other causes of low corticosteroid production (corticosteroids suppress blood eosinophil levels); reflux esophagitis (in which eosinophils will be found in the squamous epithelium of the esophagus) and eosinophilic esophagitis; and with the use of certain drugs such as penicillin. But, perhaps the most common cause for eosinophilia is an allergic condition such as asthma. In 1989, contaminated L-tryptophan supplements caused a deadly form of eosinophilia known as eosinophilia-myalgia syndrome, which was reminiscent of the Toxic Oil Syndrome in Spain in 1981.

Eosinophils play an important role in asthma as the number of accumulated eosinophils corresponds to the severity of asthmatic reaction. Eosinophilia in mice models are shown to be associated with high interleukin-5 levels. Furthermore, mucosal bronchial biopsies conducted on patients with diseases such as asthma have been found to have higher levels of interleukin-5 leading to higher levels of eosinophils. The infiltration of eosinophils at these high concentrations causes an inflammatory reaction. This ultimately leads to airway remodelling and difficulty of breathing. Eosinophils can also cause tissue damage in the lungs of asthmatic patients. High concentrations of eosinophil major basic protein and eosinophil-derived neurotoxin that approach cytotoxic levels are observed at degranulation sites in the lungs as well as in the asthmatic sputum.

Treatment

Treatments used to combat autoimmune diseases and conditions caused by eosinophils include:

- Corticosteroids – promote apoptosis. Numbers of eosinophils in blood are rapidly reduced
- Monoclonal antibody therapy – *e.g.,* mepolizumab or reslizumab against IL-5, prevents eosinophilopoiesis
- Antagonists of leukotriene synthesis or receptors
- Imatinib (STI571) – inhibits PDGF-BB in hypereosinophilic leukemia.

Monoclonal antibodies such as dupilumab and lebrikizumab target IL-13 and its receptor, which reduces eosinophilic inflammation in patients with asthma due to lowering the number of adhesion molecules present for eosinophils to bind to, thereby decreasing inflammation. Mepolizumab and benralizumab are other treatment options that target the alpha subunit of the IL-5 receptor, thereby inhibiting its function and reducing the number of developing eosinophils as well as the number of eosinophils leading to inflammation through antibody-dependent cell-mediated cytotoxicity and eosinophilic apoptosis.

ANIMAL STUDIES

Within the fat (adipose) tissue of CCR2 deficient mice, there is an increased number of eosinophils, greater alternative macrophage activation, and a propensity towards type 2 cytokine expression. Furthermore, this effect was exaggerated when the mice became obese from a high fat diet. Mouse models of eosinophilia from mice infected with T canis showed an increase in IL-5 mRNA in mice spleen. Mouse models of asthma from OVA show a higher TH2 response. When mice are administered IL-12 to induce the TH1 response, the TH2 response becomes suppressed, showing that mice that do not have TH2 cytokines are significantly less likely to express asthma symptoms.

BASOPHIL

Basophils are a type of white blood cells. Basophils are the least common of the granulocytes, representing about 0.5 to 1% of circulating white blood cells. However, they are the largest type of granulocyte. They are responsible for inflammatory reactions during immune response, as well as in the formation of acute and chronic allergic diseases, including anaphylaxis, asthma, atopic dermatitis and hay fever. They can perform phagocytosis (cell eating), produce histamine and serotonin that induce inflammation, and heparin that prevents blood clotting, although there are less than that found in Mast cell granules. It used to be thought that basophils that have migrated from blood into their resident tissues (connective tissue) are known as mast cells, but this is no longer thought to be the case. Basophils were discovered in 1879 by German physician Paul Ehrlich, who one year earlier had found a cell type present in tissues that he termed *mastzellen* (now mast cells). Ehrlich received the 1908 Nobel Prize in Physiology or Medicine for his discoveries. The name comes from the fact that these leukocytes are basophilic, *i.e.,* they are susceptible to staining by basic dyes, as shown in the picture.

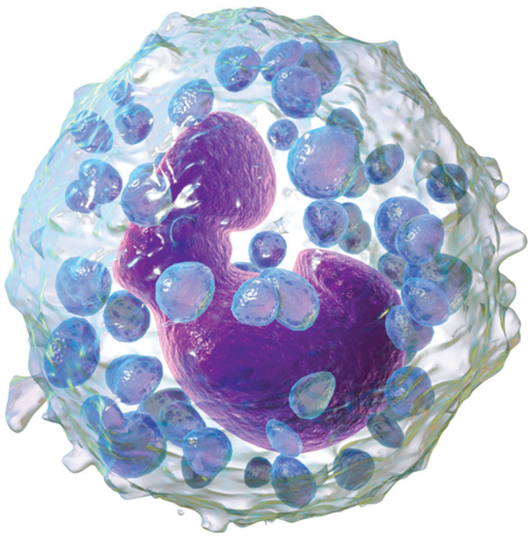


Fig. Basophil.

STRUCTURE

Basophils contain large cytoplasmic granules which obscure the cell nucleus under the microscope when stained. However, when unstained, the nucleus is visible and it usually has two lobes. The mast cell, another granulocyte, is similar in appearance and function. Both cell types store histamine, a chemical that is secreted by the cells when stimulated. However, they arise from different branches of hematopoiesis, and mast cells usually do not circulate in the blood stream, but instead are located in connective tissue. Like all circulating granulocytes, basophils can be recruited out of the blood into a tissue when needed.

FUNCTION

Basophils appear in many specific kinds of inflammatory reactions, particularly those that cause allergic symptoms. Basophils contain anticoagulant heparin, which prevents blood from clotting too quickly. They also contain the vasodilator histamine, which promotes blood flow to tissues. They can be found in unusually high numbers at sites of ectoparasite infection, *e.g.,* ticks. Like eosinophils, basophils play a role in both parasitic infections and allergies.

They are found in tissues where allergic reactions are occurring and probably contribute to the severity of these reactions. Basophils have protein receptors on their cell surface that bind IgE, an immunoglobulin involved in macroparasite defence and allergy. It is the bound IgE antibody that confers a selective response of these cells to environmental substances, for example, pollen proteins or helminth antigens. Recent studies in mice suggest that basophils may also regulate the behaviour of T cells and mediate the magnitude of the secondary immune response.

CD200

Basophil function is inhibited by CD200. Herpesvirus-6, herpesvirus-7, and herpesvirus-8 produce a CD200 homolog which also inhibits basophil function. This suggests that basophils may play a role in the immune response to these viruses.

Secretions

Basophils arise and mature in bone marrow. When activated, basophils degranulate to release histamine, proteoglycans (*e.g.,* heparin and chondroitin), and proteolytic enzymes (*e.g.,* elastase and lysophospholipase). They also secrete lipid mediators like leukotrienes (LTD-4), and several cytokines. Histamine and proteoglycans are pre-stored in the cell's granules while the other secreted substances are newly generated. Each of these substances contributes to inflammation. Recent evidence suggests that basophils are an important source of the cytokine, interleukin-4, perhaps more important than T cells. Interleukin-4 is considered one of the critical cytokines in the development of allergies and the production of IgE antibody by the immune system. There are other substances that can activate basophils to secrete which suggests that these cells have other roles in inflammation.

The degranulation of basophils can be investigated *in vitro* by using flow cytometry and the so-called basophil-activation-test (BAT). Especially, in the diagnosis of allergies including of drug reactions (*e.g.,* induced by contrast medium), the BAT is of great impact. Basopenia (a low basophil count) is difficult to demonstrate as the normal basophil count is so low; it has been reported in association with autoimmune urticaria (a chronic itching condition). Basophilia is also uncommon but may be seen in some forms of leukaemia or lymphoma.

CLINICAL SIGNIFICANCE

Immunophenotyping

Basophils of mice and humans have consistent immunophenotypes, including FcεRI, CD123, CD49b(DX-5), CD69, Thy-1.2, 2B4, CD11b, CD117(c-kit), CD24, CD19, CD80, CD14, CD23, Ly49c, CD122, CD11c, Gr-1, NK1.1, B220, CD3, γδTCR, αβTCR, α_4 and β_4-integrin negative. Recently, Heneberg proposed that basophils may be defined as the cellular population positive for CD13, CD44, CD54, CD63, CD69, CD107a, CD123, CD164, CD193/CCR3, CD203c, TLR-4, and FcεRI. When activated, some additional surface markers are known to be upregulated (CD13, CD107a, CD164), or surface-exposed (CD63, and the ectoenzyme CD203c).

LYMPHOCYTE

A lymphocyte is one of the subtypes of white blood cell in a vertebrate's immune system. Lymphocytes include natural killer cells (which function in cell-mediated, cytotoxic innate immunity), T cells (for cell-mediated, cytotoxic adaptive immunity), and B cells (for humoral, antibody-driven adaptive immunity). They are the main type of cell found in lymph, which prompted the name "lymphocyte".

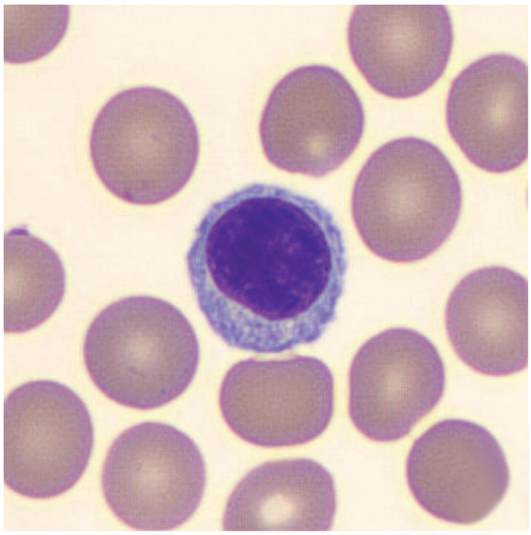


Fig. Lymphocyte.

TYPES

The three major types of lymphocyte are T cells, B cells and natural killer (NK) cells. Lymphocytes can be identified by their large nucleus.

T Cell

A T cell, or T lymphocyte, is a type of lymphocyte (a subtype of white blood cell) that plays a central role in cell-mediated immunity. T cells can be distinguished from other lymphocytes, such as B cells and natural killer cells, by the presence of a T-cell receptor on the cell surface. They are called *T cells* because they mature in the thymus from thymocytes (although some also mature in the tonsils). The several subsets of T cells each have a distinct function. The majority of human T cells rearrange their alpha and beta chains on the cell receptor and are termed alpha beta T cells (αβ T cells) and are part of the adaptive immune system. Specialized gamma delta T cells, (a small minority of T cells in the human body, more frequent in ruminants), have invariant T-cell receptors

with limited diversity, that can effectively present antigens to other T cells and are considered to be part of the innate immune system.

Types

Effector

Effector cells are the superset of all the various T cell types that actively respond immediately to a stimulus, such as co-stimulation. This includes helper, killer, regulatory, and potentially other T cell types. Memory cells are their opposite counterpart that are longer lived to target future infections as necessary.

Helper

T helper cells (T_H cells) assist other white blood cells in immunologic processes, including maturation of B cells into plasma cells and memory B cells, and activation of cytotoxic T cells and macrophages. These cells are also known as CD4 T cells because they express the CD4 glycoprotein on their surfaces. Helper T cells become activated when they are presented with peptide antigens by MHC class II molecules, which are expressed on the surface of antigen-presenting cells (APCs). Once activated, they divide rapidly and secrete small proteins called cytokines that regulate or assist in the active immune response. These cells can differentiate into one of several subtypes, including T_H1, T_H2, T_H3, T_H17, T_H9, or T_{FH}, which secrete different cytokines to facilitate different types of immune responses. Signalling from the APC directs T cells into particular subtypes.

Cytotoxic (Killer)

Cytotoxic T cells (T_C cells, CTLs, T-killer cells, killer T cells) destroy virus-infected cells and tumor cells, and are also implicated in transplant rejection. These cells are also known as CD8 T cells since they express the CD8 glycoprotein at their surfaces. These cells recognize their targets by binding to antigen associated with MHC class I molecules, which are present on the surface of all nucleated cells. Through IL-10, adenosine, and other molecules secreted by regulatory T cells, the CD8 cells can be inactivated to an anergic state, which prevents autoimmune diseases.

Memory

Antigen-naïve T cells expand and differentiate into memory and effector T cells after they encounter their cognate antigen within the context of an MHC molecule on the surface of a professional antigen presenting cell (*e.g.,* a dendritic cell). Appropriate co-stimulation must be present at the time of antigen encounter for this process to occur. Historically, memory T cells were thought to belong to either the effector or central memory subtypes, each with their own distinguishing set of cell surface markers. Subsequently, numerous new populations of memory T cells were discovered including tissue-resident memory T (Trm) cells, stem memory TSCM cells, and virtual memory T cells. The single unifying theme for all memory T cell subtypes is that they are long-lived and can quickly expand to large numbers of effector T cells upon re-exposure to their cognate antigen. By this mechanism they provide the immune system with "memory" against previously encountered pathogens. Memory T cells may be either CD4 or CD8 and usually express CD45RO.

Memory T cell subtypes:

- Central memory T cells (T_{CM} cells) express CD45RO, C-C chemokine receptor type 7 (CCR7), and L-selectin (CD62L). Central memory T cells also have intermediate to high expression of CD44. This memory subpopulation is commonly found in the lymph nodes and in the peripheral circulation. (Note- CD44 expression is usually used to distinguish murine naive from memory T cells).

- Effector memory T cells (T_{EM} cells and T_{EMRA} cells) express CD45RO but lack expression of CCR7 and L-selectin. They also have intermediate to high expression of CD44. These memory T cells lack lymph node-homing receptors and are thus found in the peripheral circulation and tissues. T_{EMRA} stands for terminally differentiated effector memory cells re-expressing CD45RA, which is a marker usually found on naive T cells.
- Tissue resident memory T cells (T_{RM}) occupy tissues (skin, lung, *etc.*.) without recirculating. One cell surface marker that has been associated with T_{RM} is the integrin $\alpha e\beta 7$.
- Virtual memory T cells differ from the other memory subsets in that they do not originate following a strong clonal expansion event. Thus, although this population as a whole is abundant within the peripheral circulation, individual virtual memory T cell clones reside at relatively low frequencies. One theory is that homeostatic proliferation gives rise to this T cell population. Although CD8 virtual memory T cells were the first to be described, it is now known that CD4 virtual memory cells also exist.

Regulatory (Suppressor)

Regulatory T cells (suppressor T cells) are crucial for the maintenance of immunological tolerance. Their major role is to shut down T cell-mediated immunity towards the end of an immune reaction and to suppress autoreactive T cells that escaped the process of negative selection in the thymus. Suppressor T cells along with Helper T cells can collectively be called Regulatory T cells due to their regulatory functions. Two major classes of CD4 T_{reg} cells have been described — FOXP3 T_{reg} cells and FOXP3 T_{reg} cells. Regulatory T cells can develop either during normal development in the thymus, and are then known as thymic Treg cells, or can be induced peripherally and are called peripherally derived Treg cells. These two subsets were previously called "naturally occurring", and "adaptive" or "induced", respectively. Both subsets require the expression of the transcription factor FOXP3 which can be used to identify the cells. Mutations of the *FOXP3* gene can prevent regulatory T cell development, causing the fatal autoimmune disease IPEX. Several other types of T cell have suppressive activity, but do not express FOXP3. These include Tr1 cells and Th3 cells, which are thought to originate during an immune response and act by producing suppressive molecules. Tr1 cells are associated with IL-10, and Th3 cells are associated with TGF-beta. Recently, Treg17 cells have been added to this list.

Natural Killer T Cell

Natural killer T cells (NKT cells – not to be confused with natural killer cells of the innate immune system) bridge the adaptive immune system with the innate immune system. Unlike conventional T cells that recognize peptide antigens presented by major histocompatibility complex (MHC) molecules, NKT cells recognize glycolipid antigen presented by a molecule called CD1d. Once activated, these cells can perform functions ascribed to both T_h and T_c cells (*i.e.,* cytokine production and release of cytolytic/cell killing molecules). They are also able to recognize and eliminate some tumor cells and cells infected with herpes viruses.

Mucosal Associated Invariant

MAIT cells display innate, effector-like qualities. In humans, MAIT cells are found in the blood, liver, lungs, and mucosa, defending against microbial activity and infection. The MHC class I-like protein, MR1, is responsible for presenting bacterially-produced vitamin B metabolites to MAIT cells. After the presentation of foreign antigen by MR1, MAIT cells secretes pro-inflammatory cytokines and are capable of lysing bacterially-infected cells. MAIT cells can also be activated through MR1-independent signaling. In addition to possessing innate-like functions, this T cell subset supports the adaptive immune response and has a memory-like phenotype.

Furthermore, MAIT cells are thought to play a role in autoimmune diseases, such as multiple sclerosis, arthritis and inflammatory bowel disease, although definitive evidence is yet to be published.

Gamma Delta T Cells

Gamma delta T cells (γδ T cells) represent a small subset of T cells that possess a distinct T cell receptor (TCR) on their surfaces. A majority of T cells have a TCR composed of two glycoprotein chains called α- and β- TCR chains. However, in γδ T cells, the TCR is made up of one γ-chain and one δ-chain. This group of T cells is much less common in humans and mice (about 2% of total T cells); and are found mostly in the gut mucosa, within a population of lymphocytes known as intraepithelial lymphocytes. In rabbits, sheep, and chickens, the number of γδ T cells can be as high as 60% of total T cells. The antigenic molecules that activate γδ T cells are still widely unknown. However, γδ T cells are not MHC-restricted and seem to be able to recognize whole proteins rather than requiring peptides to be presented by MHC molecules on APCs. Some murine γδ T cells recognize MHC class IB molecules, though. Human Vγ9/Vδ2 T cells, which constitute the major γδ T cell population in peripheral blood, are unique in that they specifically and rapidly respond to a set of non-peptidic phosphorylated isoprenoid precursors, collectively named phosphoantigens, which are produced by virtually all living cells. The most common phosphoantigens from animal and human cells (including cancer cells) are isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMPP). Many microbes produce the highly active compound hydroxy-DMAPP (HMB-PP) and corresponding mononucleotide conjugates, in addition to IPP and DMAPP. Plant cells produce both types of phosphoantigens. Drugs activating human Vγ9/Vδ2 T cells comprise synthetic phosphoantigens and aminobisphosphonates, which upregulate endogenous IPP/DMAPP.

Development

All T cells originate from haematopoietic stem cells in the bone marrow. Haematopoietic progenitors (lymphoid progenitor cells) from haematopoietic stem cells populate the thymus and expand by cell division to generate a large population of immature thymocytes. The earliest thymocytes express neither CD4 nor CD8, and are therefore classed as double-negative (CD4CD8) cells. As they progress through their development, they become double-positive thymocytes (CD4CD8), and finally mature to *single-positive* (CD4CD8 or CD4CD8) thymocytes that are then released from the thymus to peripheral tissues. There is some evidence of double-positive T-cells in the periphery, though their prevalence and function is uncertain. About 98% of thymocytes die during the development processes in the thymus by failing either positive selection or negative selection, whereas the other 2% survive and leave the thymus to become mature immunocompetent T cells. Increasing evidence indicates microRNAs, which are small non-coding regulatory RNAs, could impact the clonal selection process during thymic development. For example, miR-181a was found to play a role in the positive selection of T lymphocytes. The thymus contributes fewer cells as a person ages. As the thymus shrinks by about 3% a year throughout middle age, a corresponding fall in the thymic production of naïve T cells occurs, leaving peripheral T cell expansion to play a greater role in protecting older subjects.

Beta Selection

Common lymphoid precursor cells that migrate to the thymus become known as T-cell precursors (or thymocytes) and do not express a T cell receptor. Broadly speaking, the double negative (DN) stage is focused on producing a functional β-chain whereas the double positive (DP) stage is focused on producing a functional α-chain, ultimately producing a functional γβ T cell receptor. As the developing thymocyte progresses through the four DN stages (DN1, DN2, DN3, and DN4), the T cell expresses an invariant α-chain but rearranges the β-chain locus. If the rearranged β-chain successfully pairs with the invariant α-chain, signals are produced which

cease rearrangement of the β-chain (and silence the alternate allele) and result in proliferation of the cell. Although these signals require this pre-TCR at the cell surface, they are independent of ligand binding to the pre-TCR. These thymocytes will then express both CD4 and CD8 and progresses to the double positive (DP) stage where selection of the α-chain takes place. If a rearranged β-chain does not lead to any signalling (*e.g.,* as a result of an inability to pair with the invariant α-chain), the cell may die by neglect (lack of signalling).

Positive Selection

Positive selection "selects for" T cells capable of interacting with MHC. Positive selection involves the production of a signal by double-positive precursors that express either MHC Class I or II restricted receptors. The signal produced by these thymocytes result in RAG gene repression, long-term survival and migration into the medulla, as well as differentiation into mature T cells. The process of positive selection takes a number of days. Double-positive thymocytes (CD4/CD8) move deep into the thymic cortex, where they are presented with self-antigens. These self-antigens are expressed by thymic cortical epithelial cells on MHC molecules on the surface of cortical epithelial cells. Only those thymocytes that interact with MHC-I or MHC-II appropriately (*i.e.,* not too strongly or too weakly) will receive a vital "survival signal". All that cannot (*i.e.,* if they do not interact strongly enough, or if they bind too strongly) will die by "death by neglect" (no survival signal).

This process ensures that the selected T-cells will have an MHC affinity that can serve useful functions in the body (*i.e.,* the cells must be able to interact with MHC and peptide complexes to effect immune responses). The vast majority of all thymocytes will die during this process. A thymocyte's fate is determined during positive selection. Double-positive cells (CD4/CD8) that interact well with MHC class II molecules will eventually become CD4 cells, whereas thymocytes that interact well with MHC class I molecules mature into CD8 cells. A T cell becomes a CD4 cell by down-regulating expression of its CD8 cell surface receptors. If the cell does not lose its signal, it will continue downregulating CD8 and become a CD4, single positive cell. But, if there is a signal interruption, the cell stops downregulating CD8 and switches over to downregulating CD4 molecules, instead, eventually becoming a CD8, single positive cell. This process does not remove thymocytes that may cause autoimmunity. The potentially autoimmune cells are removed by the process of negative selection, which occurs in the thymic medulla.

Negative Selection

Negative selection removes thymocytes that are capable of strongly binding with "self" MHC peptides. Thymocytes that survive positive selection migrate towards the boundary of the cortex and medulla in the thymus. While in the medulla, they are again presented with a self-antigen presented on the MHC complex of medullary thymic epithelial cells (mTECs). mTECs must be AIRE to properly express self-antigens from all tissues of the body on their MHC class I peptides. Some mTECs are phagocytosed by thymic dendritic cells; this allows for presentation of self-antigens on MHC class II molecules (positively selected CD4 cells must interact with MHC class II molecules, thus APCs, which possess MHC class II, must be present for CD4 T-cell negative selection). Thymocytes that interact too strongly with the self-antigen receive an apoptotic signal that leads to cell death. However, some of these cells are selected to become Treg cells. The remaining cells exit the thymus as immature naïve T cells (also known as recent thymic emigrants). This process is an important component of central tolerance and serves to prevent the formation of self-reactive T cells that are capable of inducing autoimmune diseases in the host.

In summary, β-selection is the first checkpoint, where the T cells that are able to form a functional pre-TCR with an invariant alpha chain and a functional beta chain are allowed to continue development in the thymus. Next, positive selection checks that T cells have successfully rearranged their TCRα locus and are capable of recognizing peptide-MHC complexes with appropriate affinity. Negative selection in the medulla then obliterates

T cells that bind too strongly to self-antigens expressed on MHC molecules. These selection processes allow for tolerance of self by the immune system. Typical T cells that leave the thymus (via the corticomedullarly junction) are self-restricted, self-tolerant, and singly positive.

Activation

Activation of CD4 T cells occurs through the simultaneous engagement of the T-cell receptor and a co-stimulatory molecule (like CD28, or ICOS) on the T cell by the major histocompatibility complex (MHCII) peptide and co-stimulatory molecules on the APC. Both are required for production of an effective immune response; in the absence of co-stimulation, T cell receptor signalling alone results in anergy. The signalling pathways downstream from co-stimulatory molecules usually engages the PI3K pathway generating PIP3 at the plasma membrane and recruiting PH domain containing signaling molecules like PDK1 that are essential for the activation of PKCθ, and eventual IL-2 production. Optimal CD8 T cell response relies on CD4 signalling. CD4 cells are useful in the initial antigenic activation of naïve CD8 T cells, and sustaining memory CD8 T cells in the aftermath of an acute infection.

Therefore, activation of CD4 T cells can be beneficial to the action of CD8 T cells. The first signal is provided by binding of the T cell receptor to its cognate peptide presented on MHCII on an APC. MHCII is restricted to so-called professional antigen-presenting cells, like dendritic cells, B cells, and macrophages, to name a few. The peptides presented to CD8 T cells by MHC class I molecules are 8–13 amino acids in length; the peptides presented to CD4 cells by MHC class II molecules are longer, usually 12–25 amino acids in length, as the ends of the binding cleft of the MHC class II molecule are open. The second signal comes from co-stimulation, in which surface receptors on the APC are induced by a relatively small number of stimuli, usually products of pathogens, but sometimes breakdown products of cells, such as necrotic-bodies or heat shock proteins. The only co-stimulatory receptor expressed constitutively by naïve T cells is CD28, so co-stimulation for these cells comes from the CD80 and CD86 proteins, which together constitute the B7 protein, (B7.1 and B7.2, respectively) on the APC. Other receptors are expressed upon activation of the T cell, such as OX40 and ICOS, but these largely depend upon CD28 for their expression. The second signal licenses the T cell to respond to an antigen. Without it, the T cell becomes anergic, and it becomes more difficult for it to activate in future.

This mechanism prevents inappropriate responses to self, as self-peptides will not usually be presented with suitable co-stimulation. Once a T cell has been appropriately activated (*i.e.,* has received signal one and signal two) it alters its cell surface expression of a variety of proteins. Markers of T cell activation include CD69, CD71 and CD25 (also a marker for Treg cells), and HLA-DR (a marker of human T cell activation). CTLA-4 expression is also up-regulated on activated T cells, which in turn outcompetes CD28 for binding to the B7 proteins. This is a checkpoint mechanism to prevent over activation of the T cell. Activated T cells also change their cell surface glycosylation profile. The T cell receptor exists as a complex of several proteins. The actual T cell receptor is composed of two separate peptide chains, which are produced from the independent T cell receptor alpha and beta (*TCR*α and *TCR*β) genes. The other proteins in the complex are the CD3 proteins: CD3εγ and CD3γδ heterodimers and, most important, a CD3æ homodimer, which has a total of six ITAM motifs. The ITAM motifs on the CD3æ can be phosphorylated by Lck and in turn recruit ZAP-70. Lck and/or ZAP-70 can also phosphorylate the tyrosines on many other molecules, not least CD28, LAT and SLP-76, which allows the aggregation of signalling complexes around these proteins.

Phosphorylated LAT recruits SLP-76 to the membrane, where it can then bring in PLC-γ, VAV1, Itk and potentially PI3K. PLC-γ cleaves PI(4,5)P2 on the inner leaflet of the membrane to create the active intermediaries diacylglycerol (DAG), inositol-1,4,5-trisphosphate (IP3); PI3K also acts on PIP2, phosphorylating it to produce phosphatidlyinositol-3,4,5-trisphosphate (PIP3). DAG binds and activates some PKCs. Most important in T cells is PKCθ, critical for activating the transcription factors NF-κB and AP-1. IP3 is released from the membrane by PLC-γ and diffuses

rapidly to activate calcium channel receptors on the ER, which induces the release of calcium into the cytosol. Low calcium in the endoplasmic reticulum causes STIM1 clustering on the ER membrane and leads to activation of cell membrane CRAC channels that allows additional calcium to flow into the cytosol from the extracellular space. This aggregated cytosolic calcium binds calmodulin, which can then activate calcineurin. Calcineurin, in turn, activates NFAT, which then translocates to the nucleus. NFAT is a transcription factor that activates the transcription of a pleiotropic set of genes, most notable, IL-2, a cytokine that promotes long-term proliferation of activated T cells. PLCγ can also initiate the NF-κB pathway. DAG activates PKCè, which then phosphorylates CARMA1, causing it to unfold and function as a scaffold. The cytosolic domains bind an adapter BCL10 via CARD (Caspase activation and recruitment domains) domains; that then binds TRAF6, which is ubiquitinated at K63. This form of ubiquitination does not lead to degradation of target proteins. Rather, it serves to recruit NEMO, IKKα and -β, and TAB1-2/TAK1. TAK 1 phosphorylates IKK-β, which then phosphorylates IκB allowing for K48 ubiquitination: leads to proteasomal degradation. Rel A and p50 can then enter the nucleus and bind the NF-κB response element. This coupled with NFAT signaling allows for complete activation of the IL-2 gene. While in most cases activation is dependent on TCR recognition of antigen, alternative pathways for activation have been described. For example, cytotoxic T cells have been shown to become activated when targeted by other CD8 T cells leading to tolerization of the latter. In spring 2014, the T-Cell Activation in Space (TCAS) experiment was launched to the International Space Station on the SpaceX CRS-3 mission to study how "deficiencies in the human immune system are affected by a microgravity environment". T cell activation is modulated by reactive oxygen species.

Antigen Discrimination

A unique feature of T cells is their ability to discriminate between healthy and abnormal (*e.g.,* infected or cancerous) cells in the body. Healthy cells typically express a large number of self derived pMHC on their cell surface and although the T cell antigen receptor can interact with at least a subset of these self pMHC, the T cell generally ignores these healthy cells. However, when these very same cells contain even minute quantities of pathogen derived pMHC, T cells are able to become activated and initiate immune responses. The ability of T cells to ignore healthy cells but respond when these same cells contain pathogen (or cancer) derived pMHC is known as antigen discrimination. The molecular mechanisms that underlie this process are controversial.

Clinical Significance

Deficiency

Causes of T cell deficiency include lymphocytopenia of T cells and/or defects on function of individual T cells. Complete insufficiency of T cell function can result from hereditary conditions such as severe combined immunodeficiency (SCID), Omenn syndrome, and cartilage–hair hypoplasia. Causes of partial insufficiencies of T cell function include acquired immune deficiency syndrome (AIDS), and hereditary conditions such as DiGeorge syndrome (DGS), chromosomal breakage syndromes (CBSs), and B-cell and T-cell combined disorders such as ataxia-telangiectasia (AT) and Wiskott–Aldrich syndrome (WAS). The main pathogens of concern in T cell deficiencies are intracellular pathogens, including *Herpes simplex virus*, *Mycobacterium* and *Listeria*. Also, fungal infections are also more common and severe in T cell deficiencies.

Cancer

Cancer of T cells is termed T-cell lymphoma, and *accounts for perhaps one in ten cases of non-Hodgkin lymphoma. The main forms of T cell lymphoma are:*

- Extranodal T cell lymphoma

- Cutaneous T cell lymphomas: Sézary syndrome and Mycosis fungoides
- Anaplastic large cell lymphoma
- Angioimmunoblastic T cell lymphoma.

Exhaustion

T cell exhaustion is the progressive loss of T cell function. It can occur during sepsis and after other acute or chronic infections. T cell exhaustion is mediated by several inhibitory receptors including programmed cell death protein 1 (PD1), TIM3, and lymphocyte activation gene 3 protein (LAG3). CD8+ T cell exhaustion occurs in some tumours, and can be partly reversed by blocking the inhibitory receptors (*e.g.,* PD1). T cell exhaustion is associated with epigenetic changes in the T cells.

Research

Genetic Engineering

In 2015, a team of researchers led by Dr. Alexander Marson at the University of California, San Francisco successfully edited the genome of human T cells using a Cas9 ribonucleoprotein delivery method. This advancement has potential for applications in treating "cancer immunotherapies and cell-based therapies for HIV, primary immune deficiencies, and autoimmune diseases".

B Cell

B cells, also known as B lymphocytes, are a type of white blood cell of the lymphocyte subtype. They function in the humoral immunity component of the adaptive immune system by secreting antibodies. Additionally, B cells present antigen (they are also classified as professional antigen-presenting cells (APCs)) and secrete cytokines. In mammals, B cells mature in the bone marrow, which is at the core of most bones. In birds, B cells mature in the bursa of Fabricius, a lymphoid organ. (The "B" from B cells comes from the name of this organ, where it was first discovered by Chang and Glick, and not from bone marrow as commonly believed.) B cells, unlike the other two classes of lymphocytes, T cells and natural killer cells, express B cell receptors (BCRs) on their cell membrane. BCRs allow the B cell to bind to a specific antigen, against which it will initiate an antibody response.

Development

B cells develop from hematopoietic stem cells (HSCs) that originate from bone marrow. HSCs first differentiate into multipotent progenitor (MPP) cells, then common lymphoid progenitor (CLP) cells. From here, their development into B cells occurs in several stages (shown in image to the right), each marked by various gene expression patterns and immunoglobulin H chain and L chain gene loci arrangements, the latter due to B cells undergoing V(D)J recombination as they develop.

B cells undergo two types of selection while developing in the bone marrow to ensure proper development. Positive selection occurs through antigen-independent signaling involving both the pre-BCR and the BCR. If these receptors do not bind to their ligand, B cells do not receive the proper signals and cease to develop. Negative selection occurs through the binding of self-antigen with the BCR; If the BCR can bind strongly to self-antigen, then the B cell undergoes one of four fates: clonal deletion, receptor editing, anergy, or ignorance (B cell ignores signal and continues development).

This negative selection process leads to a state of central tolerance, in which the mature B cells don't bind with self antigens present in the bone marrow. To complete development, immature B cells migrate from the

bone marrow into the spleen as transitional B cells, passing through two transitional stages: T1 and T2. Throughout their migration to the spleen and after spleen entry, they are considered T1 B cells. Within the spleen, T1 B cells transition to T2 B cells. T2 B cells differentiate into either follicular (FO) B cells or marginal zone (MZ) B cells depending on signals received through the BCR and other receptors. Once differentiated, they are now considered mature B cells, or naive B cells. While immature and during the T1 phase, B cells express BCR of class IgG, but BCR expression changes to the classes IgM and IgD after transition into the T2 phase and while mature up to activation.

Activation

B cell activation occurs in the secondary lymphoid organs (SLOs), such as the spleen and lymph nodes. After B cells mature in the bone marrow, they migrate through the blood to SLOs, which receive a constant supply of antigen through circulating lymph. At the SLO, B cell activation begins when the B cell binds to an antigen via its BCR. Of the three B cell subsets, FO B cells preferentially undergo T cell-dependent activation while MZ B cells and B1 B cells preferentially undergo T cell-independent activation.

B cell activation is enhanced through the activity of CD21, a surface receptor in complex with surface proteins CD19 and CD81 (all three are collectively known as the B cell coreceptor complex). When a BCR binds an antigen tagged with a fragment of the C3 complement protein, CD21 binds the C3 fragment, co-ligates with the bound BCR, and signals are transduced through CD19 and CD81 to lower the activation threshold of the cell.

T cell-dependent Activation

Antigens that activate B cells with the help of T-cell are known as T cell-dependent (TD) antigens and include foreign proteins. They are named as such because they are unable to induce a humoral response in organisms that lack T cells. B cell response to these antigens takes multiple days, though antibodies generated have a higher affinity and are more functionally versatile than those generated from T cell-independent activation. Once a BCR binds a TD antigen, the antigen is taken up into the B cell through receptor-mediated endocytosis, degraded, and presented to T cells as peptide pieces in complex with MHC-II molecules on the cell membrane.

T helper (T_H) cells, typically follicular T helper (T_{FH}) cells, that were activated with the same antigen recognize and bind these MHC-II-peptide complexes through their T cell receptor (TCR). Following TCR-MHC-II-peptide binding, T cells express the surface protein CD40L as well as cytokines such as IL-4 and IL-21. CD40L serves as a necessary co-stimulatory factor for B cell activation by binding the B cell surface receptor CD40, which promotes B cell proliferation, immunoglobulin class switching, and somatic hypermutation as well as sustains T cell growth and differentiation. T cell-derived cytokines bound by B cell cytokine receptors also promote B cell proliferation, immunoglobulin class switching, and somatic hypermutation as well as guide differentiation. After B cells receive these signals, they are considered activated. Now activated, B cells participate in a two-step differentiation process that yields both short-lived plasmablasts for immediate protection and long-lived plasma cells and memory B cells for persistent protection. The first step, known as the extrafollicular response, occurs outside lymphoid follicles but still in the SLO.

During this step activated B cells proliferate, may undergo immunoglobulin class switching, and differentiate into plasmablasts that produce early, weak antibodies mostly of class IgM. The second step consists of activated B cells entering a lymphoid follicle and forming a germinal center (GC), which is a specialized microenvironment where B cells undergo extensive proliferation, immunoglobulin class switching, and affinity maturation directed by somatic hypermutation. These processes are facilitated by T_{FH} cells within the GC and generate both high-affinity memory B cells and long-lived plasma cells. Resultant plasma cells secrete large amounts of antibody and either stay within the SLO or, more preferentially, migrate to bone marrow.

T cell-independent Activation

Antigens that activate B cells without T cell help are known as T cell-independent (TI) antigens and include foreign polysaccharides and unmethylated CpG DNA. They are named as such because they are able to induce a humoral response in organisms that lack T cells. B cell response to these antigens is rapid, though antibodies generated tend to have lower affinity and are less functionally versatile than those generated from T cell-dependent activation. As with TD antigens, B cells activated by TI antigens need additional signals to complete activation, but instead of receiving them from T cells, they are provided either by recognition and binding of a common microbial constituent to toll-like receptors (TLRs) or by extensive crosslinking of BCRs to repeated epitopes on a bacterial cell. B cells activated by TI antigens go on to proliferate outside lymphoid follicles but still in SLOs (GCs do not form), possibly undergo immunoglobulin class switching, and differentiate into short-lived plasmablasts that produce early, weak antibodies mostly of class IgM, but also some populations of long-lived plasma cells.

Memory B Cell Activation

Memory B cell activation begins with the detection and binding of their target antigen, which is shared by their parent B cell. Some memory B cells can be activated without T cell help, such as certain virus-specific memory B cells, but others need T cell help. Upon antigen binding, the memory B cell takes up the antigen through receptor-mediated endocytosis, degrades it, and presents it to T cells as peptide pieces in complex with MHC-II molecules on the cell membrane. Memory T helper (T_H) cells, typically memory follicular T helper (T_{FH}) cells, that were derived from T cells activated with the same antigen recognize and bind these MHC-II-peptide complexes through their TCR. Following TCR-MHC-II-peptide binding and the relay of other signals from the memory T_{FH} cell, the memory B cell is activated and differentiates either into plasmablasts and plasma cells via an extrafollicular response or enter a germinal center reaction where they generate plasma cells and more memory B cells. It is unclear whether the memory B cells undergo further affinity maturation within these secondary GCs.

B Cell Types

- Plasmablast - A short-lived, proliferating antibody-secreting cell arising from B cell differentiation. Plasmablasts are generated early in an infection and their antibodies tend to have a weaker affinity towards their target antigen compared to plasma cell. Plasmablasts can result from T cell-independent activation of B cells or the extrafollicular response from T cell-dependent activation of B cells.
- Plasma cell - A long-lived, non-proliferating antibody-secreting cell arising from B cell differentiation. There is evidence that B cells first differentiate into a plasmablast-like cell, then differentiate into a plasma cell. Plasma cells are generated later in an infection and, compared to plasmablasts, have antibodies with a higher affinity towards their target antigen due to affinity maturation in the germinal center (GC) and produce more antibodies. Plasma cells typically result from the germinal center reaction from T cell-dependent activation of B cells, however they can also result from T cell-independent activation of B cells.
- Lymphoplasmacytoid cell - A cell with a mixture of B lymphocyte and plasma cell morphological features that is thought to be closely related to or a subtype of plasma cells. This cell type is found in pre-malignant and malignant plasma cell dyscrasias that are associated with the secretion of IgM monoclonal proteins; these dyscrasias include IgM monoclonal gammopathy of undetermined significance and Waldenström's macroglobulinemia.
- Memory B cell - Dormant B cell arising from B cell differentiation. Their function is to circulate through the body and initiate a stronger, more rapid antibody response (known as the anamnestic secondary antibody response) if they detect the antigen that had activated their parent B cell (memory B cells and their parent B cells share the same BCR, thus they detect the same antigen). Memory B

cells can be generated from T cell-dependent activation through both the extrafollicular response and the germinal center reaction as well as from T cell-independent activation of B_1 cells.

- Follicular (FO) B Cell (also known as a B-2 cell) - Most common type of B cell and, when not circulating through the blood, is found mainly in the lymphoid follicles of secondary lymphoid organs (SLOs). They are responsible for generating the majority of high-affinity antibodies during an infection.
- Marginal zone (MZ) B cell - Found mainly in the marginal zone of the spleen and serves as a first line of defence against blood-borne pathogens, as the marginal zone receives large amounts of blood from the general circulation. They can undergo both T cell-independent and T cell-dependent activation, but preferentially undergo T cell-independent activation.
- B-1 cell - Arises from a developmental pathway different from FO B cells and MZ B cells. In mice, they predominantly populate the peritoneal cavity and pleural cavity, generate natural antibodies (antibodies produced without infection), defend against mucosal pathogens, and primarily exhibit T cell-independent activation. A true homologue of mouse B-1 cells has not been discovered in humans, though various cell populations similar to B-1 cells have been described.
- B-2 cell - FO B cells and MZ B cells.
- Regulatory B (Breg) cell - An immunosuppressive B cell type that stops the expansion of pathogenic, pro-inflammatory lymphocytes through the secretion of IL-10, IL-35, and TGF-β. Also, it promotes the generation of regulatory T (Treg) cells by directly interacting with T cells to skew their differentiation towards Tregs. No common Breg cell identity has been described and many Breg cell subsets sharing regulatory functions have been found in both mice and humans. It is currently unknown if Breg cell subsets are developmentally linked and how exactly differentiation into a Breg cell occurs. There is evidence showing that nearly all B cell types can differentiate into a Breg cell through mechanisms involving inflammatory signals and BCR recognition.

B Cell-related Pathology

Autoimmune disease can result from abnormal B cell recognition of self-antigens followed by the production of autoantibodies. Autoimmune diseases where disease activity is correlated with B cell activity include scleroderma, multiple sclerosis, systemic lupus erythematosus, type 1 diabetes, and rheumatoid arthritis. Malignant transformation of B cells and their precursors can cause a host of cancers, including chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), hairy cell leukemia, follicular lymphoma, non-Hodgkin's lymphoma, Hodgkin's lymphoma, and plasma cell malignancies such as multiple myeloma, Waldenström's macroglobulinemia, and certain forms of amyloidosis.

Epigenetic

A study that investigated the methylome of B cells along their differentiation cycle, using whole-genome bisulfite sequencing (WGBS), showed that there is a hypomethylation from the earliest stages to the most differentiated stages. The largest methylation difference is between the stages of germinal center B cells and memory B cells. Furthermore, this study showed that there is a similarity between B cell tumors and long-lived B cells in their DNA methylation signatures.

Natural Killer Cells

Natural killer cells or NK cells are a type of cytotoxic lymphocyte critical to the innate immune system. The role NK cells play is analogous to that of cytotoxic T cells in the vertebrate adaptive immune response. NK cells provide rapid responses to viral-infected cells, acting at around 3 days after infection, and respond to tumor formation. Typically, immune cells detect major histocompatibility complex (MHC) presented on infected cell

surfaces, triggering cytokine release, causing lysis or apoptosis. NK cells are unique, however, as they have the ability to recognize stressed cells in the absence of antibodies and MHC, allowing for a much faster immune reaction. They were named "natural killers" because of the initial notion that they do not require activation to kill cells that are missing "self" markers of MHC class 1.

This role is especially important because harmful cells that are missing MHC I markers cannot be detected and destroyed by other immune cells, such as T lymphocyte cells. NK cells (belonging to the group of innate lymphoid cells) are defined as large granular lymphocytes (LGL) and constitute the third kind of cells differentiated from the common lymphoid progenitor-generating B and T lymphocytes. NK cells are known to differentiate and mature in the bone marrow, lymph nodes, spleen, tonsils, and thymus, where they then enter into the circulation. NK cells differ from natural killer T cells (NKTs) phenotypically, by origin and by respective effector functions; often, NKT cell activity promotes NK cell activity by secreting interferon gamma. In contrast to NKT cells, NK cells do not express T-cell antigen receptors (TCR) or pan T marker CD3 or surface immunoglobulins (Ig) B cell receptors, but they usually express the surface markers CD16 (FcγRIII) and CD56 in humans, NK1.1 or NK1.2 in C57BL/6 mice. The NKp46 cell surface marker constitutes, at the moment, another NK cell marker of preference being expressed in both humans, several strains of mice (including BALB/c mice) and in three common monkey species. In addition to the knowledge that natural killer cells are effectors of innate immunity, recent research has uncovered information on both activating and inhibitory NK cell receptors which play important functional roles, including self tolerance and the sustaining of NK cell activity. NK cells also play a role in the adaptive immune response: numerous experiments have demonstrated their ability to readily adjust to the immediate environment and formulate antigen-specific immunological memory, fundamental for responding to secondary infections with the same antigen. The role of NK cells in both the innate and adaptive immune responses is becoming increasingly important in research using NK cell activity as a potential cancer therapy.

NK Cell Receptors

NK cell receptors can also be differentiated based on function. NK Cells are not a subset of the T lymphocyte family. Natural cytotoxicity receptors directly induce apoptosis after binding to Fas ligand that directly indicate infection of a cell. The MHC-dependent receptors (described above) use an alternate pathway to induce apoptosis in infected cells. Natural killer cell activation is determined by the balance of inhibitory and activating receptor stimulation. For example, if the inhibitory receptor signaling is more prominent, then NK cell activity will be inhibited; similarly, if the activating signal is dominant, then NK cell activation will result. NK cell receptor types (with inhibitory, as well as some activating members) are differentiated by structure, with a few examples to follow:

Activating Receptors

- Ly49 (homodimers), relatively ancient, C-type lectin family receptors, are of multigenic presence in mice, while humans have only one pseudogenic Ly49, the receptor for classical (polymorphic) MHC I molecules.
- NCR (natural cytotoxicity receptors), a type of type 1 transmembrane proteins of the immunoglobulin superfamily, upon stimulation, mediate NK killing and release of IFNγ. They bind viral ligands such as hemagglutinins and hemagglutinin neuraminidases, some bacterial ligands and cellular ligands related with tumour growth such as PCNA.
- CD16 (FcγIIIA) plays a role in antibody-dependent cell-mediated cytotoxicity; in particular, they bind IgG.

Inhibitory Receptors

- Killer-cell immunoglobulin-like receptors (KIRs) belong to a multigene family of more recently evolved Ig-like extracellular domain receptors; they are present in non-human primates, and are the

main receptors for both classical MHC I (HLA-A, HLA-B, HLA-C) and non-classical Mamu-G (HLA-G) in primates. Some KIRs are specific for certain HLA subtypes. Most KIRs are inhibitory and dominant. Regular cells express MHC class 1, so are recognised by KIR receptors and NK cell killing is inhibited.

- CD94/NKG2 (heterodimers), a C-type lectin family receptor, is conserved in both rodents and primates and identifies non-classical (also non-polymorphic) MHC I molecules such as HLA-E. Expression of HLA-E at the cell surface is dependent on the presence of non-amer peptide epitope derived from the signal sequence of classical MHC class I molecules, which is generated by the sequential action of signal peptide peptidase and the proteasome. Though indirect, this is a way to survey the levels of classical (polymorphic) HLA molecules.
- ILT or LIR (leukocyte inhibitory receptors) — are recently discovered members of the Ig receptor family.
- Ly49 (homodimers) have both activating and inhibitory isoforms. They are highly polymorphic on the population level; though they are structurally unrelated to KIRs, they are the functional homologues of KIRs in mice, including the expression pattern. Ly49s are receptor for classical (polymorphic) MHC I molecules.

Function

Cytolytic Granule Mediated Cell Apoptosis

NK cells are cytotoxic; small granules in their cytoplasm contain proteins such as perforin and proteases known as granzymes. Upon release in close proximity to a cell slated for killing, perforin forms pores in the cell membrane of the target cell, creating an aqueous channel through which the granzymes and associated molecules can enter, inducing either apoptosis or osmotic cell lysis. The distinction between apoptosis and cell lysis is important in immunology: lysing a virus-infected cell could potentially only release the virions, whereas apoptosis leads to destruction of the virus inside. α-defensins, antimicrobial molecules, are also secreted by NK cells, and directly kill bacteria by disrupting their cell walls in a manner analogous to that of neutrophils.

Antibody-dependent Cell-mediated Cytotoxicity

Infected cells are routinely opsonized with antibodies for detection by immune cells. Antibodies that bind to antigens can be recognised by FcϒRIII (CD16) receptors expressed on NK cells, resulting in NK activation, release of cytolytic granules and consequent cell apoptosis. This is a major killing mechanism of some monoclonal antibodies like rituximab (Rituxan), ofatumumab (Azzera), and others. The contribution of antibody-dependent cell-mediated cytotoxicity to tumor cell killing can be measured with a specific test that uses NK-92 that has been transfected with a high-affinity FcR. Results are compared to the "wild type" NK-92 that does not express the FcR.

Cytokine-induced NK and Cytotoxic T Lymphocyte (CTL) Activation

Cytokines play a crucial role in NK cell activation. As these are stress molecules released by cells upon viral infection, they serve to signal to the NK cell the presence of viral pathogens in the affected area. Cytokines involved in NK activation include IL-12, IL-15, IL-18, IL-2, and CCL5. NK cells are activated in response to interferons or macrophage-derived cytokines. They serve to contain viral infections while the adaptive immune response generates antigen-specific cytotoxic T cells that can clear the infection. NK cells work to control viral infections by secreting IFNγ and TNFα. IFNγ activates macrophages for phagocytosis and lysis, and TNFα acts

to promote direct NK tumor cell killing. Patients deficient in NK cells prove to be highly susceptible to early phases of herpes virus infection.

Missing 'Self' Hypothesis

For NK cells to defend the body against viruses and other pathogens, they require mechanisms that enable the determination of whether a cell is infected or not. The exact mechanisms remain the subject of current investigation, but recognition of an "altered self" state is thought to be involved. To control their cytotoxic activity, NK cells possess two types of surface receptors: activating receptors and inhibitory receptors, including killer-cell immunoglobulin-like receptors. Most of these receptors are not unique to NK cells and can be present in some T cell subsets, as well. These inhibitory receptors recognize MHC class I alleles, which could explain why NK cells preferentially kill cells that possess low levels of MHC class I molecules. This mode of NK cell target interaction is known as "missing-self recognition", a term coined by Klas Kärre and co-workers in the late 90s. MHC class I molecules are the main mechanism by which cells display viral or tumor antigens to cytotoxic T cells. A common evolutionary adaptation to this is seen in both intracellular microbes and tumors: the chronic down-regulation of MHC I molecules, which makes affected cells invisible to T cells, allowing them to evade T cell-mediated immunity. NK cells apparently evolved as an evolutionary response to this adaptation (the loss of the MHC eliminates CD4/CD8 action, so another immune cell evolved to fulfill the function).

Tumor Cell Surveillance

Natural killer cells often lack antigen-specific cell surface receptors, so are part of innate immunity, *i.e.,* able to react immediately with no prior exposure to the pathogen. In both mice and humans, NKs can be seen to play a role in tumor immunosurveillance by directly inducing the death of tumor cells (NKs act as cytolytic effector lymphocytes), even in the absence of surface adhesion molecules and antigenic peptides. This role of NK cells is critical to immune success particularly because T cells are unable to recognize pathogens in the absence of surface antigens. Tumor cell detection results in activation of NK cells and consequent cytokine production and release. If tumor cells do not cause inflammation, they will also be regarded as self and will not induce a T cell response.

A number of cytokines are produced by NKs, including tumor necrosis factor α (TNFα), IFNγ, and interleukin (IL-10). TNFα and IL-10 act as proinflammatory and immunosuppressors, respectively. The activation of NK cells and subsequent production of cytolytic effector cells impacts macrophages, dendritic cells, and neutrophils, which subsequently enables antigen-specific T and B cell responses. Instead of acting via antigen-specific receptors, lysis of tumor cells by NK cells is mediated by alternative receptors, including NKG2D, NKp44, NKp46, NKp30, and DNAM. NKG2D is a disulfide-linked homodimer which recognizes a number of ligands, including ULBP and MICA, which are typically expressed on tumor cells. NK cells, along with macrophages and several other cell types, express the Fc receptor (FcR) molecule (FC-gamma-RIII = CD16), an activating biochemical receptor that binds the Fc portion of antibodies.

This allows NK cells to target cells against which a humoral response has been mobilized and to lyse cells through ADCC. This response depends on the affinity of the Fc receptor expressed on NK cells, which can have high, intermediate, and low affinity for the Fc portion of the antibody or IgG. This affinity is determined by the nucleotide status in position 158 of the gene, which can code phenylalanine (F allele) or valine (V allele). Individuals with high-affinity FcRgammRIII (158 V/V allele) respond better to antibody therapy. This has been shown for lymphoma patients who received the antibody Rituxan. Patients who express the 158 V/V allele had a better antitumor response. Only 15–25% of the population expressed the 158 V/V allele. To determine the ADCC contribution of monoclonal antibodies, NK-92 cells (a "pure" NK cell line) has been transfected with the gene for the high-affinity FcR.

Adaptive Features of NK Cells— "Memory-like," "Adaptive" and Memory NK Cells

The ability to generate memory cells following a primary infection and the consequent rapid immune activation and response to succeeding infections by the same antigen is fundamental to the role T and B cells play in the adaptive immune response. For many years, NK cells have been considered to be a part of the innate immune system. However, recently increasing evidence suggests that NK cells can display several features that are usually attributed to adaptive immune cells (*e.g.,* T cell responses) such as dynamic expansion and contraction of subsets, increased longevity and a form of immunological memory, characterized by a more potent response upon secondary challenge with the same antigen. In mice, the majority of research was carried out with murine cytomegalovirus (MCMV) and in models of hapten-hypersensitivity reactions. Especially, in the MCMV model, protective memory functions of MCMV-induced NK cells were discovered and direct recognition of the MCMV-ligand m157 by the receptor Ly49 was demonstrated to be crucial for the generation of adaptive NK cell responses. In humans, most studies have focused on the expansion of an NK cell subset carrying the activating receptor NKG2C. Such expansions were observed primarily in response to human cytomegalovirus (HCMV), but also in other infections including Hantavirus, Chikungunya virus, HIV, or viral hepatitis. However, whether these virus infections trigger the expansion of adaptive NKG2C+ NK cells or whether other infections result in re-activation of latent HCMV (as suggested for hepatitis), remains a field of study. Notably, further insights into the biology of adaptive NK cells are hampered by the fact that a direct viral ligand for NKG2C has not yet been identified.

NK Cell Function in Pregnancy

As the majority of pregnancies involve two parents who are not tissue-matched, successful pregnancy requires the mother's immune system to be suppressed. NK cells are thought to be an important cell type in this process. These cells are known as "uterine NK cells" (uNK cells) and they differ from peripheral NK cells. They are in the CD56 NK cell subset, potent at cytokine secretion, but with low cytotoxic ability and relatively similar to peripheral CD56 NK cells, with a slightly different receptor profile. These uNK cells are the most abundant leukocytes present *in utero* in early pregnancy, representing about 70% of leukocytes here, but from where they originate remains controversial.

These NK cells have the ability to elicit cell cytotoxicity *in vitro*, but at a lower level than peripheral NK cells, despite containing perforin. Lack of cytotoxicity *in vivo* may be due to the presence of ligands for their inhibitory receptors. Trophoblast cells downregulate HLA-A and HLA-B to defend against cytotoxic T cell-mediated death. This would normally trigger NK cells by missing self recognition; however, these cells survive. The selective retention of HLA-E (which is a ligand for NK cell inhibitory receptor NKG2A) and HLA-G (which is a ligand for NK cell inhibitory receptor KIR2DL4) by the trophoblast is thought to defend it against NK cell-mediated death. Uterine NK cells have shown no significant difference in women with recurrent miscarriage compared with controls. However, higher peripheral NK cell percentages occur in women with recurrent miscarriages than in control groups. NK cells secrete a high level of cytokines which help mediate their function. Some important cytokines they secrete include TNF-α, IL-10, IFN-γ, and TGF-β, among others. For example, IFN-γ dilates and thins the walls of maternal spiral arteries to enhance blood flow to the implantation site.

NK Cell Evasion by Tumor Cells

By shedding decoy NKG2D soluble ligands, tumor cells may avoid immune responses. These soluble NKG2D ligands bind to NK cell NKG2D receptors, activating a false NK response and consequently creating competition for the receptor site. This method of evasion occurs in prostate cancer. In addition, prostate cancer tumors can

evade CD8 cell recognition due to their ability to downregulate expression of MHC class 1 molecules. This example of immune evasion actually highlights NK cells' importance in tumor surveillance and response, as CD8 cells can consequently only act on tumor cells in response to NK-initiated cytokine production (adaptive immune response).

History

In early experiments on cell-mediated cytotoxicity against tumor target cells, both in cancer patients and animal models, investigators consistently observed what was termed a "natural" reactivity; that is, a certain population of cells seemed to be able to lyse tumor cells without having been previously sensitized to them. The first published study to assert that untreated lymphoid cells were able to confer a natural immunity to tumors was performed by Dr. Henry Smith at the University of Leeds School of Medicine in 1966, leading to the conclusion that the "phenomenon appear[ed] to be an expression of defence mechanisms to tumor growth present in normal mice." Other researchers had also made similar observations, but as these discoveries were inconsistent with the established model at the time, many initially considered these observations to be artifacts.

By 1973, 'natural killing' activity was established across a wide variety of species, and the existence of a separate lineage of cells possessing this ability was postulated. The discovery that a unique type of lymphocyte was responsible for "natural" or spontaneous cytotoxicity was made in the early 1970s by doctoral student Rolf Kiessling and postdoctoral fellow Hugh Pross, in the mouse, and by Hugh Pross and doctoral student Mikael Jondal in the human. The mouse and human work was carried out under the supervision of professors Eva Klein and Hans Wigzell, respectively, of the Karolinska Institute, Stockholm. Kiessling's research involved the well-characterized ability of T lymphocytes to lyse tumor cells against which they had been previously immunized.

Pross and Jondal were studying cell-mediated cytotoxicity in normal human blood and the effect of the removal of various receptor-bearing cells on this cytotoxicity. Later that same year, Ronald Herberman published similar data with respect to the unique nature of the mouse effector cell. The human data were confirmed, for the most part, by West *et al.* using similar techniques and the same erythroleukemic target cell line, K562. K562 is highly sensitive to lysis by human NK cells and, over the decades, the K562 chromium-release assay has become the most commonly used assay to detect human NK functional activity. Its almost universal use has meant that experimental data can be compared easily by different laboratories around the world. Using discontinuous density centrifugation, and later monoclonal antibodies, natural killing ability was mapped to the subset of large, granular lymphocytes known today as NK cells. The demonstration that density gradient-isolated large granular lymphocytes were responsible for human NK activity, made by Timonen and Saksela in 1980, was the first time that NK cells had been visualized microscopically, and was a major breakthrough in the field.

New Findings

Anticancer Therapy

Since NK cells recognize target cells when they express nonself HLA antigens (but not self), autologous (patients' own) NK cell infusions have not shown any antitumor effects. Instead, investigators are working on using allogeneic cells from peripheral blood, which requires that all T cells be removed before infusion into the patients to remove the risk of graft versus host disease, which can be fatal. This can be achieved using an immunomagnetic column (CliniMACS). In addition, because of the limited number of NK cells in blood (only 10% of lymphocytes are NK cells), their number needs to be expanded in culture. This can take a few weeks and the yield is donor-dependent.

A simpler way to obtain high numbers of pure NK cells is to expand NK-92 cells whose cells continuously grow in culture and can be expanded to clinical grade numbers in bags or bioreactors. Clinical studies have shown it to be well tolerated and some antitumor responses have been seen in patients with lung cancer, melanoma, and lymphoma. Infusions of T cells engineered to express a chimeric antigen receptor that recognizes an antigen molecule on leukemia cells could induce remissions in patients with advanced leukemia. Logistical challenges are present for expanding T cells and investigators are working on applying the same technology to peripheral blood NK cells and NK-92. In a study at Boston Children's Hospital, in coordination with Dana-Farber Cancer Institute, whereby immunocompromised mice had contracted lymphomas from EBV infection, an NK-activating receptor called NKG2D was fused with a stimulatory Fc portion of the EBV antibody. The NKG2D-Fc fusion proved capable of reducing tumor growth and prolonging survival of the recipients. In a transplantation model of LMP1-fueled lymphomas, the NKG2D-Fc fusion proved capable of reducing tumor growth and prolonging survival of the recipients.

Innate Resistance to HIV

Recent research suggests specific KIR-MHC class 1 gene interactions might control innate genetic resistance to certain viral infections, including HIV and its consequent development of AIDS. Certain HLA allotypes have been found to determine the progression of HIV to AIDS; an example is the HLA-B57 and HLA-B27 alleles, which have been found to delay progression from HIV to AIDS. This is evident because patients expressing these HLA alleles are observed to have lower viral loads and a more gradual decline in CD4 T cells numbers. Despite considerable research and data collected measuring the genetic correlation of HLA alleles and KIR allotypes, a firm conclusion has not yet been drawn as to what combination provides decreased HIV and AIDS susceptibility. NK cells can impose immune pressure on HIV, which had previously been described only for T cells and antibodies. HIV mutates to avoid NK cell activity.

Tissue-resident NK Cells

Most of our current knowledge is derived from investigations of mouse splenic and human peripheral blood NK cells. However, in recent years tissue-resident NK cell populations have been described. These specialized NK-cell subsets can play a role in organ homeostasis. For example, NK cells are enriched in the human liver with a specific phenotype and take part in the control of liver fibrosis.

DEVELOPMENT

Mammalian stem cells differentiate into several kinds of blood cell within the bone marrow. This process is called haematopoiesis. All lymphocytes originate, during this process, from a common lymphoid progenitor before differentiating into their distinct lymphocyte types. The differentiation of lymphocytes follows various pathways in a hierarchical fashion as well as in a more plastic fashion. The formation of lymphocytes is known as lymphopoiesis. B cells mature into B lymphocytes in the bursa equivalent, which in humans is the GALT, which is thought to be located in the Peyer's patches of the intestine, while T cells migrate to and mature in a distinct organ, called the thymus.

Following maturation, the lymphocytes enter the circulation and peripheral lymphoid organs (*e.g.,* the spleen and lymph nodes) where they survey for invading pathogens and/or tumor cells. The lymphocytes involved in adaptive immunity (*i.e.,* B and T cells) differentiate further after exposure to an antigen; they form effector and memory lymphocytes. Effector lymphocytes function to eliminate the antigen, either by releasing antibodies (in the case of B cells), cytotoxic granules (cytotoxic T cells) or by signaling to other cells of the immune system (helper T cells). Memory T cells remain in the peripheral tissues and circulation for an extended time ready to

respond to the same antigen upon future exposure; they live weeks to several years, which is very long compared to other leukocytes.

CHARACTERISTICS

Microscopically, in a Wright's stained peripheral blood smear, a normal lymphocyte has a large, dark-staining nucleus with little to no eosinophilic cytoplasm. In normal situations, the coarse, dense nucleus of a lymphocyte is approximately the size of a red blood cell (about 7 ìm in diameter). Some lymphocytes show a clear perinuclear zone (or halo) around the nucleus or could exhibit a small clear zone to one side of the nucleus. Polyribosomes are a prominent feature in the lymphocytes and can be viewed with an electron microscope. The ribosomes are involved in protein synthesis, allowing the generation of large quantities of cytokines and immunoglobulins by these cells. It is impossible to distinguish between T cells and B cells in a peripheral blood smear. Normally, flow cytometry testing is used for specific lymphocyte population counts. This can be used to determine the percentage of lymphocytes that contain a particular combination of specific cell surface proteins, such as immunoglobulins or cluster of differentiation (CD) markers or that produce particular proteins (for example, cytokines using intracellular cytokine staining (ICCS)). In order to study the function of a lymphocyte by virtue of the proteins it generates, other scientific techniques like the ELISPOT or secretion assay techniques can be used.

Table. Typical recognition markers for lymphocytes.

Class	Function	Proportion	Phenotypic marker(s)
Natural killer cells	Lysis of virally infected cells and tumour cells	7% (2–13%)	CD16 CD56 but not CD3
T helper cells	Release cytokines and growth factors that regulate other immune cells	46% (28–59%)	TCRαβ, CD3 and CD4
Cytotoxic T cells	Lysis of virally infected cells, tumour cells and allografts	19% (13–32%)	TCRαβ, CD3 and CD8
Gamma delta T cells	Immunoregulation and cytotoxicity	5% (2–8%)	TCRγδ and CD3
B cells	Secretion of antibodies	23% (18–47%)	MHC class II, CD19 and CD20

In the circulatory system, they move from lymph node to lymph node. This contrasts with macrophages, which are rather stationary in the nodes.

LYMPHOCYTES AND DISEASE

A lymphocyte count is usually part of a peripheral complete blood cell count and is expressed as the percentage of lymphocytes to the total number of white blood cells counted. A general increase in the number of lymphocytes is known as lymphocytosis, whereas a decrease is known as lymphocytopenia.

High

An increase in lymphocyte concentration is usually a sign of a viral infection (in some rare case, leukemias are found through an abnormally raised lymphocyte count in an otherwise normal person). A high lymphocyte count with a low neutrophil count might be caused by lymphoma. Pertussis toxin (PTx) of *Bordetella pertussis*, formerly known as lymphocytosis-promoting factor, causes a decrease in the entry of lymphocytes into lymph nodes, which can lead to a condition known as lymphocytosis, with a complete lymphocyte count of over 4000 per µl in adults or over 8000 per µl in children. This is unique in that many bacterial infections illustrate neutrophil-predominance instead.

Low

A low normal to low absolute lymphocyte concentration is associated with increased rates of infection after surgery or trauma. One basis for low T cell lymphocytes occurs when the human immunodeficiency virus

(HIV) infects and destroys T cells (specifically, the CD4 subgroup of T lymphocytes). Without the key defence that these T cells provide, the body becomes susceptible to opportunistic infections that otherwise would not affect healthy people. The extent of HIV progression is typically determined by measuring the percentage of CD4 T cells in the patient's blood – HIV ultimately progresses to acquired immune deficiency syndrome (AIDS). The effects of other viruses or lymphocyte disorders can also often be estimated by counting the numbers of lymphocytes present in the blood.

Tumor-infiltrating Lymphocytes

In some cancers, such as melanoma and colorectal cancer, lymphocytes can migrate into and attack the tumor. This can sometimes lead to regression of the primary tumor.

MONOCYTE

Monocytes are a type of *leukocyte*, or white blood cell. They are the largest type of leukocyte and can differentiate into macrophages and myeloid lineage dendritic cells. As a part of the vertebrate innate immune system monocytes also influence the process of adaptive immunity. There are at least three subclasses of monocytes in human blood based on their phenotypic receptors.

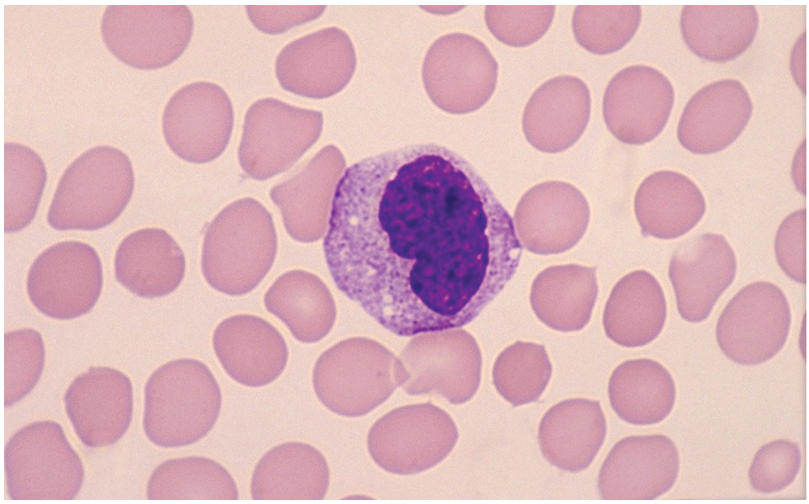


Fig. Monocyte.

STRUCTURE

Monocytes are amoeboid in appearance, and have a granulated cytoplasm. Containing unilobar nuclei, these cells are one of the types of mononuclear leukocytes which shelter azurophil granules. The archetypal geometry of the monocyte nucleus is ellipsoidal; metaphorically bean-shaped or kidney-shaped, although the most significant distinction is that the nuclear envelope should not be hyperbolically furcated into lobes. Contrast to this classification occurs in polymorphonuclear leukocytes. Monocytes compose 2% to 10% of all leukocytes in the human body and serve multiple roles in immune function. Such roles include: replenishing resident macrophages under normal conditions; migration within approximately 8–12 hours in response to inflammation signals from sites of infection in the tissues; and differentiation into macrophages or dendritic cells to effect an immune response. In an adult human, half of the monocytes are stored in the spleen. These change into macrophages after entering into appropriate tissue spaces, and can transform into foam cells in endothelium.

Subpopulations

There are at least three types of monocytes in human blood:

1. The classical monocyte is characterized by high level expression of the CD14 cell surface receptor (CD14 CD16 monocyte)
2. The non-classical monocyte shows low level expression of CD14 and additional co-expression of the CD16 receptor (CD14CD16 monocyte).
3. The intermediate monocyte with high level expression of CD14 and low level expression of CD16 (CD14CD16 monocytes).

While in humans the level of CD14 expression can be used to differentiate non-classical and intermediate monocytes, the slan cell surface marker was shown to give an unequivocal separation of the two cell types. Ghattas et al. state that the "intermediate" monocyte population is likely to be a unique subpopulation of monocytes, as opposed to a developmental step, due to their comparatively high expression of surface receptors involved in reparative processes(including vascular endothelial growth factors type 1 and 2, CXCR4, and Tie-2) as well as evidence that the "intermediate" subset is specifically enriched in the bone marrow. After stimulation with microbial products the CD14+CD16++ monocytes produce high amounts of pro-inflammatory cytokines like tumor necrosis factor and interleukin-12. Said et al. showed that activated monocytes express high levels of PD-1 which might explain the higher expression of PD-1 in CD14+CD16++ monocytes as compared to CD14++CD16- monocytes. Triggering monocytes-expressed PD-1 by its ligand PD-L1 induces IL-10 production which activates CD4 Th2 cells and inhibits CD4 Th1 cell function. In mice, monocytes can be divided in two subpopulations. Inflammatory monocytes (Cx3CR1, CCR2, Ly6C), which are equivalent to human classical CD14 CD16 monocytes and resident monocytes (Cx3CR1, CCR2, Ly6C), which are equivalent to human non-classical CD14 CD16 monocytes. Resident monocytes have the ability to patrol along the endothelium wall in the steady state and under inflammatory conditions. In man a monocyte crawling behaviour, similar to the patrolling in mice, has been demonstrated both for the classical and the non-classical monocytes.

DEVELOPMENT

Monocytes are produced by the bone marrow from precursors called monoblasts, bipotent cells that differentiated from hematopoietic stem cells. Monocytes circulate in the bloodstream for about one to three days and then typically move into tissues throughout the body where they differentiate into macrophages and dendritic cells. They constitute between three and eight percent of the leukocytes in the blood. About half of the body's monocytes are stored as a reserve in the spleen in clusters in the red pulp's Cords of Billroth. Moreover, monocytes are the largest corpuscle in blood. Monocytes which migrate from the bloodstream to other tissues will then differentiate into tissue resident macrophages or dendritic cells. Macrophages are responsible for protecting tissues from foreign substances, but are also suspected to be important in the formation of important organs like the heart and brain. They are cells that possess a large smooth nucleus, a large area of cytoplasm, and many internal vesicles for processing foreign material.

Dendritic Cells

In vitro, monocytes can differentiate into dendritic cells by adding the cytokines granulocyte macrophage colony-stimulating factor (GM-CSF) and interleukin 4.

FUNCTION

Monocytes and their macrophage and dendritic-cell progeny serve three main functions in the immune system. These are phagocytosis, antigen presentation, and cytokine production. Phagocytosis is the process of

uptake of microbes and particles followed by digestion and destruction of this material. Monocytes can perform phagocytosis using intermediary (opsonising) proteins such as antibodies or complement that coat the pathogen, as well as by binding to the microbe directly via pattern-recognition receptors that recognize pathogens. Monocytes are also capable of killing infected host cells via antibody-dependent cell-mediated cytotoxicity. Vacuolization may be present in a cell that has recently phagocytized foreign matter. Many factors produced by other cells can regulate the chemotaxis and other functions of monocytes.

These factors include most particularly chemokines such as monocyte chemotactic protein-1 (CCL2) and monocyte chemotactic protein-3 (CCL7); certain arachidonic acid metabolites such as Leukotriene B4 and members of the 5-Hydroxyicosatetraenoic acid and 5-oxo-eicosatetraenoic acid family of OXE1 receptor agonists (*e.g.,* 5-HETE and 5-oxo-ETE); and N-Formylmethionine leucyl-phenylalanine and other N-formylated oligopeptides which are made by bacteria and activate the formyl peptide receptor 1. Microbial fragments that remain after such digestion can serve as antigens. The fragments can be incorporated into MHC molecules and then trafficked to the cell surface of monocytes (and macrophages and dendritic cells). This process is called antigen presentation and it leads to activation of T lymphocytes, which then mount a specific immune response against the antigen. Other microbial products can directly activate monocytes and this leads to production of pro-inflammatory and, with some delay, of anti-inflammatory cytokines. Typical cytokines produced by monocytes are TNF, IL-1, and IL-12.

CLINICAL SIGNIFICANCE

A *monocyte count* is part of a complete blood count and is expressed either as a percentage of monocytes among all white blood cells or as absolute numbers. Both may be useful but these cells became valid diagnostic tools only when monocyte subsets are determined.

Monocytosis

Monocytosis is the state of excess monocytes in the peripheral blood. It may be indicative of various disease states.

Examples of processes that can increase a monocyte count include:

- Chronic inflammation
- Stress response
- Cushing's syndrome (hyperadrenocorticism)
- Immune-mediated disease
- Granulomatous disease
- Atherosclerosis
- Necrosis
- Red blood cell regeneration
- Viral fever
- Sarcoidosis.

A high count of CD14+CD16++ monocytes is found in severe infection (sepsis) In the field of atherosclerosis high numbers of the CD14++CD16+ intermediate monocytes were shown to be predictive of cardiovascular events in at risk populations.

Monocytopenia

Monocytopenia is a form of leukopenia associated with a deficiency of monocytes. A very low count of these cells is found after therapy with immuno-suppressive glucocorticoids. Also, non-classical slan+ monocytes are strongly reduced in patients with hereditary diffuse leukoencephalopathy with axonal spheroids (HDLS), a neurologic disease associated with mutations in the macrophage colony-stimulating factor receptor gene.

FIXED LEUCOCYTES

Some leucocytes 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.

HISTIOCYTE

A histiocyte is an animal cell that is part of the mononuclear phagocyte system (also known as the reticuloendothelial system or lymphoreticular system). The mononuclear phagocytic system is part of the organism's immune system. The histiocyte is a tissue macrophage or a dendritic cell (histio, diminutive of histo, meaning *tissue*, and cyte, meaning *cell*).

Development

Histiocytes are derived from the bone marrow by multiplication from a stem cell. The derived cells migrate from the bone marrow to the blood as monocytes. They circulate through the body and enter various organs, where they undergo differentiation into histiocytes, which are part of the mononuclear phagocytic system (MPS). However, the term *histiocyte* has been used for multiple purposes in the past, and some cells called "histocytes" do not appear to derive from monocytic-macrophage lines. (The term Histiocyte can also simply refer to a cell from monocyte origin outside the blood system, such as in a tissue (as in rheumatoid arthritis as palisading histiocytes surrounding fibrinoid necrosis of rheumatoid nodules). Some sources consider Langerhans cell derivatives to be histiocytes. The Langerhans cell histiocytosis embeds this interpretation into its name.

Structure

Histiocytes have common histological and immunophenotypical characteristics (demonstrated by immunostains). Their cytoplasm is eosinophilic and contains variable amounts of lysosomes. They bear membrane receptors for opsonins, such as IgG and the fragment C3b of complement. They express LCAs (leucocyte common antigens) CD45, CD14, CD33, and CD4 (also expressed by T helper cells).

Macrophages and Dendritic Cells

These histiocytes are part of the immune system by way of two distinct functions: phagocytosis and antigen presentation. Phagocytosis is the main process of macrophages and antigen presentation the main property of dendritic cells (so called because of their star-like cytoplasmic processes). Macrophages and dendritic cells are derived from common bone marrow precursor cells that have undergone different differentiation (as histiocytes) under the influence of various environmental (tissue location) and growth factors such as GM-CSF, TNF and IL-4. The various categories of histocytes are distinguishable by their morphology, phenotype, and size.

- Macrophages are highly variable in size and morphology, their cytoplasm contains numerous acid phosphatase laden lysosomes - in relation to their specialised phagocytic function. They express CD68.
- Dendritic cells have an indented (bean-shaped) nucleus and cytoplasm with thin processes (dendritic). Their main activity is antigen presentation; they express Factor XIIIa, CD1c, and Class II Human leukocyte antigens.

Langerhans Cells

A subset of cells differentiates into Langerhans cells; this maturation occurs in the squamous epithelium, lymph nodes, spleen, and bronchiolar epithelium. Langerhans cells are antigen-presenting cells but have

undergone further differentiation. Skin Langerhans cells express CD1a, as do cortical thymocytes (cells of the cortex of the thymus gland). They also express S-100, and their cytoplasm contains tennis-racket like ultra-structural inclusions called Birbeck granules.

Clinical Significance

Histiocytoses describe neoplasias wherein the proliferative cell is the histiocyte. The most common histiocyte disorders are Langerhans' cell histiocytosis and haemophagocytic lymphohistiocytosis.

DENDRITIC CELL

Dendritic cells (DCs) are antigen-presenting cells (also known as *accessory cells*) of the mammalian immune system. Their main function is to process antigen material and present it on the cell surface to the T cells of the immune system. They act as messengers between the innate and the adaptive immune systems. Dendritic cells are present in those tissues that are in contact with the external environment, such as the skin (where there is a specialized dendritic cell type called the Langerhans cell) and the inner lining of the nose, lungs, stomach and intestines. They can also be found in an immature state in the blood. Once activated, they migrate to the lymph nodes where they interact with T cells and B cells to initiate and shape the adaptive immune response. At certain development stages they grow branched projections, the *dendrites* that give the cell its name. While similar in appearance, these are structures distinct from the dendrites of neurons. Immature dendritic cells are also called veiled cells, as they possess large cytoplasmic 'veils' rather than dendrites.

History

Dendritic cells were first described by Paul Langerhans (hence "Langerhans cells") in the late nineteenth century. The term "dendritic cells" was coined in 1973 by Ralph M. Steinman and Zanvil A. Cohn. For discovering the central role of dendritic cells in the adaptive immune response, Steinman was awarded the Albert Lasker Award for Basic Medical Research in 2007 and the Nobel Prize in Physiology or Medicine in 2011.

Types

The morphology of dendritic cells results in a very large surface-to-volume ratio. That is, the dendritic cell has a very large surface area compared to the overall cell volume.

In vivo – *primates*

The most common division of dendritic cells is "myeloid" vs. "plasmacytoid" (lymphoid):

Name	Description	Secretion	Toll-like receptors
Conventional dendritic cell (previously called Myeloid dendritic cell) (cDC or mDC)	Most similar to monocytes. mDC are made up of at least two subsets: (1) the more common mDC-1, which is a major stimulator of T cells (2) the extremely rare mDC-2, which may have a function in fighting wound infection	Interleukin 12 (IL-12)	TLR 2, TLR 4
Plasmacytoid dendritic cell (pDC)	Look like plasma cells, but have certain characteristics similar to myeloid dendritic cells.	Can produce high amounts of interferon-alpha and were previously called *interferon-producing cells*.	TLR 7, TLR 9

The markers BDCA-2, BDCA-3, and BDCA-4 can be used to discriminate among the types. Lymphoid and myeloid DCs evolve from lymphoid and myeloid precursors, respectively, and thus are of hematopoietic origin. By contrast, follicular dendritic cells (FDC) are probably of mesenchymal rather than hematopoietic origin and do not express MHC class II, but are so named because they are located in lymphoid follicles and have long "dendritic" processes.

In Blood

The blood DCs are typically identified and enumerated in flow cytometry. Three types of DCs have been defined in human blood: the CD1c+ myeloid DCs, the CD141+ myeloid DCs and the CD303+ plasmacytoid DCs. This represents the nomenclature proposed by the nomenclature committee of the International Union of Immunological Societies. Dendritic cells that circulate in blood do not have all the typical features of their counterparts in tissue, *i.e.,* they are less mature and have no dendrites. Still, they can perform complex functions including chemokine-production (in CD1c+ myeloid DCs), cross-presentation (in CD141+ myeloid DCs), and IFNalpha production (in CD303+ plasmacytoid DCs).

In Vitro

In some respects, dendritic cells cultured in vitro do not show the same behaviour or capability as dendritic cells isolated *ex vivo*. Nonetheless, they are often used for research as they are still much more readily available than genuine DCs.

- Mo-DC or MDDC refers to cells matured from monocytes.
- HP-DC refers to cells derived from hematopoietic progenitor cells.

Non-primate

While humans and non-human primates such as rhesus macaques appear to have DCs divided into these groups, other species (such as the mouse) have different subdivisions of DCs.

Life Cycle

Formation of Immature Cells and their Maturation

Dendritic cells are derived from hematopoietic bone marrow progenitor cells. These progenitor cells initially transform into immature dendritic cells. These cells are characterized by high endocytic activity and low T-cell activation potential. Immature dendritic cells constantly sample the surrounding environment for pathogens such as viruses and bacteria. This is done through pattern recognition receptors (PRRs) such as the toll-like receptors (TLRs).

TLRs recognize specific chemical signatures found on subsets of pathogens. Immature dendritic cells may also phagocytose small quantities of membrane from live own cells, in a process called nibbling. Once they have come into contact with a presentable antigen, they become activated into mature dendritic cells and begin to migrate to the lymph node. Immature dendritic cells phagocytose pathogens and degrade their proteins into small pieces and upon maturation present those fragments at their cell surface using MHC molecules. Simultaneously, they upregulate cell-surface receptors that act as co-receptors in T-cell activation such as CD80 (B7.1), CD86 (B7.2), and CD40 greatly enhancing their ability to activate T-cells. They also upregulate CCR7, a chemotactic receptor that induces

the dendritic cell to travel through the blood stream to the spleen or through the lymphatic system to a lymph node. Here they act as antigen-presenting cells: they activate helper T-cells and killer T-cells as well as B-cells by presenting them with antigens derived from the pathogen, alongside non-antigen specific costimulatory signals. Dendritic cells can also induce T-cell tolerance (unresponsiveness). Certain C-type lectin receptors (CLRs) on the surface of dendritic cells, some functioning as PRRs, help instruct dendritic cells as to when it is appropriate to induce immune tolerance rather than lymphocyte activation. Every helper T-cell is specific to one particular antigen. Only professional antigen-presenting cells (macrophages, B lymphocytes, and dendritic cells) are able to activate a resting helper T-cell when the matching antigen is presented. However, in non-lymphoid organs, macrophages and B cells can only activate memory T cells whereas dendritic cells can activate both memory and naive T cells, and are the most potent of all the antigen-presenting cells.

In the lymph node and secondary lymphoid organs, all three cell types can activate naive T cells. Whereas mature dendritic cells are able to activate antigen-specific naive CD8 T cells, the formation of CD8 memory T cells requires the interaction of dendritic cells with CD4 helper T cells. This help from CD4 T cells additionally activates the matured dendritic cells and licenses them to efficiently induce CD8 memory T cells, which are also able to be expanded a second time. For this activation of dendritic cells, concurrent interaction of all three cell types, namely CD4 T helper cells, CD8 T cells and dendritic cells, seems to be required.

As mentioned above, mDC probably arise from monocytes, white blood cells which circulate in the body and, depending on the right signal, can turn into either dendritic cells or macrophages. The monocytes in turn are formed from stem cells in the bone marrow. Monocyte-derived dendritic cells can be generated in vitro from peripheral blood mononuclear cell (PBMCs). Plating of PBMCs in a tissue culture flask permits adherence of monocytes. Treatment of these monocytes with interleukin 4 (IL-4) and granulocyte-macrophage colony stimulating factor (GM-CSF) leads to differentiation to immature dendritic cells (iDCs) in about a week. Subsequent treatment with tumor necrosis factor (TNF) further differentiates the iDCs into mature dendritic cells. Monocytes can be induced to differentiate into dendritic cells by a self-peptide Ep1.B derived from apolipoprotein E. These are primarily tolerogenic plasmacytoid dendritic cells.

Life Span

Activated macrophages have a lifespan of only a few days though new evidence suggests that it could be extended to weeks rather than days. The lifespan of activated dendritic cells, while somewhat varying according to type and origin, is of a similar order of magnitude, but immature dendritic cells seem to be able to exist in an inactivated state for much longer.

Research Challenges

The exact genesis and development of the different types and subsets of dendritic cells and their interrelationship is only marginally understood at the moment, as dendritic cells are so rare and difficult to isolate that only in recent years they have become subject of focused research. Distinct surface antigens that characterize dendritic cells have only become known from 2000 on; before that, researchers had to work with a 'cocktail' of several antigens which, used in combination, result in isolation of cells with characteristics unique to DCs.

Cytokines

The dendritic cells are constantly in communication with other cells in the body. This communication can take the form of direct cell–cell contact based on the interaction of cell-surface proteins. An example of this includes the interaction of the membrane proteins of the B7 family of the dendritic cell with CD28 present on

the lymphocyte. However, the cell–cell interaction can also take place at a distance via cytokines. For example, stimulating dendritic cells *in vivo* with microbial extracts causes the dendritic cells to rapidly begin producing IL-12. IL-12 is a signal that helps send naive CD4 T cells towards a Th1 phenotype. The ultimate consequence is priming and activation of the immune system for attack against the antigens which the dendritic cell presents on its surface. However, there are differences in the cytokines produced depending on the type of dendritic cell. The plasmacytoid DC has the ability to produce huge amounts of type-1 IFNs, which recruit more activated macrophages to allow phagocytosis.

Disease

HIV Infection

HIV, which causes AIDS, can bind to dendritic cells via various receptors expressed on the cell. The best studied example is DC-SIGN (usually on MDC subset 1, but also on other subsets under certain conditions; since not all dendritic cell subsets express DC-SIGN, its exact role in sexual HIV-1 transmission is not clear). When the dendritic cell takes up HIV and then travels to the lymph node, the virus can be transferred to helper CD4+ T-cells, contributing to the developing infection. This infection of dendritic cells by HIV explains one mechanism by which the virus could persist after prolonged HAART. Many other viruses, such as the SARS virus seems to use DC-SIGN to 'hitchhike' to its target cells. However, most work with virus binding to DC-SIGN expressing cells has been conducted using in vitro derived cells such as moDCs. The physiological role of DC-SIGN in vivo is more difficult to ascertain.

Autoimmunity

Altered function of dendritic cells is also known to play a major or even key role in allergy and autoimmune diseases like lupus erythematosus and inflammatory bowel diseases (Crohn's disease and ulcerative colitis).

In Animals other than Humans

The above applies to humans. In other organisms, the function of dendritic cells can differ slightly. For example, in brown rats (but not mice), a subset of dendritic cells exists that displays pronounced killer cell-like activity, apparently through its entire lifespan. However, the principal function of dendritic cells as known to date is always to act as an immune sentinel. They survey the body and collect information relevant to the immune system, they are then able to instruct and direct the adaptive arms to respond to challenges. In addition, an immediate precursor to myeloid and lymphoid dendritic cells of the spleen has been identified. This precursor, termed pre-DC, lacks MHC class II surface expression, and is distinct from monocytes, which primarily give rise to DCs in non-lymphoid tissues. Dendritic cells have also been found in chickens and turtles.

MAST CELL

A mast cell (also known as a mastocyte or a labrocyte) is a type of white blood cell. Specifically, it is a type of granulocyte derived from the myeloid stem cell that is a part of the immune and neuroimmune systems and contains many granules rich in histamine and heparin. Although best known for their role in allergy and anaphylaxis, mast cells play an important protective role as well, being intimately involved in wound healing, angiogenesis, immune tolerance, defence against pathogens, and blood–brain barrier function. The mast cell is very similar in both appearance and function to the basophil, another type of white blood cell. Although mast cells were once

thought to be tissue resident basophils, it has been shown that the two cells develop from different hematopoietic lineages and thus cannot be the same cells.

Structure

Mast cells are very similar to basophil granulocytes (a class of white blood cells) in blood. Both are granulated cells that contain histamine and heparin, an anticoagulant. The Fc region of immunoglobulin E (IgE) becomes bound to mast cells and basophils and when IgE's paratopes bind to an antigen, it causes the cells to release histamine and other inflammatory mediators. These similarities have led many to speculate that mast cells are basophils that have "homed in" on tissues. Furthermore, they share a common precursor in bone marrow expressing the CD34 molecule. Basophils leave the bone marrow already mature, whereas the mast cell circulates in an immature form, only maturing once in a tissue site. The site an immature mast cell settles in probably determines its precise characteristics. The first in vitro differentiation and growth of a pure population of mouse mast cells has been carried out using conditioned medium derived from concanavalin A-stimulated splenocytes. Later, it was discovered that T cell-derived interleukin 3 was the component present in the conditioned media that was required for mast cell differentiation and growth.

Mast cells in rodents are classically divided into two subtypes: connective tissue-type mast cells and mucosal mast cells. The activities of the latter are dependent on T-cells. Mast cells are present in most tissues characteristically surrounding blood vessels and nerves, and are especially prominent near the boundaries between the outside world and the internal milieu, such as the skin, mucosa of the lungs, and digestive tract, as well as the mouth, conjunctiva, and nose.

Function

Mast cells play a key role in the inflammatory process. When activated, a mast cell can either selectively release (piecemeal degranulation) or rapidly release (anaphylactic degranulation) "mediators", or compounds that induce inflammation, from storage granules into the local microenvironment. Mast cells can be stimulated to degranulate by allergens through cross-linking with immunoglobulin E receptors (*e.g.,* FcεRI), physical injury through pattern recognition receptors for damage-associated molecular patterns (DAMPs), microbial pathogens through pattern recognition receptors for pathogen-associated molecular patterns (PAMPs), and various compounds through their associated G-protein coupled receptors (*e.g.,* morphine through opioid receptors) or ligand-gated ion channels. Complement proteins can activate membrane receptors on mast cells to exert various functions as well. Mast cells express a high-affinity receptor (FcεRI) for the Fc region of IgE, the least-abundant member of the antibodies. This receptor is of such high affinity that binding of IgE molecules is in essence irreversible. As a result, mast cells are coated with IgE, which is produced by plasma cells (the antibody-producing cells of the immune system). IgE molecules, like all antibodies, are specific to one particular antigen.

In allergic reactions, mast cells remain inactive until an allergen binds to IgE already coated upon the cell. Other membrane activation events can either prime mast cells for subsequent degranulation or act in synergy with FcåRI signal transduction. In general, allergens are proteins or polysaccharides. The allergen binds to the antigen-binding sites, which are situated on the variable regions of the IgE molecules bound to the mast cell surface. It appears that binding of two or more IgE molecules (cross-linking) is required to activate the mast cell. The clustering of the intracellular domains of the cell-bound Fc receptors, which are associated with the cross-linked IgE molecules, causes a complex sequence of reactions inside the mast cell that lead to its activation. Although this reaction is most well understood in terms of allergy, it appears to have evolved as a defence system against parasites and bacteria.

Mast Cell Mediators

A unique, stimulus-specific set of mast cell mediators is released through degranulation following the activation of cell surface receptors on mast cells.

Examples of mediators that are released into the extracellular environment during mast cell degranulation include:

- Serine proteases, such as tryptase and chymase
- Histamine (2–5 picograms per mast cell)
- Serotonin
- Proteoglycans, mainly heparin (active as anticoagulant) and some chondroitin sulfate proteoglycans
- Adenosine triphosphate (ATP)
- Lysosomal enzymes
  - β-hexosaminidase
  - β-glucuronidase
  - Arylsulfatases
- Newly formed lipid mediators (eicosanoids):
  - Thromboxane
  - Prostaglandin D2
  - Leukotriene C4
  - Platelet-activating factor
- Cytokines
  - TNF-α
  - Basic fibroblast growth factor
  - Interleukin-4
  - stem cell factor
  - Chemokines, such as eosinophil chemotactic factor
- Reactive oxygen species.

Histamine dilates post-capillary venules, activates the endothelium, and increases blood vessel permeability. This leads to local edema (swelling), warmth, redness, and the attraction of other inflammatory cells to the site of release. It also depolarizes nerve endings (leading to itching or pain). Cutaneous signs of histamine release are the "flare and wheal"-reaction.

The bump and redness immediately following a mosquito bite are a good example of this reaction, which occurs seconds after challenge of the mast cell by an allergen. The other physiologic activities of mast cells are much less-understood. Several lines of evidence suggest that mast cells may have a fairly fundamental role in innate immunity: They are capable of elaborating a vast array of important cytokines and other inflammatory mediators such as TNFa; they express multiple "pattern recognition receptors" thought to be involved in recognizing broad classes of pathogens; and mice without mast cells seem to be much more susceptible to a variety of infections. Mast cell granules carry a variety of bioactive chemicals. These granules have been found to be transferred to adjacent cells of the immune system and neurons in a process of transgranulation via mast cell pseudopodia.

In the Nervous System

Unlike other hematopoietic cells of the immune system, mast cells naturally occur in the human brain where they interact with the neuroimmune system. In the brain, mast cells are located in a number of structures that

mediate visceral sensory (*e.g.,* pain) or neuroendocrine functions or that are located along the blood–cerebrospinal fluid barrier, including the pituitary stalk, pineal gland, thalamus, and hypothalamus, area postrema, choroid plexus, and in the dural layer of the meninges near meningeal nociceptors. Mast cells serve the same general functions in the body and central nervous system, such as effecting or regulating allergic responses, innate and adaptive immunity, autoimmunity, and inflammation. Across systems, mast cells serve as the main effector cell through which pathogens can affect the gut–brain axis.

In the Gut

In the gastrointestinal tract, mucosal mast cells are located in close proximity to sensory nerve fibres, which communicate bidirectionally. When these mast cells initially degranulate, they release mediators (*e.g.,* histamine, tryptase, and serotonin) which activate, sensitize, and upregulate membrane expression of nociceptors (*i.e.,* TRPV1) on visceral afferent neurons via their receptors (respectively, HRH1, HRH2, HRH3, PAR2, 5-HT3); in turn, neurogenic inflammation, visceral hypersensitivity, and intestinal dysmotility (*i.e.,* impaired peristalsis) result. Neuronal activation induces neuropeptide (substance P and calcitonin gene-related peptide) signaling to mast cells where they bind to their associated receptors and trigger degranulation of a distinct set of mediators (β-Hexosaminidase, cytokines, chemokines, PGD2, leukotrienes, and eoxins).

Physiology

Structure of FcεR1

FcεR1 is a high affinity IgE-receptor that is expressed on the surface of the mast cell. FcεR1 is a tetramer made of one alpha (α) chain, one beta (β) chain, and two identical, disulfide-linked gamma (γ) chains. The binding site for the IgE is formed by the extracellular portion of the α chain that contains two domains that are similar to Ig. One transmembrane domain contains an aspartic acid residue, and one contains a short cytoplasmic tail. The β chain contains, a single immunoreceptor tyrosine-based activation motif ITAM, in the cytoplasmic region.

Each γ chain has one ITAM on the cytoplasmic region. The signaling cascade from the receptor is initiated when the ITAMs of the β and γ chains are phosphorylated by tyrosine. This signal is required for the activation of mast cells. Type 2 helper T cells, (Th2) and many other cell types lack the β chain, so signaling is mediated only by the β chain. This is due to the α chain containing endoplasmic reticulum retention signals that causes the α-chains to remain degraded in the ER. The assembly of the α chain with the co-transfected β and γ chains mask the ER retention and allows the α β γ complex to be exported to the golgi apparatus to the plasma membrane in rats. In humans, only the γ complex is needed to counterbalance the α chain ER retention.

Allergen Process

Allergen-mediated FcεR1 cross-linking signals are very similar to the signaling event resulting in antigen binding to lymphocytes. The Lyn tyrosine kinase is associated with the cytoplasmic end of the FcεR1 β chain. The antigen cross-links the FcεR1 molecules, and Lyn tyrosine kinase phosphorylates the ITAMs in the FcεR1 β and γ chain in the cytoplasm. Upon phosphorylation, the Syk tyrosine kinase gets recruited to the ITAMs located on the γ chains. This causes activation of the Syk tyrosine kinase, causing it to phosphorylate. Syk functions as a signal amplifying kinase activity due to the fact that it targets multiple proteins and causes their activation. This antigen stimulated phosphorylation causes the activation of other proteins in the FcεR1-mediated signaling cascade.

Degranulation and Fusion

An important adaptor protein activated by the Syk phosphorylation step is the linker for activation of T cells (LAT). LAT can be modified by phosphorylation to create novel binding sites. Phospholipase C gamma (PLCγ) becomes phosphorylated once bound to LAT, and is then used to catalyze phosphatidylinositol bisphosphate breakdown to yield inositol trisphosphate (IP3) and diacyglycerol (DAG). IP3 elevates calcium levels, and DAG activates protein kinase C (PKC). This is not the only way that PKC is made. The tyrosine kinase FYN phosphorylates Grb2-associated-binding protein 2 (Gab2), which binds to phosphoinositide 3-kinase, which activates PKC.

PKC leads to the activation of myosin light-chain phosphorylation granule movements, which disassembles the actin–myosin complexes to allow granules to come into contact with the plasma membrane. The mast cell granule can now fuse with the plasma membrane. Soluble N-ethylmaleimide sensitive fusion attachment protein receptor SNARE complex mediates this process. Different SNARE proteins interact to form different complexes that catalyze fusion. Rab3 guanosine guanosine triphosphatases and Rab-associated kinases and phosphatases regulate granule membrane fusion in resting mast cells.

Enzymes

Enzyme	Function
Lyn tyrosine kinase	Phosphorylates the ITAMs in the FcεR1 β and γ chain in the cytoplasm. It causes Syk tyrosine kinase to get recruited to the ITAMS located on the γ chains. This causes activation of the Syk tyrosine kinase, causing it to phosphorylate
Syk tyrosine kinase	Targets multiple proteins and causes their activation
Phospholipase C	Catalyzes phosphatidylinositol 4,5-bisphosphate
Inositol trisphosphate	Elevates calcium levels
Diacylglycerol	Activates protein kinase C
FYN	Phosphorylates GAB2
GAB2	Binds to phosphoinositide 3-kinase
Phosphoinositide 3-kinase	Activates protein kinase C
Protein kinase C	Activates myosin light-chain phosphorylation granule movements that disassemble the actin-myosin complexe
Rab-associated kinases and phosphatases	Regulate cell granule membrane fusion in resting mast cells.

Clinical Significance

Parasitic Infections

Mast cells are activated in response to infection by pathogenic parasites, such as certain helminths and protozoa, through IgE signaling.

Mast Cell Activation Disorders

Mast cell activation disorders are a spectrum of immune disorders that are unrelated to pathogenic infection and involve similar symptoms that arise from secreted mast cell intermediates, but differ slightly in their pathophysiology, treatment approach, and distinguishing symptoms. The classification of mast cell activation disorders was laid out in 2010.

Allergic Disease

Allergies are mediated through IgE signaling which triggers mast cell degranulation. Many forms of cutaneous and mucosal allergy are mediated in large part by mast cells; they play a central role in asthma, eczema, itch (from various causes), and allergic rhinitis and allergic conjunctivitis. Antihistamine drugs act by blocking histamine action on nerve endings. Cromoglicate-based drugs (sodium cromoglicate, nedocromil) block a calcium channel essential for mast cell degranulation, stabilizing the cell and preventing release of histamine and related mediators. Leukotriene antagonists (such as montelukast and zafirlukast) block the action of leukotriene mediators and are being used increasingly in allergic diseases. Calcium triggers the secretion of histamine from mast cells after previous exposure to sodium fluoride. The secretory process can be divided into a fluoride-activation step and a calcium-induced secretory step. It was observed that the fluoride-activation step is accompanied by an elevation of cyclic adenosine monophosphate (cAMP) levels within the cells. The attained high levels of cAMP persist during histamine release. It was further found that catecholamines do not markedly alter the fluoride-induced histamine release. It was also confirmed that the second, but not the first, step in sodium fluoride-induced histamine secretion is inhibited by theophylline. Vasodilation and increased permeability of capillaries are a result of both H1 and H2 receptor types. Stimulation of histamine activates a histamine (H2)-sensitive adenylate cyclase of oxyntic cells, and there is a rapid increase in cellular [cAMP] that is involved in activation of H^+ transport and other associated changes of oxyntic cells.

Anaphylaxis

In anaphylaxis (a severe systemic reaction to allergens, such as nuts, bee stings, or drugs), the body-wide degranulation of mast cells leads to vasodilation and, if severe, symptoms of life-threatening shock. Histamine is a vasodilatory substance released during anaphylaxis.

Autoimmunity

Mast cells may be implicated in the pathology associated with autoimmune, inflammatory disorders of the joints. They have been shown to be involved in the recruitment of inflammatory cells to the joints (*e.g.,* rheumatoid arthritis) and skin (*e.g.,* bullous pemphigoid), and this activity is dependent on antibodies and complement components.

Mastocytosis and Clonal Disorders

Mastocytosis is a rare clonal mast cell disorder involving the presence of too many mast cells (*mastocytes*) and CD34+ mast cell precursors. Mutations in c-Kit are associated with mastocytosis.

Neoplastic Disorders

Mastocytomas, or mast cell tumors, can secrete excessive quantities of degranulation products. They are often seen in dogs and cats. Other neoplastic disorders associated with mast cells include mast cell sarcoma and mast cell leukemia.

Mast Cell Activation Syndrome

Mast cell activation syndrome (MCAS) is an idiopathic immune disorder that involves recurrent and excessive mast cell degranulation and which produces symptoms that are similar to other mast cell activation disorders. The syndrome is diagnosed based upon four sets of criteria involving treatment response, symptoms, a differential diagnosis, and biomarkers of mast cell degranulation.

History

Mast cells were first described by Paul Ehrlich in his 1878 doctoral thesis on the basis of their unique staining characteristics and large granules. These granules also led him to the incorrect belief that they existed to nourish the surrounding tissue, so he named them *Mastzellen* (from German *Mast*, meaning 'fattening', as of animals). They are now considered to be part of the immune system.

Research

Autism

Research into an immunological contribution to autism suggests that autism spectrum disorder (ASD) children may present with "allergic-like" problems in the absence of elevated serum IgE and chronic urticaria, suggesting non-allergic mast cell activation in response to environmental and stress triggers. This mast cell activation could contribute to brain inflammation and neurodevelopmental problems.

Histological Staining

Toluidine blue: one of the most common stains for acid mucopolysaccharides and glycoaminoglycans, components of mast cells granules. Surface markers: cell surface markers of mast cells were discussed in detail by Heneberg, claiming that mast cells may be inadvertently included in the stem or progenitor cell isolates, since part of them is positive for the CD34 antigen. The classical mast cell markers include the high-affinity IgE receptor, CD117 (c-Kit), and CD203c (for most of the mast cell populations). Expression of some molecules may change in course of the mast cell activation.

MICROGLIA

Microglia are a type of neuroglia (glial cell) located throughout the brain and spinal cord. Microglia account for 10–15% of all cells found within the brain. As the resident macrophage cells, they act as the first and main form of active immune defence in the central nervous system (CNS). Microglia (and other neuroglia including astrocytes) are distributed in large non-overlapping regions throughout the CNS. Microglia are key cells in overall brain maintenance—they are constantly scavenging the CNS for plaques, damaged or unnecessary neurons and synapses, and infectious agents. Since these processes must be efficient to prevent potentially fatal damage, microglia are extremely sensitive to even small pathological changes in the CNS. This sensitivity is achieved in part by the presence of unique potassium channels that respond to even small changes in extracellular potassium. The brain and spinal cord, which make up the CNS, are not usually accessed directly by pathogenic factors in the body's circulation due to a series of endothelial cells known as the blood–brain barrier, or BBB. The BBB prevents most infections from reaching the vulnerable nervous tissue. In the case where infectious agents are directly introduced to the brain or cross the blood–brain barrier, microglial cells must react quickly to decrease inflammation and destroy the infectious agents before they damage the sensitive neural tissue. Due to the unavailability of antibodies from the rest of the body (few antibodies are small enough to cross the blood–brain barrier), microglia must be able to recognize foreign bodies, swallow them, and act as antigen-presenting cells activating T-cells.

Forms

Microglial cells are extremely plastic, and undergo a variety of structural changes based on location and system needs. This level of plasticity is required to fulfill the vast variety of functions that microglia perform.

The ability to transform distinguishes microglia from macrophages, which must be replaced on a regular basis, and provides them the ability to defend the CNS on extremely short notice without causing immunological disturbance. Microglia adopt a specific form, or phenotype, in response to the local conditions and chemical signals they have detected.

Sensome Genetics

The microglial sensome is a relatively new biological concept that appears to be playing a large role in neurodevelopment and neurodegeneration. The sensome refers to the unique grouping of protein transcripts used for sensing ligands and microbes. In other words, the sensome represents the genes required for the proteins used to sense molecules within the body. The sensome can be analyzed with a variety of methods including qPCR, RNA-seq, microarray analysis, and direct RNA sequencing. Genes included in the sensome code for receptors and transmembrane proteins on the plasma membrane that are more highly expressed in microglia compared to neurons. It does not include secreted proteins or transmembrane proteins specific to membrane bound organelles, such as the nucleus, mitochondria, and endoplasmic reticulum. The plurality of identified sensome genes code for pattern recognition receptors, however, there are a large variety of included genes. Microglial share a similar sensome to other macrophages, however they contain 22 unique genes, 16 of which are used for interaction with endogenous ligands. These differences create a unique microglial biomarker that includes over 40 genes including P2ry12 and HEXB. DAP12 appears to play an important role in sensome protein interaction, acting as a signalling adaptor and a regulatory protein.

The regulation of genes within the sensome must be able to change in order to respond to potential harm. Microglia can take on the role of neuroprotection or neurotoxicity in order to face these dangers. For these reasons, it is suspected that the sensome may be playing a role in neurodegeneration. Sensome genes that are upregulated with aging are mostly involved in sensing infectious microbial ligands while those that are downregulated are mostly involved in sensing endogenous ligands. This analysis suggests a glial-specific regulation favouring neuroprotection in natural neurodegeneration. This is in contrast to the shift towards neurotoxicity seen in neurodegenerative diseases. The sensome can also play a role in neurodevelopment. Early-life brain infection results in microglia that are hypersensitive to later immune stimuli. When exposed to infection, there is an upregulation of sensome genes involved in neuroinflammation and a downregulation of genes that are involved with neuroplasticity. The sensome's ability to alter neurodevelopment may however be able to combat disease. The deletion of CX3CL1, a highly expressed sensome gene, in rodent models of Rett syndrome resulted in improved health and longer lifespan. Interestingly, the downregulation of Cx_3cr1 in humans without Rett syndrome is associated with symptoms similar to schizophrenia. This suggests that the sensome not only plays a role in various developmental disorders, but also requires tight regulation in order to maintain a disease-free state.

Ramified

This form of microglial cell is commonly found at specific locations throughout the entire brain and spinal cord in the absence of foreign material or dying cells. This "resting" form of microglia is composed of long branching processes and a small cellular body. Unlike the amoeboid forms of microglia, the cell body of the ramified form remains in place while its branches are constantly moving and surveying the surrounding area. The branches are very sensitive to small changes in physiological condition and require very specific culture conditions to observe in vitro. Unlike activated or ameboid microglia, ramified microglia do not phagocytose cells and secrete fewer immunomolecules (including the MHC class I/II proteins). Microglia in this state are able to search for and identify immune threats while maintaining homeostasis in the CNS. Although this is

considered the resting state, microglia in this form are still extremely active in chemically surveying the environment. Ramified microglia can be transformed into the activated form at any time in response to injury or threat.

Reactive (Activated)

Although historically frequently used, the term "activated" microglia should be replaced by "reactive" microglia. Indeed, apparently quiescent microglia are not devoid of active functions and the "activation" term is misleading as it tends to indicate an "all or nothing" polarization of cell reactivity. The marker Iba1, which is upregulated in reactive microglia, is often used to visualize these cells.

Non-phagocytic

This state is actually part of a graded response as microglia move from their ramified form to their fully active phagocytic form. Microglia can be activated by a variety of factors including: pro-inflammatory cytokines, cell necrosis factors, lipopolysaccharide, and changes in extracellular potassium (indicative of ruptured cells). Once activated the cells undergo several key morphological changes including the thickening and retraction of branches, uptake of MHC class I/II proteins, expression of immunomolecules, secretion of cytotoxic factors, secretion of recruitment molecules, and secretion of pro-inflammatory signaling molecules (resulting in a pro-inflammation signal cascade). Activated non-phagocytic microglia generally appear as "bushy," "rods," or small ameboids depending on how far along the ramified to full phagocytic transformation continuum they are. In addition, the microglia also undergo rapid proliferation in order to increase their numbers. From a strictly morphological perspective, the variation in microglial form along the continuum is associated with changing morphological complexity and can be quantitated using the methods of fractal analysis, which have proven sensitive to even subtle, visually undetectable changes associated with different morphologies in different pathological states.

Phagocytic

Activated phagocytic microglia are the maximally immune responsive form of microglia. These cells generally take on a large, ameboid shape, although some variance has been observed. In addition to having the antigen presenting, cytotoxic and inflammatory mediating signaling of activated non-phagocytic microglia, they are also able to phagocytose foreign materials and display the resulting immunomolecules for T-cell activation. Phagocytic microglia travel to the site of the injury, engulf the offending material, and secrete pro-inflammatory factors to promote more cells to proliferate and do the same. Activated phagocytic microglia also interact with astrocytes and neural cells to fight off the infection as quickly as possible with minimal damage to the healthy brain cells.

Amoeboid

This shape allows the microglial free movement throughout the neural tissue, which allows it to fulfill its role as a scavenger cell. Amoeboid microglia are able to phagocytose debris, but do not fulfill the same antigen-presenting and inflammatory roles as activated microglia. Amoeboid microglia are especially prevalent during the development and rewiring of the brain, when there are large amounts of extracellular debris and apoptotic cells to remove. This form of microglial cell is found mainly within the perinatal white matter areas in the corpus callosum known as the "Fountains of Microglia."

Gitter Cells

Gitter cells are the eventual result of microglial cell's phagocytosis of infectious material or cellular debris. Eventually, after engulfing a certain amount of material, the phagocytic microglia becomes unable to phagocytose

any further materials. The resulting cellular mass is known as a granular corpuscle, named for its 'grainy' appearance. By looking at tissues stained to reveal gitter cells, pathologists can see post-infection areas that have healed.

Perivascular

Unlike the other types of microglia mentioned above, "perivascular" microglia refers to the location of the cell rather than its form/function. Perivascular microglia are mainly found encased within the walls of the basal lamina. They perform normal microglial functions, but unlike normal microglia they are replaced by bone marrow derived precursor cells on a regular basis and express MHC class II antigens regardless of the outside environment. Perivascular microglia also react strongly to macrophage differentiation antigens. These microglia have been shown to be essential to repair of vascular walls, as shown by Ritter's experiments and observations on ischemic retinopathy. Perivascular microglia promote endothelial cell proliferation allowing new vessels to be formed and damaged vessels to be repaired. During repair and development, myeloid recruitment and differentiation into microglial cells is highly accelerated to accomplish these tasks.

Juxtavascular

Like perivascular microglia, juxtavascular microglia can be distinguished mainly by their location. Juxtavascular microglia are found making direct contact with the basal lamina wall of blood vessels but are not found within the walls. Like perivascular cells, they express MHC class II proteins even at low levels of inflammatory cytokine activity. Unlike perivascular cells, but similar to resident microglia, juxtavascular microglia do not exhibit rapid turnover or replacement with myeloid precursor cells on a regular basis.

Functions

Microglial cells fulfill a variety of different tasks within the CNS mainly related to both immune response and maintaining homeostasis. The following are some of the major known functions carried out by these cells.

Scavenging

In addition to being very sensitive to small changes in their environment, each microglial cell also physically surveys its domain on a regular basis. This action is carried out in the ameboid and resting states. While moving through its set region, if the microglial cell finds any foreign material, damaged cells, apoptotic cells, neurofibrillary tangles, DNA fragments, or plaques it will activate and phagocytose the material or cell. In this manner microglial cells also act as "housekeepers", cleaning up random cellular debris. During developmental wiring of the brain, microglial cells play a large role regulating numbers of neural precursor cells and removing apoptotic neurons. There is also evidence that microglia can refine synaptic circuitry by engulfing and eliminating synapses. Post development, the majority of dead or apoptotic cells are found in the cerebral cortex and the subcortical white matter. This may explain why the majority of ameboid microglial cells are found within the "fountains of microglia" in the cerebral cortex.

Phagocytosis

The main role of microglia, phagocytosis, involves the engulfing of various materials. Engulfed materials generally consist of cellular debris, lipids, and apoptotic cells in the non-inflamed state, and invading virus, bacteria, or other foreign materials in the inflamed state. Once the microglial cell is "full" it stops phagocytic activity and changes into a relatively non-reactive gitter cell.

Extracellular Signaling

A large part of microglial cell's role in the brain is maintaining homeostasis in non-infected regions and promoting inflammation in infected or damaged tissue. Microglia accomplish this through an extremely complicated series of extracellular signaling molecules which allow them to communicate with other microglia, astrocytes, nerves, T-cells, and myeloid progenitor cells. As mentioned above the cytokine IFN-γ can be used to activate microglial cells.

In addition, after becoming activated with IFN-γ, microglia also release more IFN-γ into the extracellular space. This activates more microglia and starts a cytokine induced activation cascade rapidly activating all nearby microglia. Microglia-produced TNF-α causes neural tissue to undergo apoptosis and increases inflammation.

IL-8 promotes B-cell growth and differentiation, allowing it to assist microglia in fighting infection. Another cytokine, IL-1, inhibits the cytokines IL-10 and TGF-β, which downregulate antigen presentation and pro-inflammatory signaling. Additional dendritic cells and T-cells are recruited to the site of injury through the microglial production of the chemotactic molecules like MDC, IL-8, and MIP-3β. Finally, PGE_2 and other prostanoids prevent chronic inflammation by inhibiting microglial pro-inflammatory response and downregulating Th1 (T-helper cell) response.

Antigen Presentation

As mentioned above, resident non-activated microglia act as poor antigen presenting cells due to their lack of MHC class I/II proteins. Upon activation they rapidly uptake MHC class I/II proteins and quickly become efficient antigen presenters. In some cases, microglia can also be activated by IFN-γ to present antigens, but do not function as effectively as if they had undergone uptake of MHC class I/II proteins. During inflammation, T-cells cross the blood—brain barrier thanks to specialized surface markers and then directly bind to microglia in order to receive antigens. Once they have been presented with antigens, T-cells go on to fulfill a variety of roles including pro-inflammatory recruitment, formation of immunomemories, secretion of cytotoxic materials, and direct attacks on the plasma membranes of foreign cells.

Cytotoxicity

In addition to being able to destroy infectious organisms through cell to cell contact via phagocytosis, microglia can also release a variety of cytotoxic substances. Microglia in culture secrete large amounts of hydrogen peroxide and nitric oxide in a process known as 'respiratory burst'. Both of these chemicals can directly damage cells and lead to neuronal cell death. Proteases secreted by microglia catabolise specific proteins causing direct cellular damage, while cytokines like IL-1 promote demyelination of neuronal axons. Finally, microglia can injure neurons through NMDA receptor-mediated processes by secreting glutamate, aspartate and quinolinic acid. Cytotoxic secretion is aimed at destroying infected neurons, virus, and bacteria, but can also cause large amounts of collateral neural damage. As a result, chronic inflammatory response can result in large scale neural damage as the microglia ravage the brain in an attempt to destroy the invading infection.

Synaptic Stripping

In a phenomenon first noticed in spinal lesions by Blinzinger and Kreutzberg in 1968, post-inflammation microglia remove the branches from nerves near damaged tissue. This helps promote regrowth and remapping of damaged neural circuitry.

Promotion of Repair

Post-inflammation, microglia undergo several steps to promote regrowth of neural tissue. These include synaptic stripping, secretion of anti-inflammatory cytokines, recruitment of neurons and astrocytes to the damaged area, and formation of gitter cells. Without microglial cells regrowth and remapping would be considerably slower in the resident areas of the CNS and almost impossible in many of the vascular systems surrounding the brain and eyes.

Development

For a long time it was thought that microglial cells differentiate in the bone marrow from hematopoietic stem cells, the progenitors of all blood cells. However, recent studies show that microglia originate in the yolk sac during a remarkably restricted embryonal period and populate the brain mesenchyme. Additionally, they continuously renew themselves and persist throughout life without replenishment from peripheral monocytic precursors. Monocytes can also differentiate into myeloid dendritic cells and macrophages in the peripheral systems. Like macrophages in the rest of the body, microglia use phagocytic and cytotoxic mechanisms to destroy foreign materials. Microglia and macrophages both contribute to the immune response by acting as antigen presenting cells, as well as promoting inflammation and homeostatic mechanisms within the body by secreting cytokines and other signaling molecules.

In their downregulated form, microglia lack the MHC class I/MHC class II proteins, IFN-γ cytokines, CD45 antigens, and many other surface receptors required to act in the antigen-presenting, phagocytic, and cytotoxic roles that hallmark normal macrophages. Microglia also differ from macrophages in that they are much more tightly regulated spatially and temporally in order to maintain a precise immune response. Another difference between microglia and other cells that differentiate from myeloid progenitor cells is the turnover rate. Macrophages and dendritic cells are constantly being used up and replaced by myeloid progenitor cells which differentiate into the needed type. Due to the blood–brain barrier, it would be fairly difficult for the body to constantly replace microglia.

Therefore, instead of constantly being replaced with myeloid progenitor cells, the microglia maintain their status quo while in their quiescent state, and then, when they are activated, they rapidly proliferate in order to keep their numbers up. Bone chimera studies have shown, however, that in cases of extreme infection the blood–brain barrier will weaken, and microglia will be replaced with haematogenous, marrow-derived cells, namely myeloid progenitor cells and macrophages. Once the infection has decreased the disconnect between peripheral and central systems is reestablished and only microglia are present for the recovery and regrowth period.

Aging

Microglia undergo a burst of mitotic activity during injury; this proliferation is followed by apoptosis to reduce the cell numbers back to baseline. Activation of microglia places a load on the anabolic and catabolic machinery of the cells causing activated microglia to die sooner than non-activated cells. To compensate for microglial loss over time, microglia undergo mitosis and bone marrow derived progenitor cells migrate into the brain via the meninges and vasculature.

Accumulation of minor neuronal damage that occurs during normal aging can transform microglia into enlarged and activated cells. These chronic, age-associated increases in microglial activation and IL-1 expression may contribute to increased risk of Alzheimer's disease with advancing age through favouring neuritic plaque formation in susceptible patients. DNA damage might contribute to age-associated microglial activation. Another factor might be the accumulation of advanced glycation endproducts, which accumulate with aging. These

proteins are strongly resistant to proteolytic processes and promote protein cross-linking. Research has discovered dystrophic (defective development) human microglia. "These cells are characterized by abnormalities in their cytoplasmic structure, such as deramified, atrophic, fragmented or unusually tortuous processes, frequently bearing spheroidal or bulbous swellings." The incidence of dystrophic microglia increases with aging. Microglial degeneration and death have been reported in research on Prion disease, Schizophrenia and Alzheimer's disease, indicating that microglial deterioration might be involved in neurodegenerative diseases. A complication of this theory is the fact that it is difficult to distinguish between "activated" and "dystrophic" microglia in the human brain.

Clinical Significance

Microglia are the primary immune cells of the Central Nervous System, similar to peripheral macrophages. They respond to pathogens and injury by changing morphology and migrating to the site of infection/injury, where they destroy pathogens and remove damaged cells. As part of their response they secrete cytokines, chemokines, prostaglandins, and reactive oxygen species, which help to direct the immune response. Additionally, they are instrumental in the resolution of the inflammatory response, through the production of anti-inflammatory cytokines. Microglia have also been extensively studied for their harmful roles in neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Multiple sclerosis, as well as cardiac diseases, glaucoma, and viral and bacterial infections.

History

The ability to view and characterize different neural cells including microglia began in 1880 when Nissl staining was developed by Franz Nissl. Franz Nissl and F. Robertson first described microglial cells during their histology experiments. The cell staining techniques in the 1880s showed that microglia are related to macrophages. The activation of microglia and formation of ramified microglial clusters was first noted by Victor Babeº while studying a rabies case in 1897. Babeº noted the cells were found in a variety of viral brain infections but did not know what the clusters of microglia he saw were. Pío del Río Hortega, a student of Santiago Ramón y Cajal, first called the cells "microglia" around 1920. He went on to characterize microglial response to brain lesions in 1927 and note the "fountains of microglia" present in the corpus callosum and other perinatal white matter areas in 1932. After many years of research Rio-Hortega became generally considered as the "Father of Microglia." For a long period of time little improvement was made in our knowledge of microglia. Then, in 1988, Hickey and Kimura showed that perivascular microglial cells are bone-marrow derived, and express high levels of MHC class II proteins used for antigen presentation. This confirmed Pio Del Rio-Hortega's postulate that microglial cells functioned similarly to macrophages by performing phagocytosis and antigen presentation.

DISORDERS

The two commonly used categories of white blood cell disorders divide them quantitatively into those causing excessive numbers (proliferative disorders) and those causing insufficient numbers (leukopenias). Leukocytosis is usually healthy (*e.g.,* fighting an infection), but it also may be dysfunctionally proliferative. WBC proliferative disorders can be classed as myeloproliferative and lymphoproliferative. Some are autoimmune, but many are neoplastic. Another way to categorize disorders of white blood cells is qualitatively. There are various disorders in which the number of white blood cells is normal but the cells do not function normally. Neoplasia of WBCs can be benign but is often malignant. Of the various tumors of the blood and lymph, cancers of WBCs can be broadly classified as leukemias and lymphomas, although those categories overlap and are often grouped as a pair.

LEUKOPENIA

Leukopenia is a decrease in the number of white blood cells (leukocytes) found in the blood, which places individuals at increased risk of infection. Neutropenia, a subtype of leukopenia, refers to a decrease in the number of circulating neutrophil granulocytes, the most abundant white blood cells. The terms *leukopenia* and *neutropenia* may occasionally be used interchangeably, as the neutrophil count is the most important indicator of infection risk. This should not be confused with agranulocytosis.

Causes

Medical Conditions

Low white cell count may be due to acute viral infections, such as a cold or influenza. It has been associated with chemotherapy, radiation therapy, myelofibrosis, aplastic anemia (failure of white cell, red cell and platelet production), stem cell transplant, bone marrow transplant, HIV, AIDS, and steroid use. Other causes of low white blood cell count include systemic lupus erythematosus, Hodgkin's lymphoma, some types of cancer, typhoid, malaria, tuberculosis, dengue, rickettsial infections, enlargement of the spleen, folate deficiencies, psittacosis, sepsis, Sjögren's syndrome and Lyme disease. It has also been shown to be caused by deficiency in certain minerals, such as copper and zinc. Pseudoleukopenia can develop upon the onset of infection. The leukocytes (primarily neutrophils, responding to injury first) start migrating towards the site of infection, where they can be scanned. Their migration causes bone marrow to produce more WBCs to combat infection as well as to restore the leukocytes in circulation, but as the blood sample is taken upon the onset of infection, it contains low amount of WBCs, which is why it is termed "pseudoleukopenia".

Medications

Certain medications can alter the number and function of white blood cells. Medications that can cause leukopenia include clozapine, an antipsychotic medication with a rare adverse effect leading to the total absence of all granulocytes (neutrophils, basophils, eosinophils). The antidepressant and smoking addiction treatment drug bupropion HCl (Wellbutrin) can also cause leukopenia with long-term use. Minocycline, a commonly prescribed antibiotic, is another drug known to cause leukopenia. There are also reports of leukopenia caused by divalproex sodium or valproic acid (Depakote), a drug used for epilepsy (seizures), mania (with bipolar disorder) and migraine. The anticonvulsant drug, lamotrigine, has been associated with a decrease in white blood cell count. The FDA monograph for metronidazole states that this medication can also cause leukopenia, and the prescriber information suggests a complete blood count, including differential cell count, before and after, in particular, high-dose therapy. Immunosuppressive drugs, such as sirolimus, mycophenolate mofetil, tacrolimus, ciclosporin, leflunomide and TNF inhibitors, have leukopenia as a known complication. Interferons used to treat multiple sclerosis, such as interferon beta-1a and interferon beta-1b, can also cause leukopenia. Chemotherapy targets cells that grow rapidly, such as tumors, but can also affect white blood cells, because they are characterized by bone marrow as rapid growing. A common side effect of cancer treatment is neutropenia, the lowering of neutrophils (a specific type of white blood cell). Decreased white blood cell count may be present in cases of arsenic toxicity.

LYMPHOCYTOPENIA

Lymphocytopenia, or lymphopenia, is the condition of having an abnormally low level of lymphocytes in the blood. Lymphocytes are a white blood cell with important functions in the immune system. The opposite is

lymphocytosis, which refers to an excessive level of lymphocytes. Lymphocytopenia may be present as part of a pancytopenia, when the total numbers of all types of blood cells are reduced.

Classification

In some cases, lymphocytopenia can be further classified according to which kind of lymphocytes are reduced. If all three kinds of lymphocytes are suppressed, then the term is used without further qualification.

- In T lymphocytopenia, there are too few T lymphocytes, but normal numbers of other lymphocytes. It causes, and manifests as, a T cell deficiency. This is usually caused by HIV infection (resulting in AIDS), but may be Idiopathic CD4+ lymphocytopenia (ICL), which is a very rare heterogeneous disorder defined by CD4+ T-cell counts below 300 cells/ìL in the absence of any known immune deficiency condition, such as human immunodeficiency virus (HIV) infection or chemotherapy.
- In B lymphocytopenia, there are too few B lymphocytes, but possibly normal numbers of other lymphocytes. It causes, and manifests as, a humoral immune deficiency. This is usually caused by medications that suppress the immune system.
- In NK lymphocytopenia, there are too few natural killer cells, but normal numbers of other lymphocytes. This is very rare.

Causes

The most common cause of temporary lymphocytopenia is a recent infection, such as the common cold. Lymphocytopenia, but not idiopathic CD4+ lymphocytopenia, is associated with corticosteroid use, infections with HIV and other viral, bacterial, and fungal agents, malnutrition, systemic lupus erythematosus, severe stress, intense or prolonged physical exercise (due to cortisol release), rheumatoid arthritis, sarcoidosis, multiple sclerosis, and iatrogenic (caused by other medical treatments) conditions. Lymphocytopenia is a frequent, temporary result from many types of chemotherapy, such as with cytotoxic agents or immunosuppressive drugs. Some malignancies that have spread to involve the bone marrow, such as leukemia or advanced Hodgkin's disease, also cause lymphocytopenia. Another cause is infection with Influenza A virus subtype H1N1 (and other subtypes of the Influenza A virus) and is then often associated with Monocytosis; H1N1 was responsible for the Spanish flu, the 2009 flu pandemic and in 2016 for the Influenza-epidemic in Brazil. Large doses of radiation, such as those involved with nuclear accidents or medical whole body radiation, may cause lymphocytopenia.

Diagnosis

Lymphocytopenia is diagnosed when the complete blood count shows a lymphocyte count lower than the age-appropriate reference interval (for example, below 1.0 × 10(9)/L in an adult).

Prognosis

Lymphocytopenia that is caused by infections tends to resolve once the infection has cleared. Patients with idiopathic CD4+ lymphocytopenia may have either abnormally low but stable CD4+ cell counts, or abnormally low and progressively falling CD4+ cell counts; the latter condition is terminal.

Veterinary Treatment

Lymphocytopenia caused by Feline Leukemia Virus and Feline immunodeficiency virus retroviral infections is treated with Lymphocyte T-Cell Immune Modulator.

LEUKOCYTOSIS

Leukocytosis is white cells (the leukocyte count) above the normal range in the blood. It is frequently a sign of an inflammatory response, most commonly the result of infection, but may also occur following certain parasitic infections or bone tumors as well as leukemia. It may also occur after strenuous exercise, convulsions such as epilepsy, emotional stress, pregnancy and labour, anesthesia, and epinephrine administration.

There are five principle types of leukocytosis:

1. Neutrophilia (the most common form)
2. Lymphocytosis
3. Monocytosis
4. Eosinophilia
5. Basophilia.

This increase in leukocyte (primarily neutrophils) is usually accompanied by a "left upper shift" in the ratio of immature to mature neutrophils and macrophages. The proportion of immature leukocytes decreases due to proliferation and inhibition of granulocyte and monocyte precursors in the bone marrow which is stimulated by several products of inflammation including C3a and G-CSF.

Although it may indicate illness, leukocytosis is considered a laboratory finding instead of a separate disease. This classification is similar to that of fever, which is also a test result instead of a disease. "Right shift" in the ratio of immature to mature neutrophils is considered with reduced count or lack of "young neutrophils" (metamyelocytes, and band neutrophils) in blood smear, associated with the presence of "giant neutrophils". This fact shows suppression of bone marrow activity, as a hematological sign specific for pernicious anemia and radiation sickness.

A leukocyte count above 25 to 30 × 10/L is termed a *leukemoid reaction*, which is the reaction of a healthy bone marrow to extreme stress, trauma, or infection. It is different from leukemia and from leukoerythroblastosis, in which either immature white blood cells (acute leukemia) or mature, yet non-functional, white blood cells (chronic leukemia) are present in peripheral blood.

Classification

Leukocytosis can be subcategorized by the type of white blood cell that is increased in number. Leukocytosis in which neutrophils are elevated is neutrophilia; leukocytosis in which lymphocyte count is elevated is lymphocytosis; leukocytosis in which monocyte count is elevated is monocytosis; and leukocytosis in which eosinophil count is elevated is eosinophilia. An extreme form of leukocytosis, in which the WBC count exceeds 100,000/μL, is leukostasis. In this form there are so many WBCs that clumps of them block blood flow. This leads to ischemic problems including transient ischemic attack and stroke.

Causes

Leukocytosis is very common in acutely ill patients. It occurs in response to a wide variety of conditions, including viral, bacterial, fungal, or parasitic infection, cancer, hemorrhage, and exposure to certain medications or chemicals including steroids.

	Causes of leukocytosis
Neutrophilic leukocytosis (neutrophilia)	• Acute bacterial infections, especially pyogenic infections • Sterile inflammation – Tissue necrosis a. Myocardial infarction b. Burns

	Causes of leukocytosis
Eosinophilic leukocytosis (eosinophilia)	• Allergic disorders • Allergic disorders – Hay fever – Drug allergies – Allergic skin diseases a. Pemphigus b. Dermatitis herpetiformis • Parasitic infections • Some forms of malignancy – Hodgkin's lymphoma – Some forms of Non-Hodgkin lymphoma • Systemic autoimmune diseases (e.g. SLE) • Some forms of vasculitis • Cholesterol embolism (transiently)
Basophilic leukocytosis	(rare)
Basophilia	• Myeloproliferative disease, e.g. Chronic myelogenous leukemia
Monocytosis	• Chronic infections – Tuberculosis – Bacterial endocarditis – Rickettsiosis – Malaria • Systemic autoimmune diseases, *e.g.,* SLE • Inflammatory bowel diseases, *e.g.,* ulcerative colitis
Lymphocytosis	• Chronic infections – Tuberculosis – Brucellosis • Viral infections – Hepatitis – Cytomegalovirus infection – Infectious mononucleosis • Pertussis • some forms of malignancy, such as lymphocytic leukæmias.

For lung diseases such as pneumonia and tuberculosis, WBC count is very important for the diagnosis of the disease, as leukocytosis is usually present. The mechanism that causes leukocytosis can be of several forms: an increased release of leukocytes from bone marrow storage pools, decreased margination of leukocytes onto vessel walls, decreased extravasation of leukocytes from the vessels into tissues, or an increase in number of precursor cells in the marrow. Certain medications, including corticosteroids, lithium and beta agonists, may cause leukocytosis.

EOSINOPHILIA

Eosinophilia is a condition in which the eosinophil count in the peripheral blood exceeds 5.0 × 10/L (500/μL). Eosinophils usually account for less than 7% of the circulating leukocytes. A marked increase in non-blood tissue eosinophil count noticed upon histopathologic examination is diagnostic for tissue eosinophilia. Several causes are known, with the most common being some form of allergic reaction or parasitic infection. Diagnosis of eosinophilia is via a complete blood count (CBC), but diagnostic procedures directed at the underlying cause vary depending on the suspected condition(s). An absolute eosinophil count is not generally needed if the CBC shows marked eosinophilia. The location of the causal factor can be used to classify eosinophilia into two general types: extrinsic, in which the factor lies outside the eosinophil cell lineage; and intrinsic eosinophilia, which denotes etiologies within the eosiniphil cell line. Specific treatments are dictated by the causative condition,

though in idiopathic eosinophilia, the disease may be controlled with corticosteroids. Eosinophilia is not a disorder (rather, only a sign) unless it is idiopathic.

Causes

Eosinophilia can be idiopathic (primary) or, more commonly, secondary to another disease. In the Western World, allergic or atopic diseases are the most common causes, especially those of the respiratory or integumentary systems. In the developing world, parasites are the most common cause. A parasitic infection of nearly any bodily tissue can cause eosinophilia.

Diseases that feature eosinophilia as a sign include:

- Allergic disorders
  - Asthma
  - Hay fever
  - Drug allergies
  - Allergic skin diseases
    a. Pemphigus
    b. Dermatitis herpetiformis
- IgG4-related disease
- Parasitic infections
- Addison's disease and stress-induced suppression of adrenal gland function
- Some forms of malignancy
  - Acute lymphoblastic leukemia
  - Chronic myelogenous leukemia
  - Eosinophilic leukemia
  - Clonal eosinophilia
  - Hodgkin lymphoma
  - Some forms of non-Hodgkin lymphoma
  - Lymphocyte-variant hypereosinophilia
  - Systemic mastocytosis
- Systemic autoimmune diseases
  - Systemic lupus erythematosus
  - Kimura disease
  - Eosinophilic granulomatosis with polyangiitis
  - Eosinophilic fasciitis
  - Eosinophilic myositis
- Eosinophilic myocarditis
- Eosinophilic esophagitis
- Eosinophilic gastroenteritis
- Cholesterol embolism (transiently)
- Coccidioidomycosis (Valley fever), a fungal disease prominent in the US Southwest.
- Human immunodeficiency virus infection
- Interstitial nephropathy
- Hyperimmunoglobulin E syndrome, an immune disorder characterized by high levels of serum IgE
- Idiopathic hypereosinophilic syndrome.
- Congenital disorders

- Hyperimmunoglobulin E syndrome
- Omenn syndrome
- Familial eosinophilia.

Neoplastic Eosinophilia

Hodgkin lymphoma (Hodgkin's disease) often elicits severe eosinophilia; however, non-Hodgkin lymphoma and leukemia produce less marked eosinophilia. Of solid tumor neoplasms, ovarian cancer is most likely to provoke eosinophilia, though any other cancer can cause the condition. Solid epithelial cell tumors have been shown to cause both tissue and blood eosinophilia, with some reports indicating that this may be mediated by interleukin production by tumor cells, especially IL-5 or IL-3. This has also been shown to occur in Hodgkin lymphoma, in the form of IL-5 secreted by Reed-Sternberg cells. In primary cutaneous T cell lymphoma, blood and dermal eosinophilia are often seen. Lymphoma cells have also been shown to produce IL-5 in these disorders. Other types of lymphoid malignancies have been associated with eosinophilia, as in lymphoblastic leukemia with a translocation between chromosomes 5 and 14 or alterations in the genes which encode platelet-derived growth factor receptors alpha or beta. Patients displaying eosinophilia overexpress a gene encoding an eosinophil hematopoietin. A translocation between chromosomes 5 and 14 in patients with acute B lymphocytic leukemia resulted in the juxtaposition of the IL-3 gene and the immunoglobulin heavy-chain gene, causing overproduction production of IL-3, leading to blood and tissue eosinophilia.

Drug Reactions

Allergic reactions to drugs are a common cause of eosinophilia, with manifestations ranging from diffuse maculopapular rash, to severe life-threatening drug reactions with eosinophilia and systemic symptoms (DRESS). Drugs that have been shown to cause DRESS are aromatic anticonvulsants and other antiepileptics, sulfonamides, allopurinol, nonsteroidal anti-inflammatory drugs (NSAIDs), some antipsychotics such as risperidone, and certain antibiotics. Phenibut, an analogue of the neurotransmitter GABA, has also been implicated in high doses. The reaction which has been shown to be T-cell mediated may also cause eosinophilia-myalgia syndrome.

Pathophysiology

IgE mediated eosinophil production is induced by compounds released by basophils and mast cells, including eosinophil chemotactic factor of anaphylaxis, leukotriene B4, complement complex (C5-C6-C7), interleukin 5, and histamine (though this has a narrow range of concentration). Harm resulting from untreated eosinophilia potentially varies with cause. During an allergic reaction, the release of histamine from mast cells causes vasodilation which allows eosinophils to migrate from the blood and localize in affected tissues. Accumulation of eosinophils in tissues can be significantly damaging. Eosinophils, like other granulocytes, contain granules (or sacs) filled with digestive enzymes and cytotoxic proteins which under normal conditions are used to destroy parasites but in eosinophilia these agents can damage healthy tissues. In addition to these agents, the granules in eosinophils also contain inflammatory molecules and cytokines which can recruit more eosinophils and other inflammatory cells to the area and hence amplify and perpetuate the damage. This process is generally accepted to be the major inflammatory process in the pathophysiology of atopic or allergic asthma.

Diagnosis

Diagnosis is by complete blood count (CBC). However, in some cases, a more accurate absolute eosinophil count may be needed. Medical history is taken, with emphasis on travel, allergies and drug use. Specific test for

causative conditions are performed, often including chest x-ray, urinalysis, liver and kidney function tests, and serologic tests for parasitic and connective tissue diseases. The stool is often examined for traces of parasites (*i.e.,* eggs, larvae, *etc.*) though a negative test does not rule out parasitic infection; for example, trichinosis requires a muscle biopsy. Elevated serum B_{12} or low white blood cell alkaline phosphatase, or leukocytic abnormalities in a peripheral smear indicates a disorder of myeloproliferation. In cases of idiopathic eosinophilia, the patient is followed for complications. A brief trial of corticosteroids can be diagnostic for allergic causes, as the eosinophilia should resolve with suppression of the immune over-response. Neoplastic disorders are diagnosed through the usual methods, such as bone marrow aspiration and biopsy for the leukemias, MRI/CT to look for solid tumors, and tests for serum LDH and other tumor markers.

Treatment

Treatment is directed towards the underlying cause. However, in primary eosinophilia, or if the eosinophil count must be lowered, corticosteroids such as prednisone may be used. However, immune suppression, the mechanism of action of corticosteroids, can be fatal in patients with parasitosis.

COUNTING AND REFERENCE RANGES

The complete blood cell count is a blood panel that includes the overall WBC count and various subsets such as the absolute neutrophil count. Reference ranges for blood tests specify the typical counts in healthy people. TLC- (Total leucocyte count): Normal TLC in an adult person is 6000-8000WBC/mm^3 of blood. DLC- (Differential leucocyte count): Number/(%) of different type of leucocyte in per cubic mm. of blood.

NEUTROPENIA

Neutropenia or neutropaenia is an abnormally low concentration of neutrophils (a type of white blood cell) in the blood. Neutrophils make up the majority of circulating white blood cells and serve as the primary defence against infections by destroying bacteria, bacterial fragments and immunoglobulin-bound viruses in the blood. Patients with neutropenia are more susceptible to bacterial infections and, without prompt medical attention, the condition may become life-threatening (neutropenic sepsis). Neutropenia can be acute (temporary) or chronic (long lasting). The term is sometimes used interchangeably with "leukopenia" ("deficit in the number of white blood cells").

SIGNS AND SYMPTOMS

Signs and symptoms of neutropenia include fever, painful swallowing, gingival pain, skin abscesses, and otitis. These symptoms may exist because individuals with neutropenia often have infection. Children may show signs of irritability, and poor feeding. Additionally, hypotension has also been observed in individuals who suffer from this condition.

CAUSES

The causes of neutropenia can be divided between problems that are transient and those that are chronic. Causes can be divided into these groups:

- Chronic neutropenia:
  - Aplastic anemia
  - Glycogen storage disease
  - Cohen syndrome

  - Congenital immunological disorder, *e.g.,* Kostmann syndrome, GATA2 deficiency
  - Barth syndrome
  - Vitamin B_{12} deficiency
  - Pearson syndrome
  - Pudlak syndrome
- Transient neutropenia:
  - Typhoid
  - Tuberculosis
  - Cytomegalovirus
  - Propylthiouracil
  - Levamisole
  - Penicillamine
  - Trimethoprim/sulfamethoxazole
  - Clozapine
  - Valproate.

Gram-positive bacteria are present in 60–70% of bacterial infections. There are serious concerns regarding antibiotic-resistant organisms. These would include as methicillin-resistant *Staphylococcus aureus* (MRSA) or vancomycin-resistant Enterococcus (VRE).) Other causes of congenital neutropenia are Shwachman–Diamond syndrome, Cyclic neutropenia, bone marrow failure syndromes, cartilage–hair hypoplasia, reticular dysgenesis, and Barth syndrome. Viruses that infect neutrophil progenitors can also be the cause of neutropenia. Viruses identified that have an effect on neutrophils are rubella and cytomegalovirus. Though the body can manufacture a normal level of neutrophils, in some cases the destruction of excessive numbers of neutrophils can lead to neutropenia.

These are:

- Bacterial or fungal sepsis
- Necrotizing enterocolitis, circulating neutrophil population depleted due to migration into the intestines and peritoneum
- Alloimmune neonatal neutropenia, the mother produces antibodies against fetal neutrophils
- Inherited autoimmune neutropenia, mother has autoimmune neutropenia
- Autoimmune neutropenia of infancy, the sensitization to self-antigens.

PATHOPHYSIOLOGY

The pathophysiology of neutropenia can be divided into *congenital* and *acquired*. In congenital neutropenia (cyclic neutropenia) is autosomal dominant, mutations in the ELA2 gene (neutrophil elastase), is the genetic reason for this condition. Acquired neutropenia (immune-associated neutropenia) is due to anti-neutrophil antibodies that target neutrophil-specific antigens, ultimately altering neutrophil function. Furthermore, emerging research suggests neutropenia without an identifiable etiology (idiopathic neutropenia) may be the result of a low-grade, chronic inflammatory process with an abnormal excessive production of myelosuppressive cytokines in a study conducted in the island of Crete.

Neutropenia fever can complicate the treatment of cancers. Observations of pediatric patients have noted that fungal infections are more likely to develop in patients with neutropenia. Mortality increases during cancer treatments if neutropenia is also present. Congenital neutropenia is determined by blood neutrophil counts (absolute neutrophil counts or ANC) $< 0.5 \times 10$/L and recurrent bacterial infections beginning very early in childhood. Congenital neutropenia is related to alloimmunization, sepsis, maternal hypertension, twin-to-twin transfusion syndrome, and Rh hemolytic disease.

DIAGNOSIS

Neutropenia can be the result of a variety of consequences, including from certain types of drugs, environmental toxins, vitamin deficiencies, metabolic abnormalities, as well as cancer or infections. Neutropenia itself is a rare entity, but can be clinically common in oncology and immunocompromised patients as a result of chemotherapy (drug-induced neutropenia). Additionally, acute neutropenia can be commonly seen from patients recovering from a viral infection or in a post-viral state. Meanwhile, several subtypes of neutropenia exist which are rarer and chronic, including: acquired (idiopathic) neutropenia, cyclic neutropenia, autoimmune neutropenia, and congenital neutropenia. Neutropenia that is developed in response to chemotherapy typically becomes evident in seven to fourteen days after treatment. Conditions that indicate the presence of neutropenic fever are implanted devices; leukemia induction; the compromise of mucosal, mucociliary and cutaneous barriers; a rapid decline in absolute neutrophil count, duration of neutropenia >7–10 days, and other illnesses that exist in the patient.

Signs of infection in patients can be subtle. Fevers are a common and early observation. Sometimes overlooked is the presence of hypothermia, which can be present in sepsis. Physical examination and accessing the history and physical examination is focussed on sites of infection. Indwelling line sites, areas of skin breakdown, sinuses, nasopharynx, bronchi and lungs, alimentary tract, and skin are assessed. The diagnosis of neutropenia is done via the low neutrophil count detection on a complete blood count. Generally, other investigations are required to arrive at the right diagnosis.

When the diagnosis is uncertain, or serious causes are suspected, bone marrow biopsy may be necessary. Other investigations commonly performed: serial neutrophil counts for suspected cyclic neutropenia, tests for antineutrophil antibodies, autoantibody screen (and investigations for systemic lupus erythematosus), vitamin B_{12} and folate assays. Rectal examinations are usually not performed due to the increased risk of introducing bacteria into the blood stream and the possible development of rectal abscesses. A routine chest X-ray and urinalysis may be can not be relied upon or considered normal due to the absence of neutrophils.

Classification

Generally accepted reference range for absolute neutrophil count (ANC) in adults is 1500 to 8000 cells per microliter (µl) of blood. Three general guidelines are used to classify the severity of neutropenia based on the ANC (expressed below in cells/µl):

- Mild neutropenia (1000 <= ANC < 1500): minimal risk of infection
- Moderate neutropenia (500 <= ANC < 1000): moderate risk of infection
- Severe neutropenia (ANC < 500): severe risk of infection.

Each of these are either derived from laboratory tests or via the formula below:

$$\text{ANC} = \frac{(\%\,neutrophils + \%\,bands) \times (WBC)}{(100)}$$

TREATMENT

Recombinant granulocyte-colony stimulating factor preparations, such as filgrastim can be effective in patients with congenital forms of neutropenia including severe congenital neutropenia and cyclic neutropenia, the amount needed (dosage) varies considerably (depending on the individual's condition) to stabilize the neutrophil count. Guidelines for neutropenia regarding diet are currently being studied. Most cases of neonatal neutropenia are temporary. Antibiotic prophylaxis is not recommended because of the possibility of encouraging the development of multidrug-resistant bacterial strains.

Neutropenia can be treated with hematopoietic Growth Factors, granulocyte-colony stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF). These are cytokines (inflammation-inducing chemicals) that are present naturally in the body. These factors are used regularly in cancer treatment with adults and children. The factors promote neutrophil recovery following anticancer therapy. The administration of intravenous immunoglobulins (IVIGs) has had some success in treating neutropenias of alloimmune and autoimmune origins with a response rate of about 50%. Blood transfusions have not been effective.

PROGNOSIS

If left untreated, patients with fever and absolute neutrophil count <500 have a mortality of up to 70% within 24 hours. The prognosis of neutropenia depends on the cause. Antibiotic agents have improved the prognosis for individuals with severe neutropenia. Neutropenic fever in individuals treated for cancer has a mortality of 4-30%.

EPIDEMIOLOGY

Neutropenia is usually detected shortly after birth, affecting 6% to 8% of all newborns in neonatal intensive care units (NICUs). Out of the approximately 600,000 neonates annually treated in NICUs in the United States, 48,000 may be diagnosed as neutropenic. The incidence of neutropenia is greater in premature infants. Six to fifty-eight percent of preterm neonates are diagnosed with this auto-immune disease. The incidence of neutropenia correlates with decreasing birth weight. The disorder is seen up to 38% in infants that weigh less than 1000g, 13% in infants weighing less than 2500g, and 3% of term infants weighing more than 2500g. Neutropenia is often temporary, affecting most newborns in only first few days after birth. In others, it becomes more severe and chronic indicating a deficiency in innate immunity. Furthermore, the prevalence of chronic neutropenia in the general public is rare.

In a study conducted in Denmark, over 370,000 patients were assessed for the presence of neutropenia. Results published demonstrated only 1% of those evaluated were neutropenic, and were commonly seen in those suffering from HIV, viral infections, acute leukemias, and myelodsyplastic syndromes. The study concluded the presence of neutropenia is an ominous sign that warrants further investigation and follow-up.

CYCLIC NEUTROPENIA

Cyclic neutropenia (or cyclical neutropenia) is a form of neutropenia that tends to occur every three weeks and lasting three to six days at a time due to changing rates of cell production by the bone marrow. It is often present among several members of the same family. Treatment includes G-CSF and usually improves after puberty. Cyclic neutropenia is the result of autosomal dominantly inherited mutations in ELA2, the gene encoding neutrophil elastase.

CHRONIC GRANULOMATOUS DISEASE

Chronic granulomatous disease (CGD) (also known as Bridges–Good syndrome, chronic granulomatous disorder, and Quie syndrome) is a diverse group of hereditary diseases in which certain cells of the immune system have difficulty forming the reactive oxygen compounds (most importantly the superoxide radical due to defective phagocyte NADPH oxidase) used to kill certain ingested pathogens. This leads to the formation of granulomata in many organs. CGD affects about 1 in 200,000 people in the United States, with about 20 new cases diagnosed each year. This condition was first discovered in 1950 in a series of 4 boys from Minnesota, and in 1957 it was named “a fatal granulomatosus of childhood” in a publication describing their disease. The underlying cellular mechanism that causes chronic granulomatous disease was discovered in 1967, and research

since that time has further elucidated the molecular mechanisms underlying the disease. Bernard Babior made key contributions in linking the defect of superoxide production of white blood cells, to the cause of the disease. In 1986, the X-linked form of CGD was the first disease for which positional cloning was used to identify the underlying genetic mutation.

SYMPTOMS

Classically, patients with chronic granulomatous disease will suffer from recurrent bouts of infection due to the decreased capacity of their immune system to fight off disease-causing organisms.

The recurrent infections they acquire are specific and are, in decreasing order of frequency:

- Pneumonia
- Abscesses of the skin, tissues, and organs
- Suppurative arthritis
- Osteomyelitis
- Bacteremia/fungemia
- Superficial skin infections such as cellulitis or impetigo.

Most people with CGD are diagnosed in childhood, usually before age 5. Early diagnosis is important since these people can be placed on antibiotics to ward off infections before they occur. Small groups of CGD patients may also be affected by McLeod syndrome because of the proximity of the two genes on the same X-chromosome.

Atypical Infections

People with CGD are sometimes infected with organisms that usually do not cause disease in people with normal immune systems.

Among the most common organisms that cause disease in CGD patients are:

- Bacteria (particularly those that are catalase-positive)
  - *Staphylococcus aureus*.
  - *Serratia marcescens*.
  - *Listeria* species.
  - *E. coli*.
  - *Klebsiella* species.
  - *Pseudomonas cepacia, a.k.a. Burkholderia cepacia.*
  - *Nocardia*.
- Fungi
  - *Aspergillus* species. Aspergillus has a propensity to cause infection in people with CGD and of the Aspergillus species, *Aspergillus fumigatus* seems to be most common in CGD.
  - *Candida* species.

Patients with CGD can usually resist infections of catalase-negative bacteria but are susceptible to catalase-positive bacteria. Catalase is an enzyme that catalyzes the breakdown of hydrogen peroxide in many organisms. In infections caused by organisms that lack catalase (catalase-negative), the host with CGD is successfully able to "borrow" hydrogen peroxide being made by the organism and use it to fight off the infection. In infections by organisms that have catalase (catalase-positive), this "borrowing mechanism" is unsuccessful because the catalase enzyme first breaks down any hydrogen peroxide that would be borrowed from the organism. Therefore in the CGD patient, hydrogen peroxide cannot be used to make oxygen radicals to fight infection, leaving the patient vulnerable to infection by catalase-positive bacteria.

GENETICS

Most cases of chronic granulomatous disease are transmitted as a mutation on the X chromosome and are thus called an "X-linked trait". The affected gene on the X chromosome codes for the gp91 protein p91-PHOX (*p* is the weight of the protein in kDa; the *g* means glycoprotein). CGD can also be transmitted in an autosomal recessive fashion (via CYBA and NCF1) and affects other PHOX proteins. The type of mutation that causes both types of CGD are varied and may be deletions, frame-shift, nonsense, and missense. A low level of NADPH, the cofactor required for superoxide synthesis, can lead to CGD. This has been reported in women who are homozygous for the genetic defect causing glucose-6-phosphate dehydrogenase deficiency (G6PD), which is characterised by reduced NADPH levels.

PATHOPHYSIOLOGY

Phagocytes (*i.e.,* neutrophils and macrophages) require an enzyme to produce reactive oxygen species to destroy bacteria after they are ingested (phagocytosis), a process known as the respiratory burst. This enzyme is termed "phagocyte NADPH oxidase" (*PHOX*). This enzyme oxidizes NADPH and reduces molecular oxygen to produce superoxide anions, a reactive oxygen species. Superoxide is then disproportionated into peroxide and molecular oxygen by superoxide dismutase. Finally, peroxide is used by myeloperoxidase to oxidize chloride ions into hypochlorite (the active component of bleach), which is toxic to bacteria.

Thus, NADPH oxidase is critical for phagocyte killing of bacteria through reactive oxygen species. (Two other mechanisms are used by phagocytes to kill bacteria: nitric oxide and proteases, but the loss of ROS-mediated killing alone is sufficient to cause chronic granulomatous disease.) Defects in one of the four essential subunits of phagocyte NADPH oxidase (PHOX) can all cause CGD of varying severity, dependent on the defect. There are over 410 known possible defects in the PHOX enzyme complex that can lead to chronic granulomatous disease.

DIAGNOSIS

The nitroblue-tetrazolium (NBT) test is the original and most widely known test for chronic granulomatous disease. It is negative in CGD, meaning that it does not turn blue. The higher the blue score, the better the cell is at producing reactive oxygen species. This test depends upon the direct reduction of NBT to the insoluble blue compound formazan by NADPH oxidase; NADPH is oxidized in the same reaction. This test is simple to perform and gives rapid results, but only tells whether or not there is a problem with the PHOX enzymes, not how much they are affected.

A similar test uses dihydrorhodamine (DHR), in which whole blood is stained with DHR, incubated, and stimulated to produce superoxide radicals which oxidize DHR to rhodamin in cells with normal function. An advanced test called the *cytochrome C reduction assay* tells physicians how much superoxide a patient's phagocytes can produce. Once the diagnosis of CGD is established, a genetic analysis may be used to determine exactly which mutation is the underlying cause.

Classification

Chronic granulomatous disease is the name for a genetically heterogeneous group of immunodeficiencies. The core defect is a failure of phagocytic cells to kill organisms that they have engulfed because of defects in a system of enzymes that produce free radicals and other toxic small molecules.

There are several types, including:

- X-linked chronic granulomatous disease (CGD)

- Autosomal recessive cytochrome b-negative CGD
- Autosomal recessive cytochrome b-positive CGD type I
- Autosomal recessive cytochrome b-positive CGD type II
- Atypical granulomatous disease.

TREATMENT

Management of chronic granulomatous disease revolves around two goals: 1) diagnose the disease early so that antibiotic prophylaxis can be given to keep an infection from occurring, and 2) educate the patient about his or her condition so that prompt treatment can be given if an infection occurs.

Antibiotics

Physicians often prescribe the antibiotic trimethoprim-sulfamethoxazole to prevent bacterial infections. This drug also has the benefit of sparing the normal bacteria of the digestive tract. Fungal infection is commonly prevented with itraconazole, although a newer drug of the same type called voriconazole may be more effective. The use of this drug for this purpose is still under scientific investigation.

Immunomodulation

Interferon, in the form of interferon gamma-1b (Actimmune) is approved by the Food and Drug Administration for the prevention of infection in CGD. It has been shown to reduce infections in CGD patients by 70% and to decrease their severity. Although its exact mechanism is still not entirely understood, it has the ability to give CGD patients more immune function and therefore, greater ability to fight off infections. This therapy has been standard treatment for CGD for several years.

Hematopoietic Stem Cell Transplantation (HSCT)

Hematopoietic stem cell transplantation from a matched donor is curative although not without significant risk.

PROGNOSIS

There are currently no studies detailing the long term outcome of chronic granulomatous disease with modern treatment. Without treatment, children often die in the first decade of life. The increased severity of X-linked CGD results in a decreased survival rate of patients, as 20% of X-linked patients die of CGD-related causes by the age of 10, whereas 20% of autosomal recessive patients die by the age of 35. Recent experience from centers specializing in the care of patients with CGD suggests that the current mortality has fallen to under 3% and 1% respectively.

CGD was initially termed “fatal granulomatous disease of childhood” because patients rarely survived past their first decade in the time before routine use of prophylactic antimicrobial agents. The average patient now survives at least 40 years.

EPIDEMIOLOGY

CGD affects about 1 in 200,000 people in the United States, with about 20 new cases diagnosed each year. Chronic granulomatous disease affects all people of all races, however, there is limited information on prevalence outside of the United States. One survey in Sweden reported an incidence of 1 in 220,000 people, while a larger review of studies in Europe suggested a lower rate: 1 in 250,000 people.

HISTORY

This condition was first described in 1954 by Janeway, who reported five cases of the disease in children. In 1957 it was further characterized as "a fatal granulomatosus of childhood". The underlying cellular mechanism that causes chronic granulomatous disease was discovered in 1967, and research since that time has further elucidated the molecular mechanisms underlying the disease. Use of antibiotic prophylaxis, surgical abscess drainage, and vaccination led to the term "fatal" being dropped from the name of the disease as children survived into adulthood.

RESEARCH

Gene therapy is currently being studied as a possible treatment for chronic granulomatous disease. CGD is well-suited for gene therapy since it is caused by a mutation in single gene which only affects one body system (the hematopoietic system). Viruses have been used to deliver a normal gp91 gene to rats with a mutation in this gene, and subsequently the phagocytes in these rats were able to produce oxygen radicals. In 2006, two human patients with X-linked chronic granulomatous disease underwent gene therapy and blood cell precursor stem cell transplantation to their bone marrow. Both patients recovered from their CGD, clearing pre-existing infections and demonstrating increased oxidase activity in their neutrophils. However, long-term complications and efficacy of this therapy were unknown. In 2012, a 16-year-old boy with CGD was treated at the Great Ormond Street Hospital, London with an experimental gene therapy which temporarily reversed the CGD and allowed him to overcome a life-threatening lung disease.

LEUKOCYTE ADHESION DEFICIENCY

Leukocyte adhesion deficiency (LAD), is a rare autosomal recessive disorder characterized by immunodeficiency resulting in recurrent infections. LAD is currently divided into three subtypes: LAD1, LAD2, and the recently described LAD3, also known as LAD-1/variant. In LAD3, the immune defects are supplemented by a Glanzmann thrombasthenia-like bleeding tendency.

CHARACTERISTICS

LAD was first recognized as a distinct clinical entity in the 1970s. The classic descriptions of LAD included recurrent bacterial infections, defects in neutrophil adhesion, and a delay in umbilical cord sloughing. The adhesion defects result in poor leukocyte chemotaxis, particularly neutrophil, inability to form pus and neutrophilia. Individuals with LAD suffer from bacterial infections beginning in the neonatal period. Infections such as omphalitis, pneumonia, gingivitis, and peritonitis are common and often life-threatening due to the infant's inability to properly destroy the invading pathogens. These individuals do not form abscesses because granulocytes cannot migrate to the sites of infection.

CAUSE AND GENETICS

Types of leukocyte adhesion deficiency include LAD1, LAD2, and LAD3. LAD1 is the most common.

Type	OMIM	Gene
LAD1	116920	*ITGB2*
LAD2 or CDG2C	266265	*SLC35C1*
LAD3	612840	*FERMT3*

Patients with LAD1 have an inherited molecular defect that causes a deficiency of the β-2 integrin subunit, also called CD18, which is encoded by the ITGB2 gene found on chromosome 21. This subunit is involved in

the formation of the β-2 integrins (LFA-1, Integrin alphaXbeta2, and Mac-1/CR3) by dimerization with different CD11 subunits. Mutations in the ITGB2 gene lead to absent, reduced, or aberrant CD18 protein expression, causing a lack of expression in the leukocyte membrane of the β-2 integrins. The main function of these proteins is to allow neutrophils to make their way out of the blood stream to the infected tissues by adhering to different ligands expressed by the endothelium, *e.g.,* ICAM-1. In LAD-I patients, neutrophils cannot extravasate and fight against bacteria in tissues. The bacteria can then proliferate, leading to symptomatic infection, which can spread unimpeded and cause serious injury to important tissues.

DIAGNOSIS

Typically, diagnosis involves several preliminary tests of immune function, including basic evaluation of the humoral immune system and the cell-mediated immune system. A WBC differential will reveal extremely elevated levels of neutrophils (on the order of 6-10x normal) because they are unable to leave the blood vessels. In the case of LAD-I, specific diagnosis is done by flow cytometry. This technique will reveal absent or reduced CD18 expression in the leukocyte membrane. Recently, prenatal diagnosis systems has been established, allowing an early detection of the disease. LAD-II diagnosis includes the study of different glycosilated forms of the transferrin protein. In LAD-III, as platelet function is also affected, this could be used to differentiate it from the other types.

TREATMENT

Although patients can receive intensive antibiotherapy and even granulocyte transfusions from healthy donors, the only current curative therapy is the hematopoietic stem cell transplant. However, progress has been made in gene therapy, an active area of research. Both foamyviral and lentiviral vectors expressing the human ITGB2 gene under the control of different promoters have been developed and have been tested so far in preclinical LAD-I models (such as CD18-deficient mice and canine leukocyte adhesion deficiency-affected dogs).

PROGNOSIS

A 2009 study reported results from 36 children who had received a stem cell transplant. At the time of follow-up (median time 62 months), 75% of the children were still alive.

EPIDEMIOLOGY

LAD is a rare disease, with an estimated prevalence of one in 100,000 births, with no described racial or ethnic predilection. The most common type is LAD1.

MYELOPEROXIDASE DEFICIENCY

Myeloperoxidase deficiency is an autosomal recessive genetic disorder featuring deficiency, either in quantity or of function, of myeloperoxidase, an enzyme found in certain phagocytic immune cells, especially polymorphonuclear leukocytes. It can appear similar to chronic granulomatous disease on some screening tests.

PRESENTATION

Although MPO deficiency classically presents with immune deficiency (especially candida albicans infections), the majority of individuals with MPO deficiency show no signs of immunodeficiency. The lack of severe symptoms suggest that role of myeloperoxidase in the immune response must be redundant to other

mechanisms of intracellular killing of phagocytosed bacteria. Patients with MPO deficiency have a respiratory burst with a normal nitro blue tetrazolium (NBT) test because they still have NADPH oxidase activity, but do not form HClO (bleach) due to their lack of myeloperoxidase activity. This is in contrast to chronic granulomatous disease, in which the NBT test is 'negative' due to the lack of NADPH oxidase activity (positive test result means neutrophils turn blue, negative means nitroblue tetrazolium remains yellow). Patients with MPO deficiency are at increased risk for systemic candidiasis.

EPIDEMIOLOGY

- 1 in 4000 individuals have complete MPO deficiency
- 1 in 2000 have a partial defect in MPO
- Myeloperoxidase deficiency is the most common primary phagocyte disorder.

3

Bone Marrow Failure Syndromes

BONE MARROW

Bone marrow is a semi-solid tissue which may be found within the spongy or cancellous portions of bones. In birds and mammals, bone marrow is the primary site of new blood cell production or hematopoiesis. It is composed of hematopoietic cells, marrow adipose tissue, and supportive stromal cells. In adult humans, bone marrow is primarily located in the ribs, vertebrae, sternum, and bones of the pelvis. On average, bone marrow constitutes 4% of the total body mass of humans; in an adult having 65 kilograms of mass (143 lb), bone marrow typically accounts for approximately 2.6 kilograms (5.7 lb).

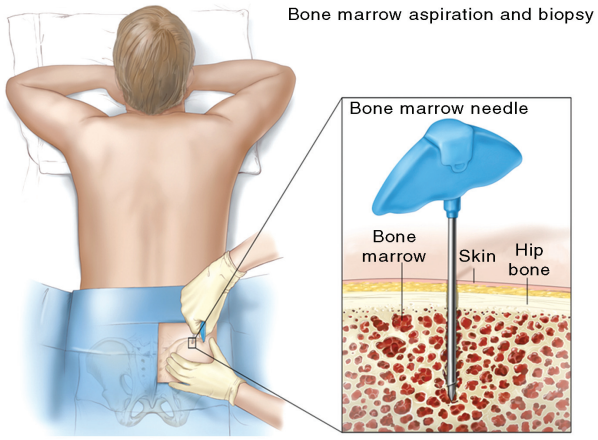


Fig. Bone marrow biopsy.

Human marrow produces approximately 500 billion blood cells per day, which join the systemic circulation via permeable vasculature sinusoids within the medullary cavity. All types of hematopoietic cells, including both myeloid and lymphoid lineages, are created in bone marrow; however, lymphoid cells must migrate to other lymphoid organs (*e.g.,* thymus) in order to complete maturation. Bone marrow transplants can be conducted to treat severe diseases of the bone marrow, including certain forms of cancer such as leukemia. Additionally, bone marrow stem cells have been successfully transformed into functional neural cells, and can also potentially be used to treat illnesses such as inflammatory bowel disease.

STRUCTURE

The composition of marrow is dynamic, as the mixture of cellular and non-cellular components (connective tissue) shifts with age and in response to systemic factors. In humans, marrow is colloquially characterized as "red" or "yellow" marrow (Latin: *medulla ossium rubra*, Latin: *medulla ossium flava*, respectively) depending

on the prevalence of hematopoetic cells vs fat cells. While the precise mechanisms underlying marrow regulation are not understood, compositional changes occur according to stereotypical patterns. For example, a newborn baby's bones exclusively contain hematopoietically active "red" marrow, and there is a progressive conversion towards "yellow" marrow with age. In adults, red marrow is found mainly in the central skeleton, such as the pelvis, sternum, cranium, ribs, vertebrae and scapulae, and variably found in the proximal epiphyseal ends of long bones such as the femur and humerus. In circumstances of chronic hypoxia, the body can convert yellow marrow back to red marrow to increase blood cell production.

Hematopoietic Components

At the cellular level, the main functional component of bone marrow includes the progenitor cells which are destined to mature into blood and lymphoid cells. Marrow contains hematopoietic stem cells which give rise to the three classes of blood cells that are found in circulation: white blood cells (leukocytes), red blood cells (erythrocytes), and platelets (thrombocytes).

Cellular constitution of the red bone marrow parenchyma			
Group	Cell type	Average fraction	Reference range
Myelopoietic cells	Myeloblasts	0.9%	0.2–1.5
	Promyelocytes	3.3%	2.1–4.1
	Neutrophilic myelocytes	12.7%	8.2–15.7
	Eosinophilic myelocytes	0.8%	0.2–1.3
	Neutrophilic metamyelocytes	15.9%	9.6–24.6
	Eosinophilic metamyelocytes	1.2%	0.4–2.2
	Neutrophilic band cells	12.4%	9.5–15.3
	Eosinophilic band cells	0.9%	0.2–2.4
	Segmented neutrophils	7.4%	6.0–12.0
	Segmented eosinophils	0.5%	0.0–1.3
	Segmented basophils and mast cells	0.1%	0.0–0.2
Erythropoietic cells	Pronormoblasts	0.6%	0.2–1.3
	Basophilic normoblasts	1.4%	0.5–2.4
	Polychromatic normoblasts	21.6%	17.9–29.2
	Orthochromatic normoblast	2.0%	0.4–4.6
Other cell types	Megakaryocytes	< 0.1%	0.0-0.4
	Plasma cells	1.3%	0.4-3.9
	Reticular cells	0.3%	0.0-0.9
	Lymphocytes	16.2%	11.1-23.2
	Monocytes	0.3%	0.0-0.8

STROMA

The stroma of the bone marrow includes all tissue not directly involved in the marrow's primary function of hematopoiesis. Stromal cells may be indirectly involved in hematopoiesis, providing a microenvironment that influences the function and differentiation of hematopoeietic cells. For instance, they generate colony stimulating factors, which have a significant effect on hematopoiesis. Cell types that constitute the bone marrow stroma include:

Fibroblast

A fibroblast is a type of biological cell that synthesizes the extracellular matrix and collagen, the structural framework (stroma) for animal tissues, and plays a critical role in wound healing. Fibroblasts are the most common cells of connective tissue in animals.

Structure

Fibroblasts have a branched cytoplasm surrounding an elliptical, speckled nucleus having two or more nucleoli. Active fibroblasts can be recognized by their abundant rough endoplasmic reticulum. Inactive fibroblasts (called fibrocytes) are smaller, spindle shaped, and have a reduced rough endoplasmic reticulum. Although disjointed and scattered when they have to cover a large space, fibroblasts, when crowded, often locally align in parallel clusters. Unlike the epithelial cells lining the body structures, fibroblasts do not form flat monolayers and are not restricted by a polarizing attachment to a basal lamina on one side, although they may contribute to basal lamina components in some situations (*e.g.,* subepithelial myofibroblasts in intestine may secrete the α-2 chain carrying component of the laminin which is absent only in regions of follicle-associated epithelia which lack the myofibroblast lining). Fibroblasts can also migrate slowly over substratum as individual cells, again in contrast to epithelial cells. While epithelial cells form the lining of body structures, it is fibroblasts and related connective tissues which sculpt the "bulk" of an organism. The life span of a fibroblast, as measured in chick embryos, is 57 ± 3 days.

Relationship with Fibrocytes

Fibroblasts and fibrocytes are two states of the same cells, the former being the activated state, the latter the less active state, concerned with maintenance and tissue metabolism. Currently, there is a tendency to call both forms fibroblasts. The suffix "-blast" is used in cellular biology to denote a stem cell or a cell in an activated state of metabolism. Fibroblasts are morphologically heterogeneous with diverse appearances depending on their location and activity. Though morphologically inconspicuous, ectopically transplanted fibroblasts can often retain positional memory of the location and tissue context where they had previously resided, at least over a few generations. This remarkable behaviour may lead to discomfort in the rare event that they stagnate there excessively.

Development

The main function of fibroblasts is to maintain the structural integrity of connective tissues by continuously secreting precursors of the extracellular matrix. Fibroblasts secrete the precursors of all the components of the extracellular matrix, primarily the ground substance and a variety of fibres. The composition of the extracellular matrix determines the physical properties of connective tissues. Like other cells of connective tissue, fibroblasts are derived from primitive mesenchyme. Thus they express the intermediate filament protein vimentin, a feature used as a marker to distinguish their mesodermal origin. However, this test is not specific as epithelial cells cultured in vitro on adherent substratum may also express vimentin after some time. In certain situations epithelial cells can give rise to fibroblasts, a process called epithelial-mesenchymal transition (EMT). Conversely, fibroblasts in some situations may give rise to epithelia by undergoing a mesenchymal to epithelial transition (MET) and organizing into a condensed, polarized, laterally connected true epithelial sheet. This process is seen in many developmental situations (*e.g.,* nephron and notocord development), as well as in wound healing and tumorigenesis.

Function

Fibroblasts make collagens, glycosaminoglycans, reticular and elastic fibres, Growing individuals' fibroblasts are dividing and synthesizing ground substance. Tissue damage stimulates fibrocytes and induces the production of fibroblasts.

Inflammation

Besides their commonly known role as structural components, fibroblasts play a critical role in an immune response to a tissue injury. They are early players in initiating inflammation in the presence of invading

microorganisms. They induce chemokine synthesis through the presentation of receptors on their surface. Immune cells then respond and initiate a cascade of events to clear the invasive microorganisms. Receptors on the surface of fibroblasts also allow regulation of hematopoietic cells and provide a pathway for immune cells to regulate fibroblasts.

Tumour Mediation

Fibroblasts, like the tumor-associated host fibroblasts (TAF), play a crucial role in immune regulation through TAF-derived extracellular matrix (ECM) components and modulators. TAF are known to be significant in the inflammatory response as well as immune suppression in tumors. TAF-derived ECM components cause alterations in the ECM composition and initiate the ECM remodeling. The ECM remodeling is described as changes in the ECM as a result of enzyme activity which can lead to degradation of the ECM. Immune regulation of tumors is largely determined by the ECM remodeling because the ECM is responsible for regulating a variety of functions, such as proliferation, differentiation, and morphogenesis of vital organs. In many tumor types, especially those related to the epithelial cells, ECM remodeling is common. Examples of TAF-derived ECM components include Tenascin and Thrombospondin-1 (TSP-1), which can be found in sites of chronic inflammation and carcinomas respectively. Immune regulation of tumors can also occur through the TAF-derived modulators. Although these modulators may sound similar to the TAF-derived ECM components, they differ in the sense that they are responsible for the variation and turnover of the ECM. Cleaved ECM molecules can play a critical role in immune regulation. Proteases like matrix metalloproteineases (MMPs) and the uPA system are known to cleave the ECM. These proteases are derived from fibroblasts.

Secondary Actions

Mouse embryonic fibroblasts (MEFs) are often used as "feeder cells" in human embryonic stem cell research. However, many researchers are gradually phasing out MEFs in favour of culture media with precisely defined ingredients of exclusively human derivation. Further, the difficulty of exclusively using human derivation for media supplements is most often solved by use of "defined media" where the supplements are synthetic and achieve the primary goal of eliminating the chance of contamination from derivative sources.

Macrophage

Macrophages are a type of white blood cell, of the immune system, that engulfs and digests cellular debris, foreign substances, microbes, cancer cells, and anything else that does not have the type of proteins specific to healthy body cells on its surface in a process called phagocytosis. These large phagocytes are found in essentially all tissues, where they patrol for potential pathogens by amoeboid movement. They take various forms (with various names) throughout the body (*e.g.,* histiocytes, Kupffer cells, alveolar macrophages, microglia, and others), but all are part of the mononuclear phagocyte system. Besides phagocytosis, they play a critical role in nonspecific defence (innate immunity) and also help initiate specific defence mechanisms (adaptive immunity) by recruiting other immune cells such as lymphocytes.

For example, they are important as antigen presenters to T cells. In humans, dysfunctional macrophages cause severe diseases such as chronic granulomatous disease that result in frequent infections. Beyond increasing inflammation and stimulating the immune system, macrophages also play an important anti-inflammatory role and can decrease immune reactions through the release of cytokines. Macrophages that encourage inflammation are called M1 macrophages, whereas those that decrease inflammation and encourage tissue repair are called M2 macrophages. This difference is reflected in their metabolism; M1 macrophages have the unique ability to metabolize arginine to the "killer" molecule nitric oxide, whereas rodent M2 macrophages have the unique ability to metabolize

arginine to the "repair" molecule ornithine. However, this dichotomy has been recently questioned as further complexity has been discovered. Human macrophages are about 21 micrometres (0.00083 in) in diameter and are produced by the differentiation of monocytes in tissues. They can be identified using flow cytometry or immunohistochemical staining by their specific expression of proteins such as CD14, CD40, CD11b, CD64, F4/80 (mice)/EMR1 (human), lysozyme M, MAC-1/MAC-3 and CD68. Macrophages were first discovered by Élie Metchnikoff, a Russian zoologist, in 1884.

Structure

Types

A majority of macrophages are stationed at strategic points where microbial invasion or accumulation of foreign particles is likely to occur. These cells together as a group are known as the mononuclear phagocyte system and were previously known as the reticuloendothelial system. Each type of macrophage, determined by its location, has a specific name:

Cell Name	Anatomical Location
Adipose tissue macrophages	Adipose tissue
Monocytes	Bone marrow/blood
Kupffer cells	Liver
Sinus histiocytes	Lymph nodes
Alveolar macrophages (dust cells)	Pulmonary alveoli of lungs
Tissue macrophages (histiocytes) leading to giant cells	Connective tissue
Microglia	Central nervous system
Hofbauer cells	Placenta
Intraglomerular mesangial cells	Kidney
Osteoclasts	Bone
Epithelioid cells	Granulomas
Red pulp macrophages (Sinusoidal lining cells)	Red pulp of spleen
Peritoneal macrophages	Peritoneal cavity
LysoMac	Peyer's patch

Investigations concerning Kupffer cells are hampered because in humans, Kupffer cells are only accessible for immunohistochemical analysis from biopsies or autopsies. From rats and mice, they are difficult to isolate, and after purification, only approximately 5 million cells can be obtained from one mouse. Macrophages can express paracrine functions within organs that are specific to the function of that organ. In the testis for example, macrophages have been shown to be able to interact with Leydig cells by secreting 25-hydroxycholesterol, an oxysterol that can be converted to testosterone by neighbouring Leydig cells. Also, testicular macrophages may participate in creating an immune privileged environment in the testis, and in mediating infertility during inflammation of the testis.

Cardiac resident macrophages participate in electrical conduction via gap junction communication with cardiac myocytes. Macrophages can be classified on basis of the fundamental function and activation. According to this grouping there are classically activated macrophages, wound-healing macrophages (alternatively activated macrophages) and regulatory macrophages (Mregs).

Development

Macrophages that reside in adult healthy tissues either derive from circulating monocytes or are established before birth and then maintained during adult life independently of monocytes. By contrast, most of the macrophages that accumulate at diseased sites typically derive from circulating monocytes. When a monocyte enters damaged tissue through the endothelium of a blood vessel, a process known as leukocyte extravasation, it undergoes a series of changes to become a macrophage. Monocytes are attracted to a damaged site by chemical

substances through chemotaxis, triggered by a range of stimuli including damaged cells, pathogens and cytokines released by macrophages already at the site. At some sites such as the testis, macrophages have been shown to populate the organ through proliferation. Unlike short-lived neutrophils, macrophages survive longer in the body, up to several months.

Function

Phagocytosis

Macrophages are professional phagocytes and are highly specialized in removal of dying or dead cells and cellular debris. This role is important in chronic inflammation, as the early stages of inflammation are dominated by neutrophils, which are ingested by macrophages if they come of age. The neutrophils are at first attracted to a site, where they proliferate, before they are phagocytized by the macrophages. When at the site, the first wave of neutrophils, after the process of aging and after the first 48 hours, stimulate the appearance of the macrophages whereby these macrophages will then ingest the aged neutrophils. The removal of dying cells is, to a greater extent, handled by *fixed macrophages*, which will stay at strategic locations such as the lungs, liver, neural tissue, bone, spleen and connective tissue, ingesting foreign materials such as pathogens and recruiting additional macrophages if needed. When a macrophage ingests a pathogen, the pathogen becomes trapped in a phagosome, which then fuses with a lysosome. Within the phagolysosome, enzymes and toxic peroxides digest the pathogen. However, some bacteria, such as *Mycobacterium tuberculosis*, have become resistant to these methods of digestion. Typhoidal *Salmonellae* induce their own phagocytosis by host macrophages in vivo, and inhibit digestion by lysosomal action, thereby using macrophages for their own replication and causing macrophage apoptosis. Macrophages can digest more than 100 bacteria before they finally die due to their own digestive compounds.

Role in Adaptive Immunity

Macrophages are versatile cells that play many roles. As scavengers, they rid the body of worn-out cells and other debris. Along with dendritic cells, they are foremost among the cells that present antigens, a crucial role in initiating an immune response. As secretory cells, monocytes and macrophages are vital to the regulation of immune responses and the development of inflammation; they produce a wide array of powerful chemical substances (monokines) including enzymes, complement proteins, and regulatory factors such as interleukin-1. At the same time, they carry receptors for lymphokines that allow them to be "activated" into single-minded pursuit of microbes and tumour cells.

After digesting a pathogen, a macrophage will present the antigen (a molecule, most often a protein found on the surface of the pathogen and used by the immune system for identification) of the pathogen to the corresponding helper T cell. The presentation is done by integrating it into the cell membrane and displaying it attached to an MHC class II molecule (MHCII), indicating to other white blood cells that the macrophage is not a pathogen, despite having antigens on its surface. Eventually, the antigen presentation results in the production of antibodies that attach to the antigens of pathogens, making them easier for macrophages to adhere to with their cell membrane and phagocytose. In some cases, pathogens are very resistant to adhesion by the macrophages.

The antigen presentation on the surface of infected macrophages (in the context of MHC class II) in a lymph node stimulates TH1 (type 1 helper T cells) to proliferate (mainly due to IL-12 secretion from the macrophage). When a B-cell in the lymph node recognizes the same unprocessed surface antigen on the bacterium with its surface bound antibody, the antigen is endocytosed and processed. The processed antigen is then presented in MHCII on the surface of the B-cell. T cells that express the T cell receptor which recognizes the antigen-MHCII

complex (with co-stimulatory factors- CD40 and CD40L) cause the B-cell to produce antibodies that help opsonisation of the antigen so that the bacteria can be better cleared by phagocytes.

Macrophages provide yet another line of defence against tumor cells and somatic cells infected with fungus or parasites. Once a T cell has recognized its particular antigen on the surface of an aberrant cell, the T cell becomes an activated effector cell, producing chemical mediators known as lymphokines that stimulate macrophages into a more aggressive form.

Macrophage subtypes:

- There are several activated forms of macrophages. In spite of a spectrum of ways to activate macrophages, there are two main groups designated M1 and M2. M1 macrophages: as mentioned earlier (previously referred to as classically activated macrophages), M1 "killer" macrophages are activated by LPS and IFN-gamma, and secrete high levels of IL-12 and low levels of IL-10. M1 macrophages have pro-inflammatory, bactericidal, and phagocytic functions. In contrast, the M2 "repair" designation (also referred to as alternatively activated macrophages) broadly refers to macrophages that function in constructive processes like wound healing and tissue repair, and those that turn off damaging immune system activation by producing anti-inflammatory cytokines like IL-10. M2 is the phenotype of resident tissue macrophages, and can be further elevated by IL-4. M2 macrophages produce high levels of IL-10, TGF-beta and low levels of IL-12. Tumor-associated macrophages are mainly of the M2 phenotype, and seem to actively promote tumor growth.
- Macrophages exist in a variety of phenotypes which are determined by the role they play in wound maturation. Phenotypes can be predominantly separated into two major categories; M1 and M2. M1 macrophages are activated by four key mediators: interferon-γ (IFN-γ), tumor necrosis factor (TNF), and damage associated pattern molecules (DAMPs). These mediator molecules create a pro-inflammatory response that in return produce pro-inflammatory cytokines like Interleukin-6 and TNF. These cytokines are essential in the initial process of wound healing. Unlike M1 macrophages, M2's secrete an anti-inflammatory response via the addition of Interleukin-4 or Interleukin-13. M2 cells are divided into four major types based on their roles: M2a, M2b, M2c, and M2d. How M2 phenotypes are determined is still up for discussion but studies have shown that their environment allows them to adjust to whichever phenotype is most appropriate to efficiently heal the wound.
- While M1 macrophages are the dominating phenotype observed in the early stages of inflammation, as the wound ages there is a significant decrease in M1 phenotype and an increase of M2 macrophages at the site. If this shift failed to occur, there would be prolonged inflammation. M2 cells are needed for production of collagen at the wound site. They are needed for revascularization and reepithethelisation. It was previously thought that an increase of M2 macrophages may decrease the time it takes for wound closure. However, studies show that rate of wound closure is not affected by an increase in M2 cells.
- M2 macrophages are needed for vascular stability. They produce vascular epithelial growth factor-A and TGF-β1. There is a phenotype shift from M1 to M2 macrophages in acute wounds, however this shift is impaired for chronic wounds. This dysregulation results in insufficient M2 macrophages and its corresponding growth factors that aid in wound repair. With a lack of these growth factors/anti-inflammatory cytokines and an overabundance of pro-inflammatory cytokines from M1 macrophages chronic wounds are unable to heal in a timely manner. Normally, after neutrophils eat debris/pathogens they perform apoptosis and are removed. At this point, inflammation is not needed and M1 undergoes a switch to M2 (anti-inflammatory). However, dysregulation occurs as the M1 macrophages are unable/do not phagocytose neutrophils that have undergone apoptosis leading to increased macrophage migration and inflammation.
- Both M1 and M2 macrophages play a role in promotion of atherosclerosis. M1 macrophages promote

atherosclerosis by inflammation. M2 macrophages can remove cholesterol from blood vessels, but when the cholesterol is oxidized, the M2 macrophages become apoptotic foam cells contributing to the atheromatous plaque of atherosclerosis.

Role in Muscle Regeneration

The first step to understanding the importance of macrophages in muscle repair, growth, and regeneration is that there are two "waves" of macrophages with the onset of damageable muscle use – subpopulations that do and do not directly have an influence on repairing muscle. The initial wave is a phagocytic population that comes along during periods of increased muscle use that are sufficient to cause muscle membrane lysis and membrane inflammation, which can enter and degrade the contents of injured muscle fibres. These early-invading, phagocytic macrophages reach their highest concentration about 24 hours following the onset of some form of muscle cell injury or reloading. Their concentration rapidly declines after 48 hours. The second group is the non-phagocytic types that are distributed near regenerative fibres. These peak between two and four days and remain elevated for several days during the hopeful muscle rebuilding. The first subpopulation has no direct benefit to repairing muscle, while the second non-phagocytic group does. It is thought that macrophages release soluble substances that influence the proliferation, differentiation, growth, repair, and regeneration of muscle, but at this time the factor that is produced to mediate these effects is unknown. It is known that macrophages' involvement in promoting tissue repair is not muscle specific; they accumulate in numerous tissues during the healing process phase following injury.

Role in Wound Healing

Macrophages are essential for wound healing. They replace polymorphonuclear neutrophils as the predominant cells in the wound by day two after injury. Attracted to the wound site by growth factors released by platelets and other cells, monocytes from the bloodstream enter the area through blood vessel walls. Numbers of monocytes in the wound peak one to one and a half days after the injury occurs. Once they are in the wound site, monocytes mature into macrophages. The spleen contains half the body's monocytes in reserve ready to be deployed to injured tissue. The macrophage's main role is to phagocytize bacteria and damaged tissue, and they also debride damaged tissue by releasing proteases. Macrophages also secrete a number of factors such as growth factors and other cytokines, especially during the third and fourth post-wound days. These factors attract cells involved in the proliferation stage of healing to the area. Macrophages may also restrain the contraction phase. Macrophages are stimulated by the low oxygen content of their surroundings to produce factors that induce and speed angiogenesis and they also stimulate cells that re-epithelialize the wound, create granulation tissue, and lay down a new extracellular matrix. By secreting these factors, macrophages contribute to pushing the wound healing process into the next phase.

Role in Limb Regeneration

Scientists have elucidated that as well as eating up material debris, macrophages are involved in the typical limb regeneration in the salamander. They found that removing the macrophages from a salamander resulted in failure of limb regeneration and a scarring response.

Role in Iron Homeostasis

As described above, macrophages play a key role in removing dying or dead cells and cellular debris. Erythrocytes have a lifespan on average of 120 days and so are constantly being destroyed by macrophages in the spleen and liver. Macrophages will also engulf macromolecules, and so play a key role in the pharmacokinetics

of parenteral irons. The iron that is released from the haemoglobin is either stored internally in ferritin or is released into the circulation via ferroportin. In cases where systemic iron levels are raised, or where inflammation is present, raised levels of hepcidin act on macrophage ferroportin channels, leading to iron remaining within the macrophages.

Clinical Significance

Due to their role in phagocytosis, macrophages are involved in many diseases of the immune system. For example, they participate in the formation of granulomas, inflammatory lesions that may be caused by a large number of diseases. Some disorders, mostly rare, of ineffective phagocytosis and macrophage function have been described, for example.

As a Host for Intracellular Pathogens

In their role as a phagocytic immune cell macrophages are responsible for engulfing pathogens to destroy them. Some pathogens subvert this process and instead live inside the macrophage. This provides an environment in which the pathogen is hidden from the immune system and allows it to replicate. Diseases with this type of behaviour include tuberculosis (caused by *Mycobacterium tuberculosis*) and leishmaniasis (caused by *Leishmania* species). In order to minimize the possibility of becoming the host of an intracellular bacteria, macrophages have evolved defence mechanisms such as induction of nitric oxide and reactive oxygen intermediates, which are toxic to microbes. Macrophages have also evolved the ability to restrict the microbe's nutrient supply and induce autophagy.

Tuberculosis:

- Once engulfed by a macrophage, the causative agent of tuberculosis, *Mycobacterium tuberculosis*, avoids cellular defences and uses the cell to replicate.

Leishmaniasis:

- Upon phagocytosis by a macrophage, the *Leishmania* parasite finds itself in a phagocytic vacuole. Under normal circumstances, this phagocytic vacuole would develop into a lysosome and its contents would be digested. *Leishmania* alter this process and avoid being destroyed; instead, they make a home inside the vacuole.

Chikungunya:

- Infection of macrophages in joints is associated with local inflammation during and after the acute phase of *Chikungunya* (caused by CHIKV or Chikungunya virus).

Others:

- Adenovirus (most common cause of pink eye) can remain latent in a host macrophage, with continued viral shedding 6–18 months after initial infection.
- *Brucella spp.* can remain latent in a macrophage via inhibition of phagosome–lysosome fusion; causes brucellosis (undulant fever).
- *Legionella pneumophila*, the causative agent of Legionnaires' disease, also establishes residence within macrophages.

Heart Disease

Macrophages are the predominant cells involved in creating the progressive plaque lesions of atherosclerosis. Focal recruitment of macrophages occurs after the onset of acute myocardial infarction. These macrophages function to remove debris, apoptotic cells and to prepare for tissue regeneration.

HIV Infection

Macrophages also play a role in human immunodeficiency virus (HIV) infection. Like T cells, macrophages can be infected with HIV, and even become a reservoir of ongoing virus replication throughout the body. HIV can enter the macrophage through binding of gp120 to CD4 and second membrane receptor, CCR5 (a chemokine receptor). Both circulating monocytes and macrophages serve as a reservoir for the virus. Macrophages are better able to resist infection by HIV-1 than CD4+ T cells, although susceptibility to HIV infection differs among macrophage subtypes.

Cancer

Macrophages can contribute to tumor growth and progression by promoting tumor cell proliferation and invasion, fostering tumor angiogenesis and suppressing antitumor immune cells. Attracted to oxygen-starved (hypoxic) and necrotic tumor cells they promote chronic inflammation. Inflammatory compounds such as tumor necrosis factor (TNF)-alpha released by the macrophages activate the gene switch nuclear factor-kappa B. NF-κB then enters the nucleus of a tumor cell and turns on production of proteins that stop apoptosis and promote cell proliferation and inflammation. Moreover, macrophages serve as a source for many pro-angiogenic factors including vascular endothelial factor (VEGF), tumor necrosis factor-alpha (TNF-alpha), Macrophage colony-stimulating factor (M-CSF/CSF1) and IL-1 and IL-6 contributing further to the tumor growth. Macrophages have been shown to infiltrate a number of tumors. Their number correlates with poor prognosis in certain cancers including cancers of breast, cervix, bladder and brain. Tumor-associated macrophages (TAMs) are thought to acquire an M2 phenotype, contributing to tumor growth and progression. Some tumors can also produce factors, including M-CSF/CSF1, MCP-1/CCL2 and Angiotensin II, that trigger the amplification and mobilization of macrophages in tumors. Research in various study models suggests that macrophages can sometimes acquire anti-tumor functions.

For example, macrophages may have cytotoxic activity to kill tumor cells directly; also the co-operation of T-cells and macrophages is important to suppress tumors. This co-operation involves not only the direct contact of T-cell and macrophage, with antigen presentation, but also includes the secretion of adequate combinations of cytokines, which enhance T-cell antitumor activity. Recent study findings suggest that by forcing IFN-á expression in tumor-infiltrating macrophages, it is possible to blunt their innate protumoral activity and reprogram the tumor microenvironment towards more effective dendritic cell activation and immune effector cell cytotoxicity. Additionally, subcapsular sinus macrophages in tumor-draining lymph nodes can suppress cancer progression by containing the spread of tumor-derived materials.

Cancer Therapy

Experimental studies indicate that macrophages can affect all therapeutic modalities, including surgery, chemotherapy, radiotherapy, immunotherapy and targeted therapy. Macrophages can influence treatment outcomes both positively and negatively. Macrophages can be protective in different ways: they can remove dead tumor cells (in a process called phagocytosis) following treatments that kill these cells; they can serve as drug depots for some anticancer drugs; they can also be activated by some therapies to promote antitumor immunity. Macrophages can also be deleterious in several ways: for example they can suppress various chemotherapies, radiotherapies and immunotherapies. Because macrophages can regulate tumor progression, therapeutic strategies to reduce the number of these cells, or to manipulate their phenotypes, are currently being tested in cancer patients.

Obesity

Increased number of pro-inflammatory macrophages within obese adipose tissue contributes to obesity complications including insulin resistance and diabetes type 2. Within the fat (adipose) tissue of CCR2 deficient

mice, there is an increased number of eosinophils, greater alternative Macrophage activation, and a propensity towards type 2 cytokine expression. Furthermore, this effect was exaggerated when the mice became obese from a high fat diet.

Intestinal Macrophages

Though very similar in structure to tissue macrophages, intestinal macrophages have evolved specific characteristics and functions given their natural environment, which is in the digestive tract. Macrophages and intestinal macrophages have high plasticity causing their phenotype to be altered by their environments. Like macrophages, intestinal macrophages are differentiated monocytes, though intestinal macrophages have to coexist with the microbiome in the intestines. This is a challenge considering the bacteria found in the gut are not recognized as "self" and could be potential targets for phagocytosis by the macrophage. To prevent the destruction of the gut bacteria, intestinal macrophages have developed key differences compared to other macrophages.

Primarily, intestinal macrophages do not induce inflammatory responses. Whereas tissue macrophages release various inflammatory cytokines, such as IL-1, IL-6 and TNF-α, intestinal macrophages do not produce or secrete inflammatory cytokines. This change is directly caused by the intestinal macrophages environment. Surrounding intestinal epithelial cells release TGF-β, which induces the change from proinflammatory macrophage to noninflammatory macrophage.

Even though the inflammatory response is downregulated in intestinal macrophages, phagocytosis is still carried out. There is no drop off in phagocytosis efficiency as intestinal macrophages are able to effectively phagocytize the bacteria, *S. typhimurium* and *E. coli*, but intestinal macrophages still do not release cytokines, even after phagocytosis. Also, intestinal macrophages do not express lipoplysaccharide (LPS), IgA, or IgG receptors. The lack of LPS receptors is important for the gut as the intestinal macrophages do not detect the microbe-associated molecular patterns (MAMPS/PAMPS) of the intestinal microbiome. Nor do they express IL-2 and IL-3 growth factor receptors.

Intestinal Macrophages Role in Disease

Intestinal macrophages have been shown to play a role in inflammatory bowel disease (IBD), such as Crohn's Disease (CD) and Ulcerative Colitis (UC). In a healthy gut, intestinal macrophages limit the inflammatory response in the gut, but in a disease-state, intestinal macrophage numbers and diversity are altered. This leads to inflammation of the gut and disease symptoms of IBD. Intestinal macrophages are critical in maintaining gut homeostasis. The presence of inflammation or pathogen alters this homeostasis, and concurrently alters the intestinal macrophages. There has yet to be a determined mechanism for the alteration of the intestinal macrophages by recruitment of new monocytes or changes in the already present intestinal macrophages.

Marrow Adipose Tissue

Marrow adipose tissue (MAT), also known as bone marrow adipose tissue (BMAT), increases in states of low bone density -osteoporosis, anorexia nervosa/caloric restriction, skeletal unweighting, anti-diabetes therapies). The marrow adipocytes originate from mesenchymal stem cell (MSC) progenitors that also give rise to osteoblasts, among other cell types.

Thus, it is thought that MAT results from preferential MSC differentiation into the adipocyte, rather than osteoblast, lineage in the setting of osteoporosis. Since MAT is increased in the setting of obesity and is suppressed by endurance exercise, or vibration, it is likely that MAT physiology- in the setting of mechanical input/exercise- approximates that of white adipose tissue (WAT).

MAT has qualities of both white and brown fat. Subcutaneous white fat contain excess energy, indicating a clear evolutionary advantage during times of scarcity. WAT is also the source of adipokines and inflammatory markers which have both positive (*e.g.,* adiponectin) and negative effects on metabolic and cardiovascular endpoints. Visceral abdominal fat (VAT) is a distinct type of WAT that is "proportionally associated with negative metabolic and cardiovascular morbidity", regenerates cortisol, and recently has been tied to decreased bone formation Both types of WAT substantially differ from brown adipose tissue (BAT) as by a group of proteins that help BAT's thermogenic role.

MAT, by its "specific marrow location, and its adipocyte origin from at least LepR+ marrow MSC is separated from non-bone fat storage by larger expression of bone transcription factors", and likely indicates a different fat phenotype. Recently, MAT was noted to "produce a greater proportion of adiponectin - an adipokine associated with improved metabolism - than WAT", suggesting an endocrine function for this depot, akin, but different, from that of WAT.

Marrow Adipose Tissue (MAT) and Bone Health

MAT increases in states of bone fragility. MAT is thought to result from preferential MSC differentiation into an adipocyte, rather than osteoblast lineage in osteoporosis based on the inverse relationship between bone and MAT in bone-fragile osteoporotic states. An increase in MAT is noted in osteoporosis clinical studies measured by MR Spectroscopy.

Estrogen therapy in postmenopausal osteoporosis reduces MAT. Antiresorptive therapies like risedronate or zoledronate also decrease MAT while increasing bone density, supporting an inverse relationship between bone quantity and MAT. During aging, bone quantity declines and fat redistributes from subcutaneous to ectopic sites such as bone marrow, muscle, and liver. Aging is associated with lower osteogenic and greater adipogenic biasing of MSC. This aging-related biasing of MSC away from osteoblast lineage may represent higher basal PPARg expression or decreased Wnt10b. Thus, bone fragility, osteoporosis, and osteoporotic fractures are thought to be linked to mechanisms which promote MAT accumulation.

- Histologic sections demonstrating Marrow Adipocytes

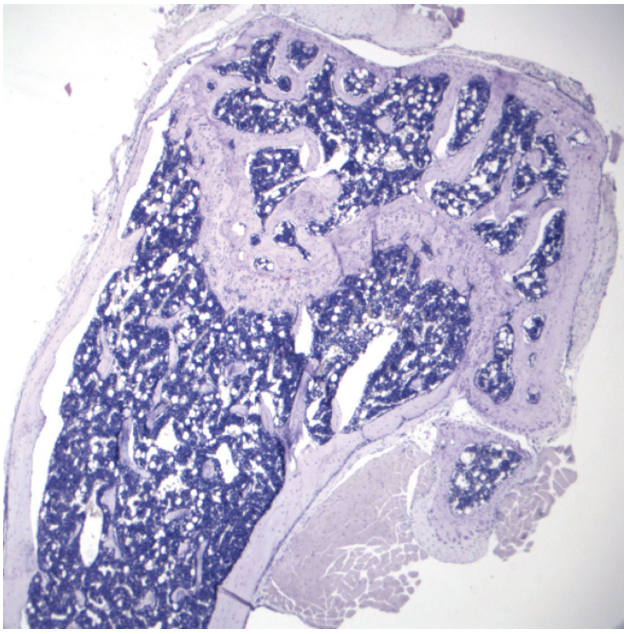


Fig. Representative distal femur histologic section of a 16-week old healthy C57BL/6 mouse demonstrating a typical quantity of marrow adipocytes.

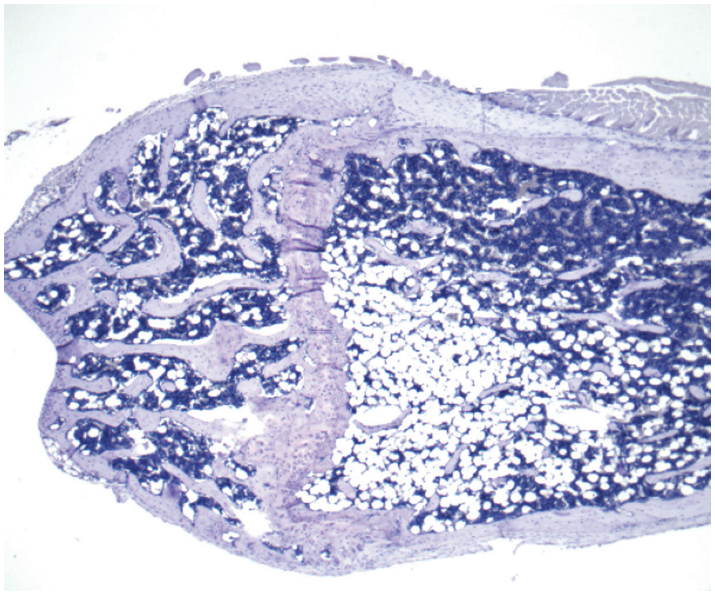


Fig. Representative distal femur histologic section of a 16-week old C57BL/6 mouse after 6 weeks of calorie restriction demonstrating an increased quantity of marrow adipocytes.

Measurement of Marrow Adipose Tissue

In order to understand the physiology of MAT, various analytic methods have been applied. Marrow adipocytes are difficult to isolate and quantify because they are interspersed with bony and hematopoietic elements. Until recently, qualitative measurements of MAT have relied on bone histology, which is subject to site selection bias and cannot adequately quantify the volume of fat in the marrow. Nevertheless, histological techniques and fixation make possible visualization of MAT, quantification of adipocyte size, and MAT's association with the surrounding endosteum, milieu of cells, and secreted factors.

Recent advances in cell surface and intracellular marker identification and single-cell analyses led to greater resolution and high-throughput *ex-vivo* quantification. Flow cytometric quantification can be used to purify adipocytes from the stromal vascular fraction of most fat depots. Early research with such machinery cited adipocytes as too large and fragile for cytometer-based purification, rendering them susceptible to lysis; however, recent advances have been made to mitigate this; nevertheless, this methodology continues to pose technical challenges and is inaccessible to much of the research community.

- Methods for Quantification of Marrow Adipose Tissue (MAT)

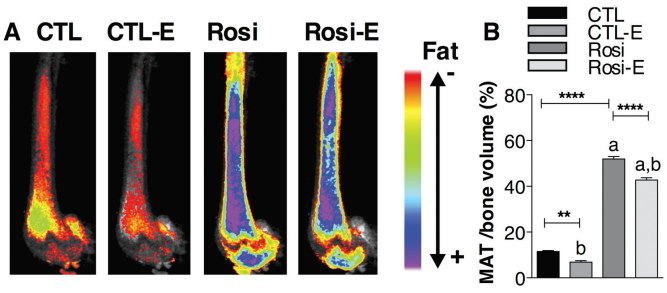


Fig. This figure demonstrates the use of the osmium- μCT method with advanced image processing to quantify MAT. In this figure, running exercise is shown to suppress MAT despite PPARγ agonist. Fat binder osmium is imaged via μCT (A) in n =5 per group overlaid images. Quantification of osmium as MAT/bone volume in the whole femur is shown. a, significant due to Rosi. b, significant due to exercise. Rosi=rosiglizaone, CTL=control, E=exercise.

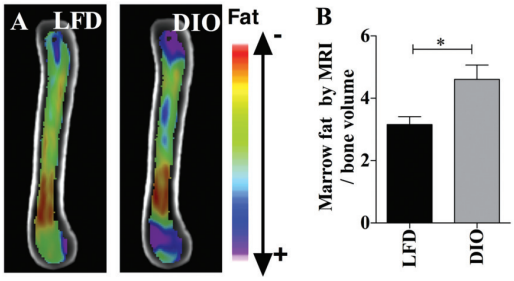


Fig. This demonstrates the use of MRI imaging (9.4T scanner) along with advanced image processing to quantify MAT. The images and graph demonstrate that MAT is higher in obese compared with lean mice. B6 mice were fed HFD from age 4 wk until age 16 wk. MAT was quantified by MRI. A) n=10 superimposed group average images are shown B) MAT normalized to bone volume in each group.

To improve quantification of MAT, novel imaging techniques have been developed as a means to visualize and quantify MAT. Although proton magnetic resonance spectroscopy (1H-MRS) has been used with success to quantify vertebral MAT in humans, it is difficult to employ in laboratory animals. Magnetic resonance imaging (MRI) provides MAT assessment in the vertebral skeleton in conjunction with μCT-based marrow density measurements. A volumetric method to identify, quantify, and localize MAT in rodent bone has been recently developed, requiring osmium staining of bones and μCT imaging, followed by advanced image analysis of osmium-bound lipid volume (in mm) relative to bone volume. This technique provides reproducible quantification and visualization of MAT, enabling the ability to consistently quantify changes in MAT with diet, exercise, and agents that constrain precursor lineage allocation. Although the osmium method is quantitatively precise, osmium is toxic and cannot be compared across batched experiments. Recently, researchers developed and validated a 9.4T MRI scanner technique that allows localization and volumetric (3D) quantification that can be compared across experiments.

Marrow Adipose Tissue and Hematopoietic Cells

Hematopoietic cells (also known as blood cells) reside in the bone marrow along with marrow adipocytes. These hematopoietic cells are derived from hematopoietic stem cells (HSC) which give rise to diverse cells: cells of the blood, immune system, as well as cells that break down bone (osteoclasts). HSC renewal occurs in the marrow stem cell niche, a microenvironment that contains cells and secreted factors that promote appropriate renewal and differentiation of HSC. The study of the stem cell niche is relevant to the field of oncology in order to improve therapy for multiple hematologic cancers. As such cancers are often treated with bone marrow transplantation, there is interest in improving the renewal of HSC. Recent work demonstrates that marrow adipocytes secrete factors that promote HSC renewal in most bones.

Osteoblast

Osteoblasts are cells with a single nucleus that synthesize bone. However, in the process of bone formation, osteoblasts function in groups of connected cells. Individual cells cannot make bone. A group of organized osteoblasts together with the bone made by a unit of cells is usually called the osteon. Osteoblasts are specialized, terminally differentiated products of mesenchymal stem cells. They synthesize dense, crosslinked collagen and specialized proteins in much smaller quantities, including osteocalcin and osteopontin, which compose the organic matrix of bone. In organized groups of connected cells, osteoblasts produce hydroxyapatite that is deposited, in a highly regulated manner, into the organic matrix forming a strong and dense mineralized tissue - the mineralized matrix. The mineralized skeleton is the main support for the bodies of air breathing vertebrates. It is an important store of minerals for physiological homeostasis including both acid-base balance and calcium or phosphate maintenance.

Bone Structure

The skeleton is a large organ that is formed and degraded throughout life in the air-breathing vertebrates. The skeleton, often referred to as the skeletal system, is important both as a supporting structure and for maintenance of calcium, phosphate, and acid-base status in the whole organism. The functional part of bone, the *bone matrix*, is entirely extracellular. The bone matrix consists of protein and mineral. The protein forms the *organic matrix.* It is synthesized and then the mineral is added. The vast majority of the organic matrix is collagen, which provides tensile strength. The matrix is mineralized by deposition of hydroxyapatite (alternative name, hydroxylapatite). This mineral is hard, and provides compressive strength. Thus, the collagen and mineral together are a composite material with excellent tensile and compressive strength, which can bend under a strain and recover its shape without damage. This is called elastic deformation. Forces that exceed the capacity of bone to behave elastically may cause failure, typically bone fractures.

Bone Remodeling

Bone is a dynamic tissue that is constantly being reshaped by osteoblasts, which produce and secrete matrix proteins and transport mineral into the matrix, and osteoclasts, which break down the tissues.

Osteoblasts

Osteoblasts are the major cellular component of bone. Osteoblasts arise from mesenchymal stem cells (MSC). MSC give rise to osteoblasts, adipocytes, and myocytes among other cell types. Osteoblast quantity is understood to be inversely proportional to that of marrow adipocytes which comprise marrow adipose tissue (MAT). Osteoblasts are found in large numbers in the periosteum, the thin connective tissue layer on the outside surface of bones, and in the endosteum. Normally, almost all of the bone matrix, in the air breathing vertebrates, is mineralized by the osteoblasts. Before the organic matrix is mineralized, it is called the osteoid. Osteoblasts buried in the matrix are called osteocytes. During bone formation, the surface layer of osteoblasts consists of cuboidal cells, called *active osteoblasts*. When the bone-forming unit is not actively synthesizing bone, the surface osteoblasts are flattened and are called *inactive osteoblasts*. Osteocytes remain alive and are connected by cell processes to a surface layer of osteoblasts. Osteocytes have important functions in skeletal maintenance.

Osteoclasts

Osteoclasts break down bone tissue, and along with osteoblasts and osteocytes form the structural components of bone. In the hollow within bones are many other cell types of the bone marrow. Components that are essential for osteoblast bone formation include mesenchymal stem cells (osteoblast precursor) and blood vessels that supply oxygen and nutrients for bone formation. Bone is a highly vascular tissue, and active formation of blood vessel cells, also from mesenchymal stem cells, is essential to support the metabolic activity of bone. The balance of bone formation and bone resorption tends to be negative with age, particularly in post-menopausal women, often leading to a loss of bone serious enough to cause fractures, which is called osteoporosis.

Osteogenesis

Bone is formed by one of two processes: endochondral ossification or intramembranous ossification. Endochondral ossification is the process of forming bone from cartilage and this is the usual method. This form of bone development is more complex; it follows the formation of a first skeleton of cartilage made by

chondrocytes, which is then removed and replaced by bone, made by osteoblasts. Intramembranous ossification is the direct ossification of mesenchyme as happens during the formation of the membrane bones of the skull and others. During osteoblast differentiation, the developing progenitor cells express the regulatory transcription factor Cbfa1/Runx2. A second required transcription factor is Sp7 transcription factor. Osteochondroprogenitor cells differentiate under the influence of growth factors, although isolated mesenchymal stem cells in tissue culture, form osteoblasts under permissive conditions that include vitamin C and substrates for alkaline phosphatase, a key enzyme that provides high concentrations of phosphate at the mineral deposition site.

Bone Morphogenetic Proteins

Key growth factors in endochondral skeletal differentiation include bone morphogenetic proteins (BMPs) that determine to a major extent where chondrocyte differentiation occurs and where spaces are left between bones. The system of cartilage replacement by bone has a complex regulatory system. BMP2 also regulates early skeletal patterning. transforming growth factor beta (TGF-β), is part of a superfamily of proteins that include BMPs, which possess common signaling elements in the TGF beta signaling pathway. TGF-β is particularly important in cartilage differentiation, which generally precedes bone formation for endochondral ossification. An additional family of essential regulatory factors is the fibroblast growth factors (FGFs) that determine where skeletal elements occur in relation to the skin.

Steroid and Protein Hormones

Many other regulatory systems are involved in the transition of cartilage to bone and in bone maintenance. A particularly important bone-targeted hormonal regulator is parathyroid hormone (PTH). Parathyroid hormone is a protein made by the parathyroid gland under the control of serum calcium activity. PTH also has important systemic functions, including to keep serum calcium concentrations nearly constant regardless of calcium intake. Increasing dietary calcium results in minor increases in blood calcium.

However, this is not a significant mechanism supporting osteoblast bone formation, except in the condition of low dietary calcium; further, abnormally high dietary calcium raises the risk of serious health consequences not directly related to bone mass including heart attack and stroke. Intermittent PTH stimulation increases osteoblast activity, although PTH is bifunctional and mediates bone matrix degradation at higher concentrations. The skeleton is also modified for reproduction and in response to nutritional and other hormone stresses; it responds to steroids, including estrogen and glucocorticoids, which are important in reproduction and energy metabolism regulation. Bone turnover involves major expenditures of energy for synthesis and degradation, involving many additional signals including pituitary hormones. Two of these are adrenocorticotropic hormone (ACTH) and follicle stimulating hormone. The physiological role for responses to these, and several other glycoprotein hormones, is not fully understood, although it is likely that ACTH is bifunctional, like PTH, supporting bone formation with periodic spikes of ACTH, but causing bone destruction in large concentrations. In mice, mutations that reduce the efficiency of ACTH-induced glucocorticoid production in the adrenals cause the skeleton to become dense (osteosclerotic bone).

Organization and Ultrastructure

In well-preserved bone studied at high magnification via electron microscopy, individual osteoblasts are shown to be connected by tight junctions, which prevent extracellular fluid passage and thus create a bone compartment separate from the general extracellular fluid. The osteoblasts are also connected by gap junctions, small pores that connect osteoblasts, allowing the cells in one cohort to function as a unit. The gap junctions also connect deeper layers of cells to the surface layer (*osteocytes* when surrounded by bone). This was

demonstrated directly by injecting low molecular weight fluorescent dyes into osteoblasts and showing that the dye diffused to surrounding and deeper cells in the bone-forming unit. Bone is composed of many of these units, which are separated by impermeable zones with no cellular connections, called cement lines.

Collagen and Accessory Proteins

Almost all of the organic (non-mineral) component of bone is dense collagen type I, which forms dense crosslinked ropes that give bone its tensile strength. By mechanisms still unclear, osteoblasts secrete layers of oriented collagen, with the layers parallel to the long axis of the bone alternating with layers at right angles to the long axis of the bone every few micrometers. Defects in collagen type I cause the commonest inherited disorder of bone, called osteogenesis imperfecta. Minor, but important, amounts of small proteins, including osteocalcin and osteopontin, are secreted in bone's organic matrix. Osteocalcin is not expressed at significant concentrations except in bone, and thus osteocalcin is a specific marker for bone matrix synthesis. These proteins link organic and mineral component of bone matrix. The proteins are necessary for maximal matrix strength due to their intermediate localization between mineral and collagen. However, in mice where expression of osteocalcin or osteopontin was eliminated by targeted disruption of the respective genes (knockout mice), accumulation of mineral was not notably affected, indicating that organization of matrix is not significantly related to mineral transport.

Bone versus Cartilage

The primitive skeleton is cartilage, a solid avascular (without blood vessels) tissue in which individual cartilage-matrix secreting cells, or chondrocytes, occur. Chondrocytes do not have intercellular connections and are not coordinated in units. Cartilage is composed of a network of collagen type II held in tension by water-absorbing proteins, hydrophilic proteoglycans. This is the adult skeleton in cartilaginous fishes such as sharks. It develops as the initial skeleton in more advanced classes of animals. In air-breathing vertebrates, cartilage is replaced by cellular bone. A transitional tissue is mineralized cartilage. Cartilage mineralizes by massive expression of phosphate-producing enzymes, which cause high local concentrations of calcium and phosphate that precipitate. This mineralized cartilage is not dense or strong. In the air breathing vertebrates it is used as a scaffold for formation of cellular bone made by osteoblasts, and then it is removed by osteoclasts, which specialize in degrading mineralized tissue. Osteoblasts produce an advanced type of bone matrix consisting of dense, irregular crystals of hydroxyapatite, packed around the collagen ropes. This is a strong composite material that allows the skeleton to be shaped mainly as hollow tubes. Reducing the long bones to tubes reduces weight while maintaining strength.

Mineralization of Bone

The mechanisms of mineralization are not fully understood. Fluorescent, low-molecular weight compounds such as tetracycline or calcein bind strongly to bone mineral, when administered for short periods. They then accumulate in narrow bands in the new bone. These bands run across the contiguous group of bone-forming osteoblasts. They occur at a narrow (sub-micrometer) mineralization front. Most bone surfaces express no new bone formation, no tetracycline uptake and no mineral formation. This strongly suggests that facilitated or active transport, coordinated across the bone-forming group, is involved in bone formation, and that only cell-mediated mineral formation occurs. That is, dietary calcium does not create mineral by mass action. The mechanism of mineral formation in bone is clearly distinct from the phylogenetically older process by which cartilage is mineralized: tetracycline does not label mineralized cartilage at narrow bands or in specific sites, but diffusely, in keeping with a passive mineralization mechanism.

Osteoblasts separate bone from the extracellular fluid by tight junctions by regulated transport. Unlike in cartilage, phosphate and calcium cannot move in or out by passive diffusion, because the tight osteoblast junctions isolate the bone formation space. Calcium is transported across osteoblasts by facilitated transport (that is, by passive transporters, which do not pump calcium against a gradient). In contrast, phosphate is actively produced by a combination of secretion of phosphate-containing compounds, including ATP, and by phosphatases that cleave phosphate to create a high phosphate concentration at the mineralization front. Alkaline phosphatase is a membrane-anchored protein that is a characteristic marker expressed in large amounts at the apical (secretory) face of active osteoblasts. At least one more regulated transport process is involved. The stoichiometry of bone mineral basically is that of hydroxyapatite precipitating from phosphate, calcium, and water at a slightly alkaline pH:

$$6\,HPO_4^{2-} + 2\,H_2O + 10\,Ca^{2+} \rightleftharpoons Ca_{10}(PO_4)_6(OH)_2 + 8H^+$$

In a closed system as mineral precipitates, acid accumulates, rapidly lowering the pH and stopping further precipitation. Cartilage presents no barrier to diffusion and acid therefore diffuses away, allowing precipitation to continue. In the osteon, where matrix is separated from extracellular fluid by tight junctions, this cannot occur. In the controlled, sealed compartment, removing H drives precipitation under a wide variety of extracellular conditions, as long as calcium and phosphate are available in the matrix compartment. The mechanism by which acid transits the barrier layer remains uncertain. Osteoblasts have capacity for Na/H exchange via the redundant Na/H exchangers, NHE1 and NHE6. This H exchange is a major element in acid removal, although the mechanism by which H is transported from the matrix space into the barrier osteoblast is not known. In bone removal, a reverse transport mechanism uses acid delivered to the mineralized matrix to drive hydroxyapatite into solution.

Osteocyte Feedback

Feedback from yes physical activity maintains bone mass, while feedback from osteocytes limits the size of the bone-forming unit. An important additional mechanism is secretion by osteocytes, buried in the matrix, of sclerostin, a protein that inhibits a pathway that maintains osteoblast activity. Thus, when the osteon reaches a limiting size, it inactivates bone synthesis.

Morphology and Histological Staining

Hematoxylin and eosin staining (H&E) shows that the cytoplasm of active osteoblasts is slightly basophilic due to the substantial presence of rough endoplasmic reticulum. The active osteoblast produces substantial collagen type I. About 10% of the bone matrix is collagen with the balance mineral.

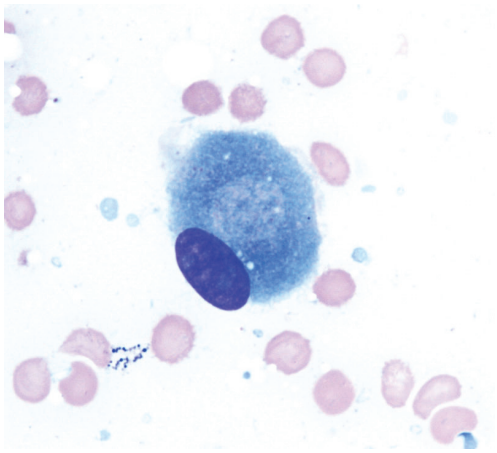


Fig. Osteoblast (Wright Giemsa stain, 100x)

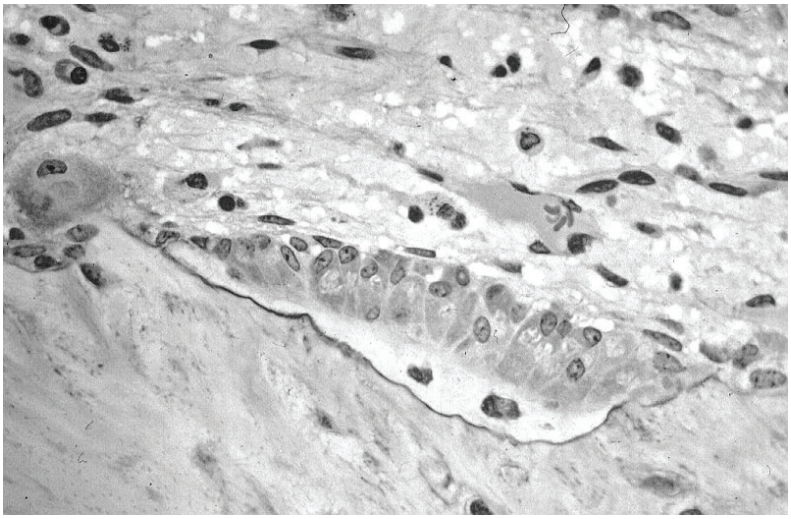


Fig. Light micrograph of decalcified (a process that removes the mineral) cancellous bone displaying osteoblasts actively synthesizing osteoid, containing two osteocytes.

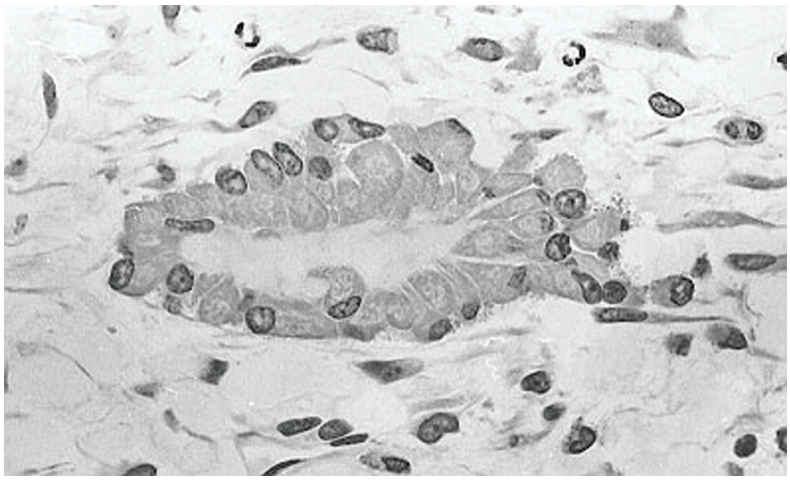


Fig. Light micrograph of undecalcified tissue displaying osteoblasts actively synthesizing osteoid (center).

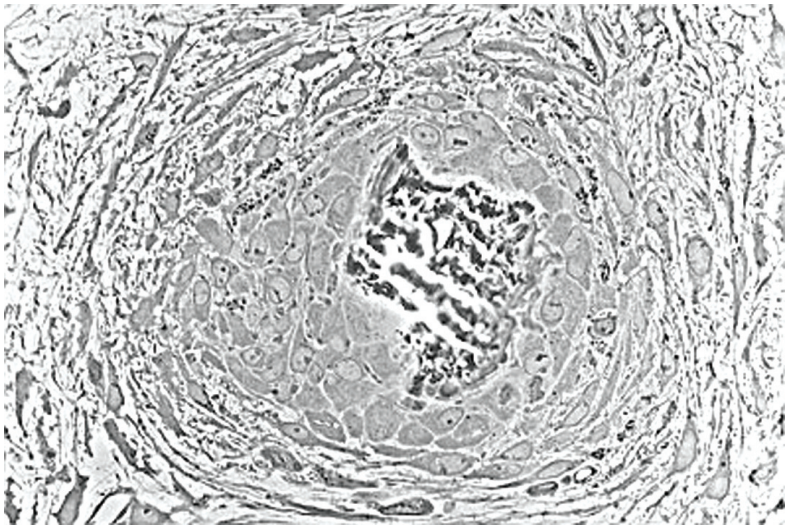


Fig. Light micrograph of undecalcified tissue displaying osteoblasts actively synthesizing rudimentary bone tissue (center).

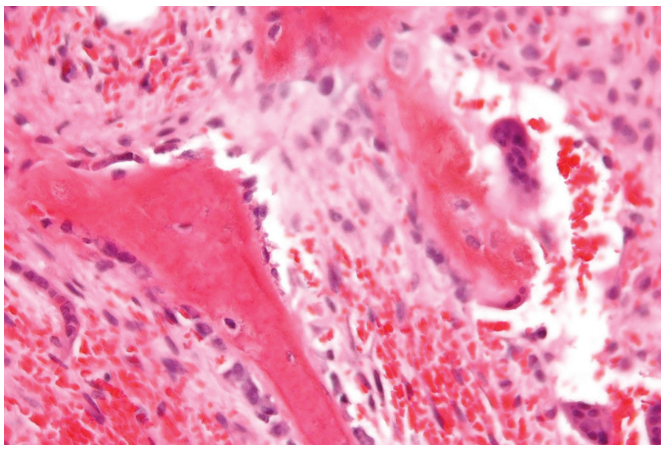


Fig. Osteoblasts lining bone (H&E stain).

The osteoblast's nucleus is spherical and large. An active osteoblast is characterized morphologically by a prominent Golgi apparatus that appears histologically as a clear zone adjacent to the nucleus. The products of the cell are mostly for transport into the osteoid, the non-mineralized matrix. Active osteoblasts can be labeled by antibodies to Type-I collagen, or using naphthol phosphate and the diazonium dye fast blue to demonstrate alkaline phosphatase enzyme activity directly.

Osteoclast

An osteoclast is a type of bone cell that breaks down bone tissue. This function is critical in the maintenance, repair, and remodelling of bones of the vertebralskeleton. The osteoclast disassembles and digests the composite of hydrated protein and mineral at a molecular level by secreting acid and a collagenase, a process known as *bone resorption*. This process also helps regulate the level of blood calcium. An odontoclast is an osteoclast associated with absorption of the roots of deciduous teeth.

Structure

An osteoclast is a large multinucleated cell and human osteoclasts on bone typically have five nuclei and are about 150-200 μm in diameter. When osteoclast-inducing cytokines are used to convert macrophages to osteoclasts, very large cells that may reach 100 μm in diameter occur. These may have dozens of nuclei, and typically express major osteoclast proteins but have significant differences from cells in living bone because of the not-natural substrate. The size of the multinucleated assembled osteoclast allows it to focus the ion transport, protein secretory and vesicular transport capabilities of many macrophages on a localized area of bone.

Location

In bone, osteoclasts are found in pits in the bone surface which are called resorption bays, or Howship's lacunae. Osteoclasts are characterized by a cytoplasm with a homogeneous, "foamy" appearance. This appearance is due to a high concentration of vesicles and vacuoles. These vacuoles include lysosomes filled with acid phosphatase. This permits characterization of osteoclasts by their staining for high expression of tartrate resistant acid phosphatase (TRAP) and cathepsin K. Osteoclast rough endoplasmic reticulum is sparse, and the Golgi complex is extensive. At a site of active bone resorption, the osteoclast forms a specialized cell membrane, the "ruffled border," that opposes the surface of the bone tissue. This extensively folded or ruffled border facilitates bone removal by dramatically increasing the cell surface for secretion and uptake of the resorption compartment contents and is a morphologic characteristic of an osteoclast that is actively resorbing bone.

Development

Since their discovery in 1873 there has been considerable debate about their origin. Three theories were dominant: from 1949 to 1970 the connective tissue origin was popular, which stated that osteoclasts and osteoblasts are of the same lineage, and osteoblasts fuse together to form osteoclasts. After years of controversy it is now clear that these cells develop from the self fusion of macrophages. It was in the beginning of 1980 that the monocyte phagocytic system was recognized as precursor of osteoclasts. Osteoclast formation requires the presence of RANKL (receptor activator of nuclear factor κβ ligand) and M-CSF (Macrophage colony-stimulating factor). These membrane-bound proteins are produced by neighbouring stromal cells and osteoblasts, thus requiring direct contact between these cells and osteoclast precursors. M-CSF acts through its receptor on the osteoclast, c-fms (colony-stimulating factor 1 receptor), a transmembrane tyrosine kinase-receptor, leading to secondary messenger activation of tyrosine kinase Src. Both of these molecules are necessary for osteoclastogenesis and are widely involved in the differentiation of monocyte/macrophage derived cells.

RANKL is a member of the tumour necrosis family (TNF), and is essential in osteoclastogenesis. RANKL knockout mice exhibit a phenotype of osteopetrosis and defects of tooth eruption, along with an absence or deficiency of osteoclasts. RANKL activates NF-κβ (nuclear factor-κβ) and NFATc1 (nuclear factor of activated t cells, cytoplasmic, calcineurin-dependent 1) through RANK. NF-κβ activation is stimulated almost immediately after RANKL-RANK interaction occurs and is not upregulated. NFATc1 stimulation, however, begins ~24–48 hours after binding occurs and its expression has been shown to be RANKL dependent. Osteoclast differentiation is inhibited by osteoprotegerin (OPG), which is produced by osteoblasts and binds to RANKL thereby preventing interaction with RANK. It may be important to note that while osteoclasts are derived from the hematopoietic lineage, osteoblasts are derived from mesenchymal stem cells.

Function

Once activated, osteoclasts move to areas of microfracture in the bone by chemotaxis. Osteoclasts lie in a small cavity called Howship's lacunae, formed from the digestion of the underlying bone. The sealing zone is the attachment of the osteoclast's plasma membrane to the underlying bone. Sealing zones are bounded by belts of specialized adhesion structures called podosomes. Attachment to the bone matrix is facilitated by integrin receptors, such as αvβ3, via the specific amino acid motif Arg-Gly-Asp in bone matrix proteins, such as osteopontin. The osteoclast releases hydrogen ions through the action of carbonic anhydrase ($H_2O + CO_2 \rightarrow HCO_3 + H$) through the ruffled border into the resorptive cavity, acidifying and aiding dissolution of the mineralized bone matrix into Ca, H_3PO_4, H_2CO_3, water and other substances. Dysfunction of the carbonic anhydrase has been documented to cause some forms of osteopetrosis.

Hydrogen ions are pumped against a high concentration gradient by proton pumps, specifically a unique vacuolar-ATPase. This enzyme has been targeted in the prevention of osteoporosis. In addition, several hydrolytic enzymes, such as members of the cathepsin and matrix metalloprotease (MMP) groups, are released to digest the organic components of the matrix. These enzymes are released into the compartment by lysosomes. Of these hydrolytic enzymes, cathepsin K is of most importance.

Cathepsin K and other Cathepsins

Cathepsin K is a collagenolytic, papain-like, cysteine protease that is mainly expressed in osteoclasts, and is secreted into the resorptive pit. Cathepsin K is the major protease involved in the degradation of type I collagen and other noncollagenous proteins. Mutations in the cathepsin K gene are associated with pycnodysostosis, a hereditary osteopetrotic disease, characterised by a lack of functional cathepsin K expression. Knockout studies of cathepsin K in mice lead to an osteopetrotic phenotype, which, is partially compensated by increased expression

of proteases other that cathepsin K and enhanced osteoclastogenesis. Cathepsin K has an optimal enzymatic activity in acidic conditions.

It is synthesized as a proenzyme with a molecular weight of 37kDa, and upon activation by autocatalytic cleavage, is transformed into the mature, active form with a molecular weight of ~27kDa. Upon polarization of the osteoclast over the site of resorption, cathepsin K is secreted from the ruffled border into the resorptive pit. Cathepsin K transmigrates across the ruffled border by intercellular vesicles and is then released by the functional secretory domain. Within these intercellular vesicles, cathepsin K, along with reactive oxygen species generated by TRAP, further degrades the bone extracellular matrix. Several other cathepsins are expressed in osteoclasts including cathepsins B, C, D, E, G, and L. The function of these cysteine and aspartic proteases is generally unknown within bone, and they are expressed at much lower levels than cathepsin K. Studies on cathepsin L knockout mice have been mixed, with a report of reduced trabecular bone in homozygous and heterozygous cathepsin L knockout mice compared to wild-type and another report finding no skeletal abnormalities.

Matrix Metalloproteinases

The matrix metalloproteinases (MMPs) comprise a family of more than 20 zinc-dependent endopeptidases. The role of matrix metalloproteinases (MMPs) in osteoclast biology is ill-defined, but in other tissue they have been linked with tumor promoting activities, such as activation of growth factors and are required for tumor metastasis and angiogenesis. MMP-9 is associated with the bone microenvironment. It is expressed by osteoclasts, and is known to be required for osteoclast migration and is a powerful gelatinase. Transgenic mice lacking MMP-9 develop defects in bone development, intraosseous angiogenesis, and fracture repair. MMP-13 is believed to be involved in bone resorption and in osteoclast differentiation, as knockout mice revealed decreased osteoclast numbers, osteopetrosis, and decreased bone resorption. MMPs expressed by the osteoclast include MMP-9, -10, -12, and -14. apart from MMP-9, little is known about their relevance to the osteoclast, however, high levels of MMP-14 are found at the sealing zone.

Osteoclast Physiology

In the 1980s and 90s the physiology of typical osteoclasts was studied in detail. With the isolation of the ruffled border, ion transport across it was studied directly in biochemical detail. Energy-dependent acid transport was verified and the postulated proton pump purified. With the successful culture of osteoclasts, it became apparent that they are organized to support the massive transport of protons for acidification of the resorption compartment and solubilization of the bone mineral. This includes ruffled border Cl permeability to control membrane potential and basolateral Cl/HCO_3 exchange to maintain cytosolic pH in physiologically acceptable ranges. The effectiveness of its ion secretion depends upon the osteoclast forming an effective seal around the resorption compartment. The positioning of this “sealing zone” appears to be mediated by integrins expressed on the osteoclast surface. With the sealing zone in place, the multinucleated osteoclast reorganizes itself. Developing the highly invaginated ruffled membrane apposing the resorption compartment allows massive secretory activity. In addition, it permits the vesicular transcytosis of the mineral and degraded collagen from the ruffled border to the free membrane of the cell, and its release into the extracellular compartment. This activity completes the bone resorption, and both the mineral components and collagen fragments are released to the general circulation.

Regulation

Osteoclasts are regulated by several hormones, including parathyroid hormone (PTH) from the parathyroid gland, calcitonin from the thyroid gland, and growth factor interleukin 6 (IL-6). This last hormone, IL-6, is one of

the factors in the disease osteoporosis, which is an imbalance between bone resorption and bone formation. Osteoclast activity is also mediated by the interaction of two molecules produced by osteoblasts, namely osteoprotegerin and RANK ligand. Note that these molecules also regulate differentiation of the osteoclast.

Odontoclast

An odontoclast is an osteoclast associated with absorption of the roots of deciduous teeth.

Alternate use of Term

An osteoclast can also be an instrument used to fracture and reset bones (the origin is Greek *osteon*: bone and *klastos*: broken). To avoid confusion, the cell was originally termed osotoclast. When the surgical instrument went out of use, the cell became known by its present name.

Clinical Significance

Giant osteoclasts can occur in some diseases, including Paget's disease of bone and bisphosphonate toxicity.

Endothelium

Endothelium refers to cells that line the interior surface of blood vessels and lymphatic vessels, forming an interface between circulating blood or lymph in the lumen and the rest of the vessel wall. It is a thin layer of simple, or single-layered, squamous cells called endothelial cells. Endothelial cells in direct contact with blood are called vascular endothelial cells, whereas those in direct contact with lymph are known as lymphatic endothelial cells. Vascular endothelial cells line the entire circulatory system, from the heart to the smallest capillaries. These cells have unique functions in vascular biology. These functions include fluid filtration, such as in the glomerulus of the kidney, blood vessel tone, hemostasis, neutrophil recruitment, and hormone trafficking. Endothelium of the interior surfaces of the heart chambers is called endocardium.

Structure

Endothelium is mesodermal in origin. Both blood and lymphatic capillaries are composed of a single layer of endothelial cells called a monolayer. In straight sections of a blood vessel, vascular endothelial cells typically align and elongate in the direction of fluid flow.

Terminology

The foundational model of anatomy makes a distinction between endothelial cells and epithelial cells on the basis of which tissues they develop from, and states that the presence of vimentin rather than keratin filaments separate these from epithelial cells. Many considered the endothelium a specialized epithelial tissue.

Function

Endothelial cells are involved in many aspects of vascular biology, including:

- Barrier function - the endothelium acts as a semi-selective barrier between the vessel lumen and surrounding tissue, controlling the passage of materials and the transit of white blood cells into and out of the bloodstream. Excessive or prolonged increases in permeability of the endothelial monolayer, as in cases of chronic inflammation, may lead to tissue edema/swelling.

- Blood clotting (thrombosis&fibrinolysis). The endothelium normally provides a non-thrombogenic surface because it contains, for example, heparan sulfate which acts as a cofactor for activating antithrombin, a protease that inactivates several factors in the coagulation cascade.
- Inflammation
- Formation of new blood vessels (angiogenesis)
- Vasoconstriction and vasodilation, and hence the control of blood pressure
- Repair of damaged or diseased organs via an injection of blood vessel cells
- Angiopoietin-2 works with VEGF to facilitate cell proliferation and migration of endothelial cells.

Clinical Significance

Endothelial dysfunction, or the loss of proper endothelial function, is a hallmark for vascular diseases, and is often regarded as a key early event in the development of atherosclerosis. Impaired endothelial function, causing hypertension and thrombosis, is often seen in patients with coronary artery disease, diabetes mellitus, hypertension, hypercholesterolemia, as well as in smokers. Endothelial dysfunction has also been shown to be predictive of future adverse cardiovascular events, and is also present in inflammatory disease such as rheumatoid arthritis and systemic lupus erythematosus. One of the main mechanisms of endothelial dysfunction is the diminishing of nitric oxide, often due to high levels of asymmetric dimethylarginine, which interfere with the normal L-arginine-stimulated nitric oxide synthesis and so leads to hypertension. The most prevailing mechanism of endothelial dysfunction is an increase in reactive oxygen species, which can impair nitric oxide production and activity via several mechanisms. The signalling protein ERK5 is essential for maintaining normal endothelial cell function. A further consequence of damage to the endothelium is the release of pathological quantities of von Willebrand factor, which promote platelet aggregation and adhesion to the subendothelium, and thus the formation of potentially fatal thrombi.

FUNCTION

Mesenchymal Stem Cell

Mesenchymal stem cells are multipotent stromal cells that can differentiate into a variety of cell types, including osteoblasts (bone cells), chondrocytes (cartilage cells), myocytes (muscle cells) and adipocytes (fat cells which give rise to marrow adipose tissue).

Structure

Definition

While the terms mesenchymal stem cell (MSC) and marrow stromal cell have been used interchangeably for many years, neither term is sufficiently descriptive:

- Mesenchyme is embryonic connective tissue that is derived from the mesoderm and that differentiates into hematopoietic and connective tissue, whereas MSCs do not differentiate into hematopoietic cells.
- Stromal cells are connective tissue cells that form the supportive structure in which the functional cells of the tissue reside. While this is an accurate description for one function of MSCs, the term fails to convey the relatively recently discovered roles of MSCs in the repair of tissue.
- The term encompasses multipotent cells derived from other non-marrow tissues, such as placenta, umbilical cord blood, adipose tissue, adult muscle, corneal stroma or the dental pulp of deciduous baby teeth. The cells do not have the capacity to reconstitute an entire organ.

Morphology

Mesenchymal stem cells are characterized morphologically by a small cell body with a few cell processes that are long and thin. The cell body contains a large, round nucleus with a prominent nucleolus, which is surrounded by finely dispersed chromatin particles, giving the nucleus a clear appearance. The remainder of the cell body contains a small amount of Golgi apparatus, rough endoplasmic reticulum, mitochondria and polyribosomes. The cells, which are long and thin, are widely dispersed and the adjacent extracellular matrix is populated by a few reticular fibrils but is devoid of the other types of collagen fibrils.

Location

Bone Marrow

Bone marrow was the original source of MSCs, and still is the most frequently utilized. These bone marrow stem cells do not contribute to the formation of blood cells and so do not express the hematopoietic stem cell marker CD34. They are sometimes referred to as *bone marrow stromal stem cells*.

Cord Cells

The youngest and most primitive MSCs can be obtained from umbilical cord tissue, namely Wharton's jelly and the umbilical cord blood. However MSCs are found in much higher concentration in the Wharton's jelly compared to cord blood, which is a rich source of hematopoietic stem cells. The umbilical cord is available after a birth. It is normally discarded and poses no risk for collection. These MSCs may prove to be a useful source of MSCs for clinical applications due to their primitive properties.

Adipose Tissue

Adipose tissue is a rich source of MSCs (or adipose-derived mesenchymal stem cells, AdMSCs).

Molar Cells

The developing tooth bud of the mandibular third molar is a rich source of MSCs. While they are described as multipotent, it is possible that they are pluripotent. They eventually form enamel, dentin, blood vessels, dental pulp and nervous tissues. These stem cells are capable of producing hepatocytes.

Amniotic Fluid

Stem cells are present in amniotic fluid. As many as 1 in 100 cells collected during amniocentesis are pluripotent mesenchymal stem cells.

Function

Differentiation Capacity

MSCs have a great capacity for self-renewal while maintaining their multipotency. Beyond that, there is little that can be definitively said. The standard test to confirm multipotency is differentiation of the cells into osteoblasts, adipocytes and chondrocytes as well as myocytes and neurons. MSCs have been seen to even differentiate into neuron-like cells, but there is lingering doubt whether the MSC-derived neurons are functional. The degree to

which the culture will differentiate varies among individuals and how differentiation is induced, *e.g.,* chemical vs. mechanical; and it is not clear whether this variation is due to a different amount of "true" progenitor cells in the culture or variable differentiation capacities of individuals' progenitors. The capacity of cells to proliferate and differentiate is known to decrease with the age of the donor, as well as the time in culture. Likewise, whether this is due to a decrease in the number of MSCs or a change to the existing MSCs is not known.

Immunomodulatory Effects

Numerous studies have demonstrated that human MSCs avoid allorecognition, interfere with dendritic cell and T-cell function and generate a local immunosuppressive microenvironment by secreting cytokines. Other studies contradict some of these findings, reflecting both the highly heterogeneous nature of MSC isolates and the considerable differences between isolates generated by the many different methods under development.

Clinical Significance

Mesenchymal stem cells in the body can be activated and mobilized if needed. However, the efficiency is low. For instance, damage to muscles heals very slowly but further study into mechanisms of MSC action may provide avenues for increasing their capacity for tissue repair.

Autoimmune Disease

Clinical studies investigating the efficacy of mesenchymal stem cells in treating diseases are in preliminary development, particularly for understanding autoimmune diseases, graft versus host disease, Crohn's disease, multiple sclerosis, systemic lupus erythematosus and systemic sclerosis. As of 2014, no high-quality clinical research provides evidence of efficacy, and numerous inconsistencies and problems exist in the research methods.

Other Diseases

Many of the early clinical successes using intravenous transplantation came in systemic diseases such as graft versus host disease and sepsis. Direct injection or placement of cells into a site in need of repair may be the preferred method of treatment, as vascular delivery suffers from a "pulmonary first pass effect" where intravenous injected cells are sequestered in the lungs.

Detection

The International Society for Cellular Therapy (ISCT) has proposed a set of standards to define MSCs. A cell can be classified as an MSC if it shows plastic adherent properties under normal culture conditions and has a fibroblast-like morphology. In fact, some argue that MSCs and fibroblasts are functionally identical. Furthermore, MSCs can undergo osteogenic, adipogenic and chondrogenic differentiation ex-vivo. The cultured MSCs also express on their surface CD73, CD90 and CD105, while lacking the expression of CD11b, CD14, CD19, CD34, CD45, CD79a and HLA-DR surface markers.

Research

Culturing

The majority of modern culture techniques still take a colony-forming unit-fibroblasts (CFU-F) approach, where raw unpurified bone marrow or ficoll-purified bone marrow Mononuclear cell are plated directly into

cell culture plates or flasks. Mesenchymal stem cells, but not red blood cells or haematopoetic progenitors, are adherent to tissue culture plastic within 24 to 48 hours. However, at least one publication has identified a population of non-adherent MSCs that are not obtained by the direct-plating technique. Other flow cytometry-based methods allow the sorting of bone marrow cells for specific surface markers, such as STRO-1. STRO-1+ cells are generally more homogenous and have higher rates of adherence and higher rates of proliferation, but the exact differences between STRO-1+ cells and MSCs are not clear. Methods of immunodepletion using such techniques as MACS have also been used in the negative selection of MSCs. The supplementation of basal media with fetal bovine serum or human platelet lysate is common in MSC culture. Prior to the use of platelet lysates for MSC culture, the pathogen inactivation process is recommended to prevent pathogen transmission.

Clinical Trials of Cryopreserved MSCs

Scientists have reported that MSCs when transfused immediately a few hours post thawing may show reduced function or show decreased efficacy in treating diseases as compared to those MSCs which are in log phase of cell growth, so cryopreserved MSCs should be brought back into log phase of cell growth in *in vitro* culture before these are administered for clinical trials or experimental therapies, re-culturing of MSCs will help in recovering from the shock the cells get during freezing and thawing. Various clinical trials on MSCs have failed which used cryopreserved product immediately post thaw as compared to those clinical trials which used fresh MSCs.

History

In 1924, Russian-born morphologist Alexander A. Maximow used extensive histological findings to identify a singular type of precursor cell within mesenchyme that develops into different types of blood cells. Scientists Ernest A. McCulloch and James E. Till first revealed the clonal nature of marrow cells in the 1960s. An *ex vivo* assay for examining the clonogenic potential of multipotent marrow cells was later reported in the 1970s by Friedenstein and colleagues. In this assay system, stromal cells were referred to as colony-forming unit-fibroblasts (CFU-f). The first clinical trials of MSCs were completed in 1995 when a group of 15 patients were injected with cultured MSCs to test the safety of the treatment. Since then, over 200 clinical trials have been started. However, most are still in the safety stage of testing. Subsequent experimentation revealed the plasticity of marrow cells and how their fate is determined by environmental cues. Culturing marrow stromal cells in the presence of osteogenic stimuli such as *ascorbic acid*, *inorganic phosphate* and *dexamethasone* could promote their differentiation into osteoblasts. In contrast, the addition of *transforming growth factor-beta* (TGF-b) could induce chondrogenic markers.

Bone Marrow Barrier

The blood vessels of the bone marrow constitute a barrier, inhibiting immature blood cells from leaving the marrow. Only mature blood cells contain the membrane proteins, such as aquaporin and glycophorin, that are required to attach to and pass the blood vessel endothelium. Hematopoietic stem cells may also cross the bone marrow barrier, and may thus be harvested from blood.

Lymphatic Role

The red bone marrow is a key element of the lymphatic system, being one of the primary lymphoid organs that generate lymphocytes from immature hematopoietic progenitor cells. The bone marrow and thymus constitute the primary lymphoid tissues involved in the production and early selection of lymphocytes. Furthermore, bone marrow performs a valve-like function to prevent the backflow of lymphatic fluid in the lymphatic system.

Compartmentalization

Biological compartmentalization is evident within the bone marrow, in that certain cell types tend to aggregate in specific areas. For instance, erythrocytes, macrophages, and their precursors tend to gather around blood vessels, while granulocytes gather at the borders of the bone marrow.

SOCIETY AND CULTURE

Animal bone marrow has been used in cuisine worldwide for millennia, such as the famed Milanese Ossobuco.

CLINICAL SIGNIFICANCE

Disease

The normal bone marrow architecture can be damaged or displaced by aplastic anemia, malignancies such as multiple myeloma, or infections such as tuberculosis, leading to a decrease in the production of blood cells and blood platelets. The bone marrow can also be affected by various forms of leukemia, which attacks its hematologic progenitor cells. Furthermore, exposure to radiation or chemotherapy will kill many of the rapidly dividing cells of the bone marrow, and will therefore result in a depressed immune system. Many of the symptoms of radiation poisoning are due to damage sustained by the bone marrow cells. To diagnose diseases involving the bone marrow, a bone marrow aspiration is sometimes performed. This typically involves using a hollow needle to acquire a sample of red bone marrow from the crest of the ilium under general or local anesthesia.

Application of Stem Cells in Therapeutics

Bone marrow derived stem cells have a wide array of application in regenerative medicine.

Imaging

Medical imaging may provide a limited amount of information regarding bone marrow. Plain film x-rays pass through soft tissues such as marrow and do not provide visualization, although any changes in the structure of the associated bone may be detected. CT imaging has somewhat better capacity for assessing the marrow cavity of bones, although with low sensitivity and specificity. For example, normal fatty "yellow" marrow in adult long bones is of low density (-30 to -100 Hounsfield units), between subcutaneous fat and soft tissue. Tissue with increased cellular composition, such as normal "red" marrow or cancer cells within the medullary cavity will measure variably higher in density.

MRI is more sensitive and specific for assessing bone bone composition. MRI enables assessment of the average molecular composition of soft tissues, and thus provides information regarding the relative fat content of marrow. In adult humans, "yellow" fatty marrow is the dominant tissue in bones, particularly in the (peripheral) appendicular skeleton. Because fat molecules have a high T1-relaxivity, T1-weighted imaging sequences show "yellow" fatty marrow as bright (hyperintense). Furthermore, normal fatty marrow loses signal on fat-saturation sequences, in a similar pattern to subcutaneous fat.

When "yellow" fatty marrow becomes replaced by tissue with more cellular composition, this change is apparent as decreased brightness on T1-weighted sequences. Both normal "red" marrow and pathologic marrow lesions (such as cancer) are darker than "yellow" marrow on T1-weight sequences, although can often be distinguished by comparison with the MR signal intensity of adjacent soft tissues. Normal "red" marrow is typically equivalent or brighter than skeletal muscle or intervertebral disc on T1-weighted sequences. Fatty marrow

change, the inverse of red marrow hyperplasia, can occur with normal aging, though it can also be seen with certain treatments such as radiation therapy. Diffuse marrow T1 hypointensity without contrast enhancement or cortical discontinuity suggests red marrow conversion or myelofibrosis. Falsely normal marrow on T1 can be seen with diffuse multiple myeloma or leukemic infiltration when the water to fat ratio is not sufficiently altered, as may be seen with lower grade tumors or earlier in the disease process.

Histology

Bone marrow examination refers to the pathologic analysis of samples of bone marrow obtained by bone marrow biopsy (often called a trephine biopsy) and bone marrow aspiration. Bone marrow examination is used in the diagnosis of a number of conditions, including leukemia, multiple myeloma, lymphoma, anemia, and pancytopenia. The bone marrow produces the cellular elements of the blood, including platelets, red blood cells and white blood cells. While much information can be gleaned by testing the blood itself (drawn from a vein by phlebotomy), it is sometimes necessary to examine the source of the blood cells in the bone marrow to obtain more information on hematopoiesis; this is the role of bone marrow aspiration and biopsy.

Components of the Procedure

Bone marrow samples can be obtained by aspiration and trephine biopsy. Sometimes, a bone marrow examination will include both an aspirate and a biopsy. The aspirate yields semi-liquid bone marrow, which can be examined by a pathologist under a light microscope and analyzed by flow cytometry, chromosome analysis, or polymerase chain reaction (PCR). Frequently, a trephine biopsy is also obtained, which yields a narrow, cylindrically shaped solid piece of bone marrow, 2mm wide and 2 cm long (80 µL), which is examined microscopically (sometimes with the aid of immunohistochemistry) for cellularity and infiltrative processes. An aspiration, using a 20 mL syringe, yields approximately 300 µL of bone marrow. A volume greater than 300 µL is not recommended, since it may dilute the sample with peripheral blood.

Comparison		
	Aspiration	Biopsy
Advantages	• Fast • Gives relative quantity of different cell types • Gives material to further study, e.g. molecular genetics and flow cytometry	• Gives cell and stroma constitution • Represents all cells • Explains cause of "dry tap" (aspiration gives no blood cells)
Drawbacks	Does not represent all cells	Slow processing

Aspiration does not always represent all cells since some such as lymphoma stick to the trabecula, and would thus be missed by a simple aspiration.

Site of Procedure

Bone marrow aspiration and trephine biopsy are usually performed on the back of the hipbone, or posterior iliac crest. An *aspirate* can also be obtained from the sternum (breastbone). For the sternal aspirate, the patient lies on their back, with a pillow under the shoulder to raise the chest. A *trephine biopsy* should never be performed on the sternum, due to the risk of injury to blood vessels, lungs or the heart. Bone marrow aspiration may also be performed on the tibial (shinbone) site in children up to 2 years of age while spinous process aspiration is frequently done in a lumbar puncture position and on the L3-L4 vertebrae. Anesthesia is used to reduce surface pain at the spot where the needle is inserted. Pain may result from the procedure's insult to the marrow, which cannot be anesthetized, as well as short periods of pain from the anesthetic process itself. The experience is not uniform; different patients report different levels of pain, and some do not report any pain at certain expected points.

How the Test is Performed

A bone marrow biopsy may be done in a health care provider's office or in a hospital. Informed consent for the procedure is typically required. The patient is asked to lie on their abdomen (prone position) or on their side (lateral decubitus position). The skin is cleansed, and a local anesthetic such as lidocaine or procaine is injected to numb the area. Patients may also be pretreated with analgesics and/or anti-anxiety medications, although this is not a routine practice.

Typically, the aspirate is performed first. An aspirate needle is inserted through the skin using manual pressure and force until it abuts the bone. Then, with a twisting motion of clinician's hand and wrist, the needle is advanced through the bony cortex (the hard outer layer of the bone) and into the marrow cavity. Once the needle is in the marrow cavity, a syringe is attached and used to aspirate ("suck out") liquid bone marrow. A twisting motion is performed during the aspiration to avoid excess content of blood in the sample, which might be the case if an excessively large sample from one single point is taken. Subsequently, the biopsy is performed if indicated. A different, larger trephine needle is inserted and anchored in the bony cortex. The needle is then advanced with a twisting motion and rotated to obtain a solid piece of bone marrow. This piece is then removed along with the needle.

The entire procedure, once preparation is complete, typically takes 10–15 minutes. If several samples are taken, the needle is removed between the samples to avoid blood coagulation. After the procedure is complete, the patient is typically asked to lie flat for 5–10 minutes to provide pressure over the procedure site. After that, assuming no bleeding is observed, the patient can get up and go about their normal activities. Paracetamol (aka acetaminophen) or other simple analgesics can be used to ease soreness, which is common for 2–3 days after the procedure. Any worsening pain, redness, fever, bleeding or swelling may suggest a complication. Patients are also advised to avoid washing the procedure site for at least 24 hours after the procedure is completed.

Contraindications

There are few contraindications to bone marrow examination. It is important to note that thrombocytopenia or bleeding disorders are *not* contraindications as long as the procedure is performed by a skilled clinician. Bone marrow aspiration and biopsy can be safely performed even in the setting of extreme thrombocytopenia (low platelet count). If there is a skin or soft tissue infection over the hip, a different site should be chosen for bone marrow examination.

Complications

While mild soreness lasting 12–24 hours is common after a bone marrow examination, serious complications are extremely rare. In a large review, an estimated 55,000 bone marrow examinations were performed, with 26 serious adverse events (0.05%), including one fatality. The same author collected data on over 19,000 bone marrow examinations performed in the United Kingdom in 2003, and found 16 adverse events (0.08% of total procedures), the most common of which was bleeding. In this report, complications, while rare, were serious in individual cases.

Donation and Transplantation

In a bone marrow transplant, hematopoietic stem cells are removed from a person and infused into another person (allogenic) or into the same person at a later time (autologous). If the donor and recipient are compatible, these infused cells will then travel to the bone marrow and initiate blood cell production. Transplantation from one person to another is conducted for the treatment of severe bone marrow diseases, such as congenital defects,

autoimmune diseases or malignancies. The patient's own marrow is first killed off with drugs or radiation, and then the new stem cells are introduced. Before radiation therapy or chemotherapy in cases of cancer, some of the patient's hematopoietic stem cells are sometimes harvested and later infused back when the therapy is finished to restore the immune system. Bone marrow stem cells can be induced to become neural cells to treat neurological illnesses, and can also potentially be used for the treatment of other illnesses, such as inflammatory bowel disease. In 2013, following a clinical trial, scientists proposed that bone marrow transplantation could be used to treat HIV in conjunction with antiretroviral drugs; however, it was later found that HIV remained in the bodies of the test subjects.

Harvesting

The stem cells are typically harvested directly from the red marrow in the iliac crest, often under general anesthesia. The procedure is minimally invasive and does not require stitches afterwards. Depending on the donor's health and reaction to the procedure, the actual harvesting can be an outpatient procedure, or can require 1–2 days of recovery in the hospital. Another option is to administer certain drugs that stimulate the release of stem cells from the bone marrow into circulating blood. An intravenous catheter is inserted into the donor's arm, and the stem cells are then filtered out of the blood. This procedure is similar to that used in blood or platelet donation. In adults, bone marrow may also be taken from the sternum, while the tibia is often used when taking samples from infants. In newborns, stem cells may be retrieved from the umbilical cord.

FOSSIL RECORD

The earliest fossilised evidence of bone marrow was discovered in 2014 in *Eusthenopteron*, a lobe-finned fish which lived during the Devonian period approximately 370 million years ago. Scientists from Uppsala University and the European Synchrotron Radiation Facility used X-ray synchrotron microtomography to study the fossilised interior of the skeleton's humerus, finding organised tubular structures akin to modern vertebrate bone marrow. *Eusthenopteron* is closely related to the early tetrapods, which ultimately evolved into the land-dwelling mammals and lizards of the present day.

APLASTIC ANEMIA

Aplastic anemia is a rare disease in which the bone marrow and the hematopoietic stem cells that reside there are damaged. This causes a deficiency of all three blood cell types (pancytopenia): red blood cells (anemia), white blood cells (leukopenia), and platelets (thrombocytopenia). *Aplastic* refers to inability of the stem cells to generate mature blood cells. It is more frequent in people in their teens and twenties, but is also common among the elderly. It can be caused by heredity, immune disease, or exposure to chemicals, drugs, or radiation. However, in about half the cases, the cause is unknown.

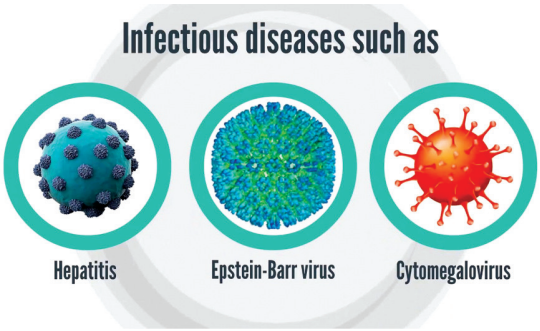


Fig. Aplastic Anaemia.

The definitive diagnosis is by bone marrow biopsy; normal bone marrow has 30–70% blood stem cells, but in aplastic anemia, these cells are mostly gone and replaced by fat. First line treatment for aplastic anemia consists of immunosuppressive drugs, typically either anti-lymphocyte globulin or anti-thymocyte globulin, combined with corticosteroids and ciclosporin. Hematopoietic stem cell transplantation is also used, especially for patients under 30 years of age with a related matched marrow donor. The disease is also known as the cause of death of Eleanor Roosevelt and Marie Curie.

SIGNS AND SYMPTOMS

Anemia may lead to malaise, pallor and associated symptoms such as palpitations. Low platelet counts (thrombocytopenia) if present is associated with an increased risk of hemorrhage, bruising and petechiae. Low white blood cell counts (leukocytopenia) if present leads to an increased risk of infections which can be severe.

CAUSES

Aplastic anemia can be caused by exposure to certain chemicals, drugs, radiation, infection, immune disease; in about half the cases, a definitive cause is unknown. It is not a familial line hereditary condition, nor is it contagious. It can be acquired due to exposure to other conditions but if a person develops the condition, their offspring would not develop it by virtue of their gene connection. Aplastic anemia is also sometimes associated with exposure to toxins such as benzene, or with the use of certain drugs, including chloramphenicol, carbamazepine, felbamate, phenytoin, quinine, and phenylbutazone. Many drugs are associated with aplasia mainly according to case reports, but at a very low probability. As an example, chloramphenicol treatment is followed by aplasia in less than one in 40,000 treatment courses, and carbamazepine aplasia is even rarer.

Exposure to ionizing radiation from radioactive materials or radiation-producing devices is also associated with the development of aplastic anemia. Marie Curie, famous for her pioneering work in the field of radioactivity, died of aplastic anemia after working unprotected with radioactive materials for a long period of time; the damaging effects of ionizing radiation were not then known. Aplastic anemia is present in up to 2% of patients with acute viral hepatitis.

One known cause is an autoimmune disorder in which white blood cells attack the bone marrow. Short-lived aplastic anemia can also be a result of parvovirus infection. In humans, the P antigen (also known as globoside), one of the many cellular receptors that contribute to a person's blood type, is the cellular receptor for parvovirus B19 virus that causes erythema infectiosum (fifth disease) in children. Because it infects red blood cells as a result of the affinity for the P antigen, Parvovirus causes complete cessation of red blood cell production. In most cases, this goes unnoticed, as red blood cells live on average 120 days, and the drop in production does not significantly affect the total number of circulating red blood cells. In people with conditions where the cells die early (such as sickle cell disease), however, parvovirus infection can lead to severe anemia. More frequently parvovirus B19 is associated with aplastic crisis which involves only the red blood cells (despite the name). Aplastic anemia involves all different cell lines. In some animals, aplastic anemia may have other causes. For example, in the ferret (*Mustela putorius furo*), it is caused by estrogen toxicity, because female ferrets are induced ovulators, so mating is required to bring the female out of heat. Intact females, if not mated, will remain in heat, and after some time the high levels of estrogen will cause the bone marrow to stop producing red blood cells.

DIAGNOSIS

The condition needs to be differentiated from pure red cell aplasia. In aplastic anemia, the patient has pancytopenia (*i.e.,* leukopenia and thrombocytopenia) resulting in decrease of all formed elements. In contrast, pure red cell aplasia is characterized by reduction in red cells only. The diagnosis can only be confirmed on bone

marrow examination. Before this procedure is undertaken, a patient will generally have had other blood tests to find diagnostic clues, including a complete blood count, renal function and electrolytes, liver enzymes, thyroid function tests, vitamin B_{12} and folic acid levels.

The following tests aid in determining differential diagnosis for aplastic anemia:

1. Bone marrow aspirate and biospy: to rule out other causes of pancytopenia (*i.e.,* neoplastic infiltration or significant myelofibrosis).
2. History of iatrogenic exposure to cytotoxic chemotherapy: can cause transient bone marrow suppression
3. X-rays, computed tomography (CT) scans, or ultrasound imaging tests: enlarged lymph nodes (sign of lymphoma), kidneys and bones in arms and hands (abnormal in Fanconi anemia)
4. Chest X-ray: infections
5. Liver tests: liver diseases
6. Viral studies: viral infections
7. Vitamin B_{12} and folate levels: vitamin deficiency
8. Blood tests for paroxysmal nocturnal hemoglobinuria
9. Test for antibodies: immune competency.

TREATMENT

Treating immune-mediated aplastic anemia involves suppression of the immune system, an effect achieved by daily medicine intake, or, in more severe cases, a bone marrow transplant, a potential cure. The transplanted bone marrow replaces the failing bone marrow cells with new ones from a matching donor. The multipotent stem cells in the bone marrow reconstitute all three blood cell lines, giving the patient a new immune system, red blood cells, and platelets. However, besides the risk of graft failure, there is also a risk that the newly created white blood cells may attack the rest of the body ("graft-versus-host disease"). In young patients with an HLA matched sibling donor, bone marrow transplant can be considered as first-line treatment, patients lacking a matched sibling donor typically pursue immunosuppression as a first-line treatment, and matched unrelated donor transplants are considered a second-line therapy.

Medical therapy of aplastic anemia often includes a course of antithymocyte globulin (ATG) and several months of treatment with ciclosporin to modulate the immune system. Chemotherapy with agents such as cyclophosphamide may also be effective but has more toxicity than ATG. Antibody therapy, such as ATG, targets T-cells, which are believed to attack the bone marrow. Corticosteroids are generally ineffective, though they are used to ameliorate serum sickness caused by ATG. Normally, success is judged by bone marrow biopsy 6 months after initial treatment with ATG. One prospective study involving cyclophosphamide was terminated early due to a high incidence of mortality, due to severe infections as a result of prolonged neutropenia. In the past, before the above treatments became available, patients with low leukocyte counts were often confined to a sterile room or bubble (to reduce risk of infections), as in the case of Ted DeVita.

Follow-up

Regular full blood counts are required on a regular basis to determine whether the patient is still in a state of remission. Many patients with aplastic anemia also have clones of cells characteristic of the rare disease paroxysmal nocturnal hemoglobinuria (PNH, anemia with thrombopenia and/or thrombosis), sometimes referred to as AA/PNH. Occasionally PNH dominates over time, with the major manifestation intravascular hemolysis. The overlap of AA and PNH has been speculated to be an escape mechanism by the bone marrow against destruction by the immune system. Flow cytometry testing is performed regularly in people with previous aplastic anemia to monitor for the development of PNH.

PROGNOSIS

Untreated, severe aplastic anemia has a high risk of death. Modern treatment, by drugs or stem cell transplant, has a five-year survival rate that exceeds 85%, with younger age associated with higher survival. Survival rates for stem cell transplant vary depending on age and availability of a well-matched donor. Five-year survival rates for patients who receive transplants have been shown to be 82% for patients under age 20, 72% for those 20–40 years old, and closer to 50% for patients over age 40. Success rates are better for patients who have donors that are matched siblings and worse for patients who receive their marrow from unrelated donors. Older people (who are generally too frail to undergo bone marrow transplants), and people who are unable to find a good bone marrow match, undergoing immune suppression have five-year survival rates of up to 75%. Relapses are common. Relapse following ATG/ciclosporin use can sometimes be treated with a repeated course of therapy. In addition, 10-15% of severe aplastic anemia cases evolve into MDS and leukemia. According to a study, for children who underwent immunosuppressive therapy, about 15.9% of children who responded to immunosuppressive therapy encountered relapse. Milder disease can resolve on its own.

DIAMOND–BLACKFAN ANEMIA

Diamond–Blackfan anemia (DBA) is a congenital erythroid aplasia that usually presents in infancy. DBA causes low red blood cell counts (anemia), without substantially affecting the other blood components (the platelets and the white blood cells), which are usually normal. This is in contrast to Shwachman–Bodian–Diamond syndrome, in which the bone marrow defect results primarily in neutropenia, and Fanconi anemia, where all cell lines are affected resulting in pancytopenia. A variety of other congenital abnormalities may also occur in DBA.

Signs/Symptoms

Diamond–Blackfan anemia is characteriz d by normocytic or macrocytic anemia (low red blood cell counts) with decreased erythroid progenitor cells in the bone marrow. This usually develops during the neonatal period. About 47% of affected individuals also have a variety of congenital abnormalities, including craniofacial malformations, thumb or upper limb abnormalities, cardiac defects, urogenital malformations, and cleft palate. Low birth weight and generalized growth delay are sometimes observed. DBA patients have a modest risk of developing leukemia and other malignancies.

Genetics

Most pedigrees suggest an autosomal dominant mode of inheritance with incomplete penetrance. Approximately 10–25% of DBA occurs with a family history of disease. About 25-50% of the causes of DBA have been tied to abnormal ribosomal protein genes. The disease is characterized by genetic heterogeneity, affecting different ribosomal gene loci: Exceptions to this paradigm have been demonstrated, such as with rare mutations of transcription factor GATA1 and advanced alternative splicing of a gene involved in iron metabolism, SLC49A1 (FLVCR1).

In 1997, a patient was identified who carried a rare balanced chromosomal translocation involving chromosome 19 and the X chromosome. This suggested that the affected gene might lie in one of the two regions that were disrupted by this cytogenetic anomaly. Linkage analysis in affected families also implicated this region in disease, and led to the cloning of the first DBA gene. About 20–25% of DBA cases are caused by mutations in the ribosome protein S19 (RPS19) gene on chromosome 19 at cytogenetic position 19q13.2. Some previously undiagnosed relatives of DBA patients were found to carry mutations, and also had increased adenosine deaminase levels in their red blood cells, but had no other overt signs of disease.

DBA types					
Name	Chromosome	Genotype	Phenotype	Protein	Disruption(cite)(cite)
DBA1	19q13.2	603474	105650	RPS19	30S to 18S(cite)
DBA2	8p23-p22	unknown	606129		
DBA3	10q22-q23	602412	610629	RPS24	30S to 18S(cite)
DBA4	15q	180472	612527	RPS17	30S to 18S
DBA5	3q29-qter	180468	612528	RPL35A	32S to 5.8S/28S(cite)
DBA6	1p22.1	603634	612561	RPL5	32S to 5.8S/28S
DBA7	1p36.1-p35	604175	612562	RPL11	32S to 5.8S/28S
DBA8	2p25	603658	612563	RPS7	30S to 18S
DBA9	6p	603632	613308	RPS10	30S to 18S
DBA10	12q	603701	613309	RPS26	30S to 18S
DBA11	17p13	603704	614900	RPL26	30S to 18S
DBA12	3p24	604174	615550	RPL15	45S to 32S
DBA13	14q	603633	615909	RPS29	
"other"				TSR2,RPS28, GATA1 SLC49A1 (FLVCR1)	

A subsequent study of families with no evidence of RPS19 mutations determined that 18 of 38 families showed evidence for involvement of an unknown gene on chromosome 8 at 8p23.3-8p22. The precise genetic defect in these families has not yet been delineated. Malformations are seen more frequently with DBA6 RPL5 and DBA7 RPL11 mutations. The genetic abnormalities underpinning the combination of DBA with Treacher Collins syndrome (TCS)/mandibulofacial dysostosis (MFD) phenotypes are heterogeneous, including RPS26 (the known DBA10 gene), TSR2 which encodes a direct binding partner of RPS26, and RPS28.

Molecular Basis

The phenotype of DBA patients suggests a hematological stem cell defect specifically affecting the erythroid progenitor population. Loss of ribosomal function might be predicted to affect translation and protein biosynthesis broadly and impact many tissues. However, DBA is characterized by dominant inheritance, and arises from partial loss of ribosomal function, so it is possible that erythroid progenitors are more sensitive to this decreased function, while most other tissues are less affected.

Diagnosis

Typically, a diagnosis of DBA is made through a blood count and a bone marrow biopsy. A diagnosis of DBA is made on the basis of anemia, low reticulocyte (immature red blood cells) counts, and diminished erythroid precursors in bone marrow. Features that support a diagnosis of DBA include the presence of congenital abnormalities, macrocytosis, elevated fetal hemoglobin, and elevated adenosine deaminase levels in red blood cells. Most patients are diagnosed in the first two years of life. However, some mildly affected individuals only receive attention after a more severely affected family member is identified. About 20–25% of DBA patients may be identified with a genetic test for mutations in the RPS19 gene.

Treatment

Corticosteroids can be used to treat anemia in DBA. In a large study of 225 patients, 82% initially responded to this therapy, although many side effects were noted. Some patients remained responsive to steroids, while efficacy waned in others. Blood transfusions can also be used to treat severe anemia in DBA. Periods of remission may occur, during which transfusions and steroid treatments are not required. Bone marrow transplantation

(BMT) can cure hematological aspects of DBA. This option may be considered when patients become transfusion-dependent because frequent transfusions can lead to iron overloading and organ damage. However, adverse events from BMTs may exceed those from iron overloading. A 2007 study showed the efficacy of leucine and isoleucine supplementation in one patient. Larger studies are being conducted.

History

First noted by Hugh W. Josephs in 1936, the condition is however named for the pediatricians Louis K. Diamond and Kenneth Blackfan, who described congenital hypoplastic anemia in 1938. Responsiveness to corticosteroids was reported in 1951. In 1961, Diamond and colleagues presented longitudinal data on 30 patients and noted an association with skeletal abnormalities. In 1997, a region on chromosome 19 was determined to carry a gene mutated in some DBA. In 1999, mutations in the ribosomal protein S19 gene (RPS19) were found to be associated with disease in 42 of 172 DBA patients. In 2001, a second DBA gene was localized to a region of chromosome 8, and further genetic heterogeneity was inferred. Additional genes were subsequently identified.

Notable Cases

A girl named Audrey Nethery of Louisville, Kentucky has a large online following from her singing and dancing videos and has brought public attention to the very rare disease. “The tiny dancer’s zest for the feel-happy, cool move packed, music pumping workout (Zumba) has inspired millions of people to fall in love with her. Subsequently, all the unexpected attention on Audrey has given her family a great opportunity to raise much needed awareness and funds for Diamond Blackfan Anemia (DBA).”

MCFALL V. SHIMP

McFall v. Shimp, 10 Pa. D. & C. 3d 90 (July 26, 1978) was an Allegheny County, Pennsylvania, court case. The court ruled that it is unacceptable to force another person to donate body parts, even in a situation of medical necessity. Thirty-nine-year-old unmarried asbestos worker Robert McFall suffered “from a rare bone marrow disease” called aplastic anemia, where the patient’s bone marrow fails to manufacture certain necessary blood components. Without an urgent bone marrow transfusion, McFall would soon die. McFall’s first cousin, a 42-year-old crane worker named David Shimp, was the only available bone marrow match for McFall at the time, but Shimp refused to donate his bone marrow, which would have dramatically increased the odds of saving McFall’s life (with Shimp’s bone marrow donation, doctors estimated that McFall would have had a 50% to 60% chance of surviving). McFall then sued Shimp in order to force him to donate his bone marrow. When the case ended up in court, Judge John P. Flaherty Jr. stated that Shimp’s position was “morally indefensible,” but simultaneously refused to force Shimp to donate his bone marrow. Judge Flaherty also stated that forcing a person to submit to an intrusion of his body in order to donate bone marrow “would defeat the sanctity of the individual and would impose a rule which would know no limits, and one could not imagine where the line would be drawn.” McFall attempted to cite a 700-year-old statute of Edward I, pointing out that the court was a successor to the Court of Chancery, although the statute was not found to have any authority after a diligent search. Robert McFall died of a massive hemorrhage on August 10, 1978, about half a month after this court case was decided against him. Robert McFall’s sister, Beverly Hope, stated that McFall forgave Shimp near the very end of his life and asked his family to forgive Shimp for refusing to donate his bone marrow as well. David Shimp generally refused to talk with reporters, but he did state that his decision not to donate bone marrow was “common sense” in an interview with the Pittsburgh Press. In addition to being cited in analyses of tissue donation from a legal point of view, its ruling on the compelled use of the body of a non-consenting person to benefit another person has also been cited in legal analysis of the abortion debate and of women’s rights during pregnancy.

CONGENITAL AMEGAKARYOCYTIC THROMBOCYTOPENIA

Congenital amegakaryocytic thrombocytopenia (CAMT) is a rare inherited disorder.

Fig. Congenital amegakaryocytic thrombocytopenia.

PRESENTATION

The primary manifestations are thrombocytopenia and megakaryocytopenia, or low numbers of platelets and megakaryocytes. There is an absence of megakaryocytes in the bone marrow with no associated physical abnormalities.

CAUSE

The cause for this disorder appears to be a mutation in the gene for the TPO receptor, *c-mpl*, despite high levels of serum TPO. In addition, there may be abnormalities with the central nervous system including the cerebrum and cerebellum which could cause symptoms.

TREATMENT

The primary treatment for CAMT is bone marrow transplantation. Bone Marrow/Stem Cell Transplant is the only thing that ultimately cures this genetic disease. Frequent platelet transfusions are required to ensure that platelet levels do not fall to dangerous levels, although this is not always the case. It is known for patients to continue to create very small numbers of platelets over time.

DYSKERATOSIS CONGENITA

Dyskeratosis congenita (DKC), also called Zinsser-Cole-Engman syndrome, is a rare progressive congenital disorder with a highly variable phenotype. The entity was classically defined by the triad of abnormal skin pigmentation, nail dystrophy, and leukoplakia of the oral mucosa, but these components do not always occur. DKC is characterized by short telomeres. Some of the manifestations resemble premature aging (similar to

progeria). The disease initially mainly affects the skin, but a major consequence is progressive bone marrow failure which occurs in over 80%, causing early mortality.

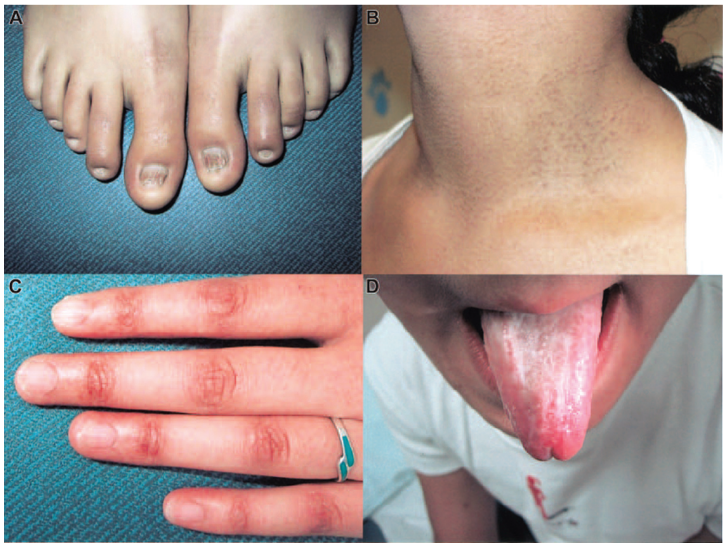


Fig. Dyskeratosis Congenita.

CHARACTERISTICS

DKC can be characterized by cutaneous pigmentation, premature graying, dystrophy of the nails, leukoplakia of the oral mucosa, continuous lacrimation due to atresia of the lacrimal ducts, often thrombocytopenia, anemia, testicular atrophy in the male carriers, and predisposition to cancer. Many of these symptoms are characteristic of geriatrics, and those carrying the more serious forms of the disease often have significantly shortened lifespans.

Clinical Features

- Age: The mucocutaneous features of DKC typically develop between ages 5 and 15 years. The median age of onset of the peripheral cytopenia is 10 years.
- Sex: The male-to-female ratio is approximately 3:1.
- Physical: The triad of reticulated hyperpigmentation of the skin, nail dystrophy, and leukoplakia characterizes DKC. The syndrome is clinically heterogeneous; in addition to the diagnostic mucocutaneous features and bone marrow failure, affected individuals can have a variety of other clinical features.
- Cutaneous findings: The primary finding is abnormal skin pigmentation, with tan-to-gray hyperpigmented or hypopigmented macules and patches in a mottled or reticulated pattern. Reticulated pigmentation occurs in approximately 90% of patients. Poikilodermatous changes with atrophy and telangiectasia are common. The cutaneous presentation may clinically and histologically resemble graft versus host disease. The typical distribution involves the sun-exposed areas, including the upper trunk, neck, and face. Other cutaneous findings may include alopecia of the scalp, eyebrows, and eyelashes; premature graying of the hair; hyperhidrosis; hyperkeratosis of the palms and soles; and adermatoglyphia (loss of dermal ridges on fingers and toes).
- Nail findings: Nail dystrophy is seen in approximately 90% of patients, with fingernail involvement often preceding toenail involvement. Progressive nail dystrophy begins with ridging and longitudinal splitting. Progressive atrophy, thinning, pterygium, and distortion eventuate in small, rudimentary, or absent nails.

- Mucosal findings: Mucosal leukoplakia occurs in approximately 80% of patients and typically involves the buccal mucosa, tongue, and oropharynx. The leukoplakia may become verrucous, and ulceration may occur. Patients also may have an increased prevalence and severity of periodontal disease.
- Other mucosal sites may be involved (*e.g.,* esophagus, urethral meatus, glans penis, lacrimal duct, conjunctiva, vagina, anus). Constriction and stenosis can occur at these sites, with subsequent development of dysphagia, dysuria, phimosis, and epiphora.
- Bone marrow failure: Approximately 90% have peripheral cytopenia of one or more lineages. In some cases, this is the initial presentation, with a median age of onset of 10 years. Bone marrow failure is a major cause of death, with approximately 70% of deaths related to bleeding and opportunistic infections as a result of bone marrow failure.
- Pulmonary complications: Approximately 20% of individuals with DKC develop pulmonary complications, including pulmonary fibrosis and abnormalities of pulmonary vasculature. The recommendation is that DKC patients avoid taking drugs with pulmonary toxicity (*e.g.,* busulfan) and that they have their lungs shielded from radiation during bone marrow transplant(BMT).
- Increased risk of malignancy: Patients have an increased prevalence of malignant mucosal neoplasms, particularly squamous cell carcinoma of the mouth, nasopharynx, esophagus, rectum, vagina, or cervix. These often occur within sites of leukoplakia. The prevalence of squamous cell carcinoma of the skin is also increased. Other malignancies reported include Hodgkin lymphoma, adenocarcinoma of the gastrointestinal tract, and bronchial and laryngeal carcinoma. Malignancy tends to develop in the third decade of life.
- Neurologic system findings: Patients may have learning difficulties and mental retardation.
- Ophthalmic system findings: DKC reportedly is associated with conjunctivitis, blepharitides, and pterygium. Lacrimal duct stenosis resulting in epiphora (*i.e.,* excessive tearing) occurs in approximately 80% of patients.
- Skeletal system findings: Patients may have mandibular hypoplasia, osteoporosis, avascular necrosis, and scoliosis.
- Gastrointestinal system findings: These may include esophageal webs, hepatosplenomegaly, enteropathy, and cirrhosis.
- Genitourinary system findings:: Hypospastic testes, hypospadias, and ureteral stenosis are reported.
- Female carriers: Female carriers of DKC may have subtle clinical features. One study showed that 3 of 20 female carriers had clinical features that included a single dystrophic nail, a patch of hypopigmentation, or mild leukoplakia.

PATHOPHYSIOLOGY

Dyskeratosis congenita is a disorder of poor telomere maintenance mainly due to a number of gene mutations that give rise to abnormal ribosome function, termed ribosomopathy. Specifically, the disease is related to one or more mutations which directly or indirectly affect the vertebrate telomerase RNA component (TERC). Telomerase is a reverse transcriptase which maintains a specific repeat sequence of DNA, the telomere, during development.

Telomeres are placed by telomerase on both ends of linear chromosomes as a way to protect linear DNA from general forms of chemical damage and to correct for the chromosomal end-shortening that occurs during normal DNA replication. This end-shortening is the result of the eukaryotic DNA polymerases having no mechanism for synthesizing the final nucleotides present on the end of the "lagging strand" of double stranded DNA. DNA polymerase can only synthesize new DNA from an old DNA strand in the 5'→3' direction. Given that DNA has two strands that are complementary, one strand must be 5'→3' while the other is 3'→5'. This

inability to synthesize in the 3'→5' directionality is compensated with the use of Okazaki fragments, short pieces of DNA that are synthesized 5'→3' from the 3'→5' as the replication fork moves. As DNA polymerase requires RNA primers for DNA binding in order to commence replication, each Okazaki fragment is thus preceded by an RNA primer on the strand being synthesized. When the end of the chromosome is reached, the final RNA primer is placed upon this nucleotide region, and it is inevitably removed. Unfortunately once the primer is removed, DNA polymerase is unable to synthesize the remaining bases.

Sufferers of DKC have been shown to have a reduction in TERC levels invariably affecting the normal function of telomerase which maintains these telomeres. With TERC levels down, telomere maintenance during development suffers accordingly. In humans, telomerase is inactive in most cell types after early development (except in extreme cases such as cancer). Thus, if telomerase is not able to efficiently affect the DNA in the beginning of life, chromosomal instability becomes a grave possibility in individuals much earlier than would be expected. A study shows that proliferative defects in DC skin keratinocytes are corrected by expression of the telomerase reverse transcriptase, TERT, or by activation of endogenous telomerase through expression of papillomavirus E6/E7 of the telomerase RNA component, TERC.

GENETICS

Of the components of the telomerase RNA component (TERC), one of key importance is the box H/ACA domain. This H/ACA domain is responsible for maturation and stability of TERC and therefore of telomerase as a whole. The mammalian H/ACA ribonucleoprotein contains four protein subunits: dyskerin, Gar1, Nop10, and Nhp2. Mutations in Nop10, Nhp2 and dyskerin1 have all been shown to lead to DKC-like symptoms.

X-linked

The best characterized form of dyskeratosis congenita is a result of one or more mutations in the long arm of the X chromosome in the gene DKC1. This results in the X-linked recessive form of the disease wherein the major protein affected is dyskerin. Of the five mutations described by Heiss and colleagues in *Nature Genetics*, four were single nucleotide polymorphisms all resulting in the change of highly conserved amino acids. One case was an in-frame deletion resulting in the loss of a leucine residue, also conserved in mammals. In three of the cases, the specific amino acids affected (phenylalanine, proline, glycine) are found in the same locus in humans as they are in yeast (*S. Cerevisiae*) and the brown rat (*R. Norvegicus*). This establishes the sequence conservation and importance of dyskerin within the eukaryotes.

The relevant nature of dyskerin throughout most species is to catalyze the post-transcriptional pseudouridylation of specific uridines found in non-coding RNAs, such as ribosomal RNA (rRNA). Cbf5, the yeast analog of human dyskerin, is indeed known to be associated with the processing and maturation of rRNA. In humans this role can be attributed to dyskerin. Thus, the X-linked form of this disease may result in specific issues related to dysfunctional rRNA and perhaps a graver phenotype. Within the vertebrates, as opposed to single celled eukaryotes, dyskerin is a key component of the telomerase RNA component (TERC) in the form of the H/ACA motif. This X-linked variety, like the Nop10 and Nhp2 mutations, demonstrates shortened telomeres as a result of lower TERC concentrations.

Autosomal Dominant

Genes: TERC, TERT, TINF2 The evidence supporting the importance of the H/ACA domain in human telomerase is abundant. At least one study has shown that these mutations affect telomerase activity by negatively affecting pre-RNP assembly and maturation of human telomerase RNA. Nonetheless, mutations which directly affect the telomerase RNA components would presumably exist and should also cause premature aging or DKC-like symptoms.

Indeed, three families with mutations in the human TERC gene have been studied with intriguing results. In two of these families, two family-specific single nucleotide polymorphisms were present while in the other there persisted a large-scale deletion (821 base pairs of DNA) on chromosome 3 which includes 74 bases coding for a section of the H/ACA domain. These three different mutations result in a mild form of dyskeratosis congenita which uniquely follows an autosomal dominant pattern of inheritance. Premature graying, early dental loss, predisposition to skin cancer, as well as shortening of telomere length continue to be characteristic of this disease.

Autosomal Recessive

Genes: The true phenotype of DKC individuals may depend upon which protein has incurred a mutation. One documented autosomal recessive mutation in a family that carries DKC has been found in Nop10. Specifically, the mutation is a change of base from cytosine to thymine in a highly conserved region of the Nop10 sequence. This mutation, on chromosome 15, results in an amino acid change from arginine to tryptophan. Homozygous recessive individuals show the symptoms of dyskeratosis congenita in full. As compared to age-matched normal individuals, those suffering from DKC have telomeres of a much shorter length. Furthermore, heterozygotes, those who have one normal allele and one coding for the disease, also show relatively shortened telomeres. The cause of this was determined to be a reduction in TERC levels in those with the Nop10 mutation. With TERC levels down, telomere maintenance, especially in development, would be presumed to suffer accordingly. This would lead to the telomere shortening described. Nhp2 mutations are similar in characterization to Nop10. These mutations are also autosomal recessive with three specific single-nucleotide polymorphisms being recognized which result in dyskeratosis congenita. Also like Nop10, individuals with these Nhp2 mutations have a reduction in the amount of telomerase RNA component (TERC) present in the cell. Again it can be presumed that a reduction in TERC results in aberrant telomere maintenance and thus shortened telomeres. Those homozygous recessive for mutations in Nhp2 do show shorter telomeres when compared with age-matched normal individuals.

PREDISPOSITION TO CANCER

Susceptibility to cancer seems counterintuitive because in many known cancers reactivation of telomerase is actually a required step for malignancy to evolve. In a disease where telomerase is affected, it does not seem to follow that cancer would be a complication to result. The authors note the paradoxical nature of cancer predisposition in individuals who seem to lack one of the required components for cancer to form. It is thought that without functional telomerase, chromosomes will likely be attached together at their ends through the non-homologous end joining pathway. If this proves to be a common enough occurrence, malignancy even without telomerase present is possible.

RESEARCH

Recent research has used induced pluripotent stem cells to study disease mechanisms in humans, and discovered that the reprogramming of somatic cells restores telomere elongation in dyskeratosis congenita (DKC) cells despite the genetic lesions that affect telomerase. The reprogrammed DKC cells were able to overcome a critical limitation in TERC levels and restored function (telomere maintenance and self-renewal). Therapeutically, methods aimed at increasing TERC expression could prove beneficial in DKC.

FANCONI ANEMIA

Fanconi anaemia (FA) is a rare genetic disease resulting in impaired response to DNA damage. Although it is a very rare disorder, study of this and other bone marrow failure syndromes has improved scientific understanding

of the mechanisms of normal bone marrow function and development of cancer. Among those affected, the majority 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 people have congenital defects, commonly short stature, abnormalities of the skin, arms, head, eyes, kidneys, and ears, and developmental disabilities. Around 75% of people have some form of endocrine problems, with varying degrees of severity. FA is the result of a genetic defect in a cluster of proteins responsible for DNA repair via homologous recombination. 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 crosslinking, such as mitomycin C.

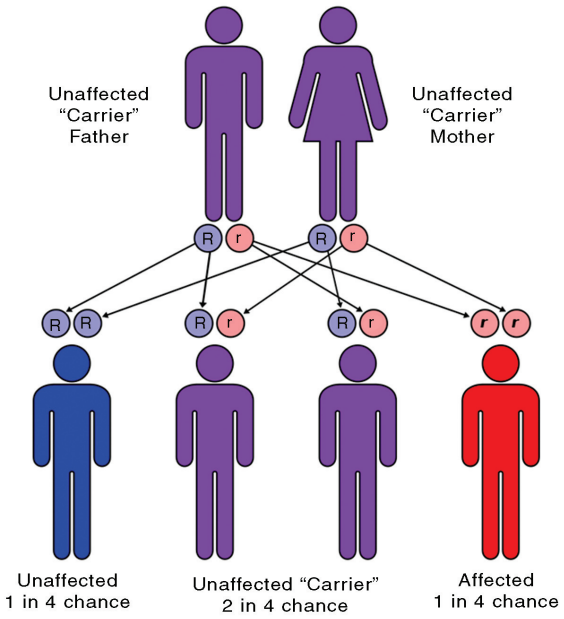


Fig. Fanconi anemia.

The typical age of death was 30 years in 2000. FA occurs in about one per 130,000 births, with a higher frequency in Ashkenazi Jews in Israel and Afrikaners in South Africa. 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.

SIGNS AND SYMPTOMS

FA is characterized by bone marrow failure, AML, solid tumors, and developmental abnormalities. Classic features include abnormal thumbs, absent radii, short stature, skin hyperpigmentation, including café au lait spots, abnormal facial features (triangular face, microcephaly), abnormal kidneys, and decreased fertility. Many FA patients (about 30%) do not have any of the classic physical findings, but Diepoxybutane chromosome fragility assay showing increased chromosomal breaks can make the diagnosis. About 80% of FA will develop bone marrow failure by age 20. The first sign of a hematologic problem is usually petechiae and bruises, with later onset of pale appearance, feeling tired, and infections. Because macrocytosis usually precedes a low platelet count, patients with typical congenital anomalies associated with FA should be evaluated for an elevated red blood cell mean corpuscular volume.

GENETICS

FA is primarily an autosomal recessive genetic disorder. This means that two mutated alleles (one from each parent) are required to cause the disease. The risk is 25% that each subsequent child will have FA. About 2% of

FA cases are X-linked recessive, which means that if the mother carries one mutated Fanconi anemia allele on one X chromosome, a 50% chance exists that male offspring will present with Fanconi anemia.

Scientists have identified 17 FA or FA-like genes: *FANCA, FANCB, FANCC, FANCD1 (BRCA2), FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ (BRIP1), FANCL, FANCM, FANCN (PALB2), FANCP (SLX4), FANCS (BRCA1), RAD51C* and *XPF*. *FANCB* is the one exception to FA being autosomal recessive, as this gene is on the X chromosome. These genes are involved in DNA repair. The carrier frequency in the Ashkenazi Jewish population is about one in 90. Genetic counseling and genetic testing are recommended for families who may be carriers of Fanconi anemia. Because of the failure of hematologic components to develop—white blood cells, red blood cells, and platelets—the body's capabilities to fight infection, deliver oxygen, and form clots are all diminished.

PATHOGENESIS

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. However, a few cases have occurred 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/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. This is in contrast to Diamond–Blackfan anemia, which affects only erythrocytes, and Shwachman–Diamond syndrome, which primarily causes neutropenia. 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 DNA repair, specifically homologous recombination, and given the current knowledge about dynamic cell division in the bone marrow, finding patients are more likely to develop bone marrow failure, myelodysplastic syndromes, and acute myeloid leukemia (AML) is not surprising.

Myelodysplastic Syndromes

MDSs, 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, blast cells, is always less than 20%, with 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 (unless prompt allogeneic hematopoietic progenitor cell transplant is an option).

Acute Myeloid Leukemia

FA patients are at elevated risk for the development of 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. Many MDS patients' diseases evolve into AML if they survive long enough. Furthermore, the risk of developing AML increases with the onset of bone-marrow failure. Although 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. Historically, even with a marrow transplant, about a quarter of FA patients diagnosed with MDS/ALS have died from MDS/ALS-related causes within two years, although more recent published evidence suggests that earlier allogeneic hematopoietic progenitor cell transplantation in children with FA is leading to better outcomes over time.

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 radiation or immunosuppressants, thus increased 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, two-year probability of survival can be as high as 89%. However, if the transplant is performed at ages older than 10, two-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

There are 19 genes responsible for FA, one of them being 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 (such as mitomycin C or cisplatin) or reactive oxygen species (ROS) that is detected by the FANCM protein. 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.

Spermatogenesis

In humans, infertility is one of the characteristics of individuals with mutational defects in the FANC genes. In mice, spermatogonia, preleptotene spermatocytes, and spermatocytes in the meiotic stages of leptotene, zygotene and early pachytene are enriched for FANC proteins. This finding suggests that recombinational repair processes mediated by the FANC proteins are active during germ cell development, particularly during meiosis, and that defects in this activity can lead to infertility.

Neural Stem Cell Homeostasis

Microphthalmia and microcephaly are frequent congenital defects in FA patients. The loss of FANCA and FANCG in mice causes neural progenitor apoptosis both during early developmental neurogenesis and later during adult neurogenesis. This leads to depletion of the neural stem cell pool with aging. Much of the Fanconi anemia phenotype might be interpreted as a reflection of premature aging of stem cells.

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 donors exist, a savior sibling can be conceived by preimplantation genetic diagnosis (PGD) to match the recipient's HLA type.

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. A significant number of Fanconi patients have kidney problems, trouble with their eyes, developmental retardation and other serious defects, such as microcephaly (small head).

MYELODYSPLASTIC SYNDROME

Myelodysplastic syndromes (MDS) are a group of cancers in which immature blood cells in the bone marrow do not mature and therefore do not become healthy blood cells. Early on there are typically no symptoms. Later symptoms may include feeling tired, shortness of breath, easy bleeding, or frequent infections. Some types may develop into acute myeloid leukemia. Risk factors include previous chemotherapy or radiation therapy, exposure to certain chemicals such as tobacco smoke, pesticides, and benzene, and exposure to heavy metals such as mercury or lead. Problems with blood cell formation result in some combination of low red blood cells, low

platelets, and low white blood cells. Some types have an increase in immature blood cells, called blasts, in the bone marrow or blood. The types of MDS are based on specific changes in the blood cells and bone marrow.

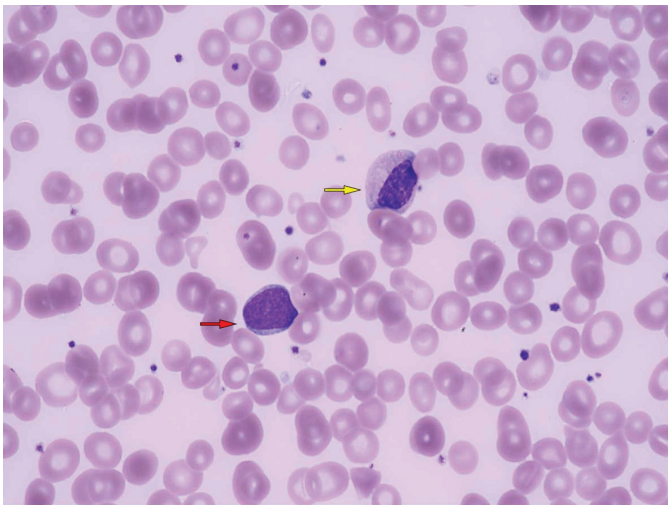


Fig. Myelodysplastic syndrome.

Treatments may include supportive care, drug therapy, and stem cell transplantation. Supportive care may include blood transfusions, medications to increase the making of red blood cells, and antibiotics. Drug therapy may include the medication lenalidomide, antithymocyte globulin, and azacitidine. Certain people can be cured with chemotherapy followed by a stem-cell transplant from a donor. Abou t seven per 100,000 people are affected with about four per 100,000 people newly acquiring the condition each year. The typical age of onset is 70 years. The outlook depends on the type of cells affected, the number of blasts in the bone marrow or blood, and the changes present in the chromosomes of the affected cells. The typical survival rate following diagnosis is 2.5 years. The conditions were first recognized in the early 1900s. The current name came into use in 1976.

SIGNS AND SYMPTOMS

Signs and symptoms are nonspecific and generally related to the blood cytopenias:

- Anemia (low RBC count or reduced hemoglobin) – chronic tiredness, shortness of breath, chilled sensation, sometimes chest pain
- Neutropenia (low neutrophil count) – increased susceptibility to infection
- Thrombocytopenia (low platelet count) – increased susceptibility to bleeding and ecchymosis (bruising), as well as subcutaneous hemorrhaging resulting in purpura or petechiae.

Many individuals are asymptomatic, and blood cytopenia or other problems are identified as a part of a routine blood count:

- Neutropenia, anemia, and thrombocytopenia
- Splenomegaly or rarely hepatomegaly
- Abnormal granules in cells, abnormal nuclear shape and size
- Chromosome abnormality, including chromosomal translocations and abnormal chromosome number.

Although some risk exists for developing acute myelogenous leukemia, about 50% of deaths occur as a result of bleeding or infection. However, leukemia that occurs as a result of myelodysplasia is notoriously resistant to treatment. Anemia dominates the early course. Most symptomatic patients complain of the gradual onset of fatigue and weakness, dyspnea, and pallor, but at least half the patients are asymptomatic and their MDS is discovered only incidentally on routine blood counts. Previous chemotherapy or radiation exposure is an important fact in the person's medical history. Fever and weight loss should point to a myeloproliferative rather than myelodysplastic process.

CAUSE

Some people have a history of exposure to chemotherapy (especially alkylating agents such as melphalan, cyclophosphamide, busulfan, and chlorambucil) or radiation (therapeutic or accidental), or both (*e.g.,* at the time of stem cell transplantation for another disease). Workers in some industries with heavy exposure to hydrocarbons such as the petroleum industry have a slightly higher risk of contracting the disease than the general population. Xylene and benzene exposure has been associated with myelodysplasia. Vietnam veterans exposed to Agent Orange are at risk of developing MDS. A link may exist between the development of MDS "in atomic-bomb survivors 40 to 60 years after radiation exposure" (in this case, referring to people who were in close proximity to the dropping of the atomic bomb in Hiroshima and Nagasaki during World War II). Children with Down syndrome are susceptible to MDS, and a family history may indicate a hereditary form of sideroblastic anemia or Fanconi anemia.

PATHOPHYSIOLOGY

MDS most often develops without an identifiable cause. Risk factors include exposure to an agent known to cause DNA damage, like radiation, benzene, and certain chemotherapies; other risk factors have been inconsistently reported. It can be difficult to prove a connection between a suspected exposure and the development of MDS, but the presence of genetic abnormalities may provide some supportive information. Secondary MDS can occur as a late toxicity of cancer therapy (therapy associated MDS, t-MDS). MDS after exposure to radiation or Alkylating agents such as busulfan, nitrosourea, or procarbazine, typically occurs 3-7 years after exposure and frequently demonstrates loss of chromosome 5 or 7. MDS after exposure to DNA topoisomerase II inhibitors occurs after a shorter latency of only 1-3 years and can have a 11q23 translocation. Other pre-existing bone marrow disorders like acquired aplastic anemia following immunosuppressive treatment and Fanconi anemia can evolve into MDS.

MDS is thought to arise from mutations in the multipotent bone marrow stem cell, but the specific defects responsible for these diseases remain poorly understood. Differentiation of blood precursor cells is impaired, and a significant increase in levels of apoptotic cell death occurs in bone marrow cells. Clonal expansion of the abnormal cells results in the production of cells which have lost the ability to differentiate. If the overall percentage of bone marrow myeloblasts rises over a particular cutoff (20% for WHO and 30% for FAB), then transformation to acute myelogenous leukemia (AML) is said to have occurred. The progression of MDS to AML is a good example of the multistep theory of carcinogenesis in which a series of mutations occurs in an initially normal cell and transforms it into a cancer cell. While recognition of leukemic transformation was historically important, a significant proportion of the morbidity and mortality attributable to MDS results not from transformation to AML, but rather from the cytopenias seen in all MDS patients. While anemia is the most common cytopenia in MDS patients, given the ready availability of blood transfusion, MDS patients rarely suffer injury from severe anemia. The two most serious complications in MDS patients resulting from their cytopenias are bleeding (due to lack of platelets) or infection (due to lack of white blood cells). Long-term transfusion of packed red blood cells leads to iron overload.

Genetics

The recognition of epigenetic changes in DNA structure in MDS has explained the success of two (namely the hypomethylating agents 5-azacytidine and decitabine) of three (the third is lenalidomide) commercially available medications approved by the U.S. Food and Drug Administration to treat MDS. Proper DNA methylation is critical in the regulation of proliferation genes, and the loss of DNA methylation control can lead to uncontrolled cell growth and cytopenias. The recently approved DNA methyltransferase inhibitors take advantage of this

mechanism by creating a more orderly DNA methylation profile in the hematopoietic stem cell nucleus, and thereby restoring normal blood counts and retarding the progression of MDS to acute leukemia. Some authors have proposed that the loss of mitochondrial function over time leads to the accumulation of DNA mutations in hematopoietic stem cells, and this accounts for the increased incidence of MDS in older patients. Researchers point to the accumulation of mitochondrial iron deposits in the ringed sideroblast as evidence of mitochondrial dysfunction in MDS.

5q- Syndrome

Since at least 1974, the deletion in the long arm of chromosome 5 has been known to be associated with dysplastic abnormalities of hematopoietic stem cells. By 2005, lenalidomide, a chemotherapy drug, was recognized to be effective in MDS patients with the 5q- syndrome, and in December 2005, the US FDA approved the drug for this indication. Patients with isolated 5q-, low IPSS risk, and transfusion dependence respond best to lenalidomide. Typically, prognosis for these patients is favourable, with a 63-month median survival. Lenalidomide has dual action, by lowering the malignant clone number in patients with 5q-, and by inducing better differentiation of healthy erythroid cells, as seen in patients without 5q deletion.

Splicing Factor Mutations

Mutations in splicing factors have been found in 40-80% of cases with myelodysplastic syndrome, particularly in those with ringed sideroblasts.

IDH1 *and* IDH2 *Mutations*

Mutations in the genes encoding for isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) occur in 10-20% of patients with myelodysplastic syndrome, and confer a worsened prognosis in low-risk MDS. Because the incidence of *IDH1/2* mutations increases as the disease malignancy increases, these findings together suggest that *IDH1/2* mutations are important drivers of progression of MDS to a more malignant disease state.

DIAGNOSIS

The elimination of other causes of cytopenias, along with a dysplastic bone marrow, is required to diagnose a myelodysplastic syndrome, so differentiating MDS from anemia, thrombocytopenia, and leukopenia is important.

A typical diagnostic investigation includes:

- Full blood count and examination of blood film: The blood film morphology can provide clues about hemolytic anemia, clumping of the platelets leading to spurious thrombocytopenia, or leukemia.
- Blood tests to eliminate other common causes of cytopenias, such as lupus, hepatitis, B_{12}, folate, or other vitamin deficiencies, renal failure or heart failure, HIV, hemolytic anemia, monoclonal gammopathy: Age-appropriate cancer screening should be considered for all anemic patients.
- Bone marrow examination by a hematopathologist: This is required to establish the diagnosis, since all hematopathologists consider dysplastic marrow the key feature of myelodysplasia.
- Cytogenetics or chromosomal studies: This is ideally performed on the bone marrow aspirate. Conventional cytogenetics require a fresh specimen, since live cells are induced to enter metaphase to allow chromosomes to be seen.
- Interphase fluorescence in situ hybridization testing, usually ordered together with conventional cytogenetic testing, offers rapid detection of several chromosome abnormalities associated with MDS, including del 5q, -7, +8, and del 20q.

- Virtual karyotyping can be done for MDS, which uses computational tools to construct the karyogram from disrupted DNA. Virtual karyotyping does not require cell culture and has dramatically higher resolution than conventional cytogenetics, but cannot detect balanced translocations.
- Flow cytometry is helpful to establish the presence of any lymphoproliferative disorder in the marrow.
- Testing for copper deficiency should not be overlooked, as it can morphologically resemble MDS in bone marrow biopsies.

The features generally used to define a MDS are blood cytopenias, ineffective hematopoiesis, dyserythropoiesis, dysgranulopoiesis, dysmegakaropoiesis, and increased myeloblasts. Dysplasia can affect all three lineages seen in the bone marrow. The best way to diagnose dysplasia is by morphology and special stains (PAS) used on the bone marrow aspirate and peripheral blood smear. Dysplasia in the myeloid series is defined by:

- Granulocytic series:
  1. Hypersegmented neutrophils (also seen in vit B_{12}/folate deficiency)
  2. Hyposegmented neutrophils (pseudo Pelger-Huet)
  3. Hypogranular neutrophils or pseudo Chediak-Higashi (large azurophilic granules)
  4. Auer rods - automatically RAEB II (if blast count < 5% in the peripheral blood and < 10% in the bone marrow aspirate); also note Auer rods may be seen in mature neutrophils in AML with translocation t(8;21)
  5. Dimorphic granules (basophilic and eosinophilic granules) within eosinophils
- Erythroid series:
  1. Binucleated erythroid percursors and karyorrhexis
  2. Erythroid nuclear budding
  3. Erythroid nuclear strings or internuclear bridging (also seen in congenital dyserythropoietic anemias)
  4. Loss of e-cadherin in normoblasts is a sign of aberrancy.
  5. Periodic acid-Schiff (PAS) (globular in vacuoles or diffuse cytoplasmic staining) within erythroid precursors in the bone marrow aspirate (has no bearing on paraffin-fixed bone-marrow biopsy). Note: one can see PAS vacuolar positivity in L1 and L2 blasts (FAB classification; the L1 and L2 nomenclature is not used in the WHO classification)
  6. Ringed sideroblasts (10 or more iron granules encircling one-third or more of the nucleus) seen on Prussian blue iron stain (>15% ringed sideroblasts when counted among red cell precursors for refractory anemia with ring sideroblasts)
- Megakaryocytic series (can be the most subjective):
  1. Hyposegmented nuclear features in platelet producing megakaryocytes (lack of lobation)
  2. Hypersegmented (osteoclastic appearing) megakaryocytes
  3. Ballooning of the platelets.

Other stains can help in special cases (PAS and napthol ASD chloroacetate esterase positivity) in eosinophils is a marker of abnormality seen in chronic eosinophilic leukemia and is a sign of aberrancy. On the bone marrow biopsy, high-grade dysplasia (RAEB-I and RAEB-II) may show atypical localization of immature precursors which are islands of immature precursors cells (myeloblasts and promyelocytes) localized to the center of the intertrabecular space rather than adjacent to the trabeculae or surrounding arterioles. This morphology can be difficult to differentiate from treated leukemia and recovering immature normal marrow elements. Also topographic alteration of the nucleated erythroid cells can be seen in early myelodysplasia (RA and RARS), where normoblasts are seen next to bony trabeculae instead of forming normal interstitially placed erythroid islands.

Differential Diagnosis

3Myelodysplasia is a diagnosis of exclusion and must be made after proper determination of iron stores, vitamin deficiencies, and nutrient deficiencies are ruled out. Also, congenital diseases such as congenital

dyserythropoietic anemia (CDA I through IV) have been recognized, Pearson's syndrome (sideroblastic anemia), Jordans anomaly - vacuolization in all cell lines may be seen in Chanarin-Dorfman syndrome, aminolevulinic acid enzyme deficiency, and other more esoteric enzyme deficiencies are known to give a pseudomyelodysplastic picture in one of the cell lines; however, all three cell lines are never morphologically dysplastic in these entities with the exception of chloramphenicol, arsenic toxicity, and other poisons.

All of these conditions are characterized by abnormalities in the production of one or more of the cellular components of blood (red cells, white cells other than lymphocytes, and platelets or their progenitor cells, megakaryocytes).

Classification

French-American-British (FAB) Classification

In 1974 and 1975, a group of pathologists from France, the US, and Britain produced the first widely used classification of these diseases. This French-American-British classification was published in 1976, and revised in 1982. It was used by pathologists and clinicians for almost 20 years. Cases were classified into five categories:

ICD-O	Name	Description
M9980/3	Refractory anemia (RA)	characterized by less than 5% primitive blood cells (myeloblasts) in the bone marrow and pathological abnormalities primarily seen in red cell precursors
M9982/3	Refractory anemia with ring sideroblasts (RARS)	also characterized by less than 5% myeloblasts in the bone marrow, but distinguished by the presence of 15% or greater of red cell precursors in the marrow being abnormal iron-stuffed cells called "ringed sideroblasts"
M9983/3	Refractory anemia with excess blasts (RAEB)	characterized by 5-19% myeloblasts in the marrow
M9984/3	Refractory anemia with excess blasts in transformation (RAEB-T)	characterized by 5%-19% myeloblasts in the marrow (>20% blasts is defined as acute myeloid leukemia)
M9945/3	Chronic myelomonocytic leukemia (CMML), not to be confused with chronic myelogenous leukemia or CML	characterized by less than 20% myeloblasts in the bone marrow and greater than 1*10/L monocytes (a type of white blood cell) circulating in the peripheral blood.

The best prognosis is seen with RA and RARS, where some nontransplant patients live more than a decade (the average is on the order of three to five years, although long-term remission is possible if a bone marrow transplant is successful). The worst outlook is with RAEB-T, where the mean life expectancy is less than 1 year. About one-quarter of patients develop overt leukemia. The others die of complications of low blood count or unrelated disease. The International Prognostic Scoring System is another tool for determining the prognosis of MDS, published in *Blood* in 1997. This system takes into account the percentage of blasts in the marrow, cytogenetics, and number of cytopenias.

World Health Organization

In the late 1990s, a group of pathologists and clinicians working under the World Health Organization (WHO) modified this classification, introducing several new disease categories and eliminating others. Most recently, the WHO has evolved a new classification scheme (2008) which is based more on genetic findings. However, morphology of the cells in the peripheral blood, bone marrow aspirate, and bone marrow biopsy are still the screening tests used to decide which classification is best and which cytogenetic aberrations may be related.

The list of dysplastic syndromes under the new WHO system includes:

Old System	New System
Refractory anemia (RA)	Refractory cytopenia with unilineage dysplasia (Refractory anemia, Refractory neutropenia, and Refractory thrombocytopenia)
Refractory anemia with ringed sideroblasts (RARS)	Refractory anemia with ring sideroblasts (RARS) Refractory anemia with ring sideroblasts - thrombocytosis (RARS-t) (provisional entity) which is in essence a myelodysplastic/ myeloproliferative disorder and usually has a JAK2 mutation (janus kinase) - New WHO classification 2008 Refractory cytopenia with multilineage dysplasia (RCMD) includes the subset Refractory cytopenia with multilineage dysplasia and ring sideroblasts (RCMD-RS). RCMD includes patients with pathological changes not restricted to red cells (*i.e.,* prominent white cell precursor and platelet precursor (megakaryocyte) dysplasia.
Refractory anemia with excess blasts (RAEB)	Refractory anemias with excess blasts I and II. RAEB was divided into RAEB-I (5-9% blasts) and RAEB-II (10-19%) blasts, which has a poorer prognosis than RAEB-I. Auer rods may be seen in RAEB-II which may be difficult to distinguish from acute myeloid leukemia.
Refractory anemia with excess blasts in transformation (RAEB-T)	This category was eliminated; such patients are now considered to have acute leukemia. 5q- syndrome, typically seen in older women with normal or high platelet counts and isolated deletions of the long arm of chromosome 5 in bone marrow cells, was added to the classification.
Chronic myelomonocytic leukemia (CMML)	CMML was removed from the myelodysplastic syndromes and put in a new category of myelodysplastic-myeloproliferative overlap syndromes. Myelodysplasia unclassifiable (seen in those cases of megakaryocyte dysplasia with fibrosis and others) Refractory cytopenia of childhood (dysplasia in childhood) - New in WHO classification 2008.

Note: Not all physicians concur with this reclassification, because the underlying pathology of this diseases is not well understood.

Myelodysplastic Syndrome Unclassified

The WHO has proposed a criterion for diagnosis and classification of MDS that may apply to most cases. However, occasional cases are difficult to classify into defined categories because of one or more unusual features:

- Rare cases with less than 5% blast will present with Auer rods. These cases usually have the features of RAMD.
- Occasionally, cases of MDS present with isolated neutropenia or thrombocytopenia without anemia and with dysplastic changes confined to the single lineage. The term refractory neutropenia and refractory thrombocytopenia have sometimes been used to describe these cases. A diagnosis of MDS in patients with neutropenia or thrombocytopenia without anemia should be made with caution.
- Patients with RA or RAEB occasionally present with leukocytosis or thrombocytosis instead of the usual cytopenia.

MANAGEMENT

The goals of therapy are to control symptoms, improve quality of life, improve overall survival, and decrease progression to AML. The IPSS scoring system can help triage patients for more aggressive treatment (*i.e.,* bone marrow transplant) as well as help determine the best timing of this therapy. Supportive care with blood products and hematopoietic growth factors (*e.g.,* erythropoietin) is the mainstay of therapy. The regulatory environment

for the use of erythropoietins is evolving, according to a recent US Medicare National coverage determination. No comment on the use of hematopoeitic growth factors for MDS was made in that document though.

Three agents have been approved by the FDA for the treatment of MDS:

1. 5-azacytidine: 21-month median survival
2. Decitabine: Complete response rate reported as high as 43%. A phase I study has shown efficacy in AML when decitabine is combined with valproic acid.
3. Lenalidomide: Effective in reducing red blood cell transfusion requirement in patients with the chromosome 5q deletion subtype of MDS.

Chemotherapy with the hypomethylating agents 5-azacytidine and decitabine has been shown to decrease blood transfusion requirements and to retard the progression of MDS to AML. Lenalidomide was approved by the FDA in December 2005 only for use in the 5q- syndrome. In the United States, treatment of MDS with lenalidomide costs about $9,200 per month. Stem cell transplantation, particularly in younger (*i.e.,* less than 40 years of age) and more severely affected patients, offers the potential for curative therapy. Success of bone marrow transplantation has been found to correlate with severity of MDS as determined by the IPSS score, with patients having a more favourable IPSS score tending to have a more favourable outcome with transplantation.

Iron Levels

Iron overload can develop in MDS as a result of the RBC transfusions which are a major part of the supportive care for anemic MDS patients. Although the specific therapies patients receive may alleviate the RBC transfusion need in some cases, many MDS patients may not respond to these treatments, thus may develop iron overload from repeated RBC transfusions. Patients requiring relatively large numbers of RBC transfusions can experience the adverse effect of chronic iron overload on their liver, heart, and endocrine functions. The resulting organ dysfunction from transfusional iron overload might be a contributor to increased illness and death in early-stage MDS. For patients requiring many RBC transfusions, serum ferritin levels, number of RBC transfusions received, and associated organ dysfunction (heart, liver, and pancreas) should be monitored to determine iron levels.

Monitoring serum ferritin may also be useful, aiming to decrease ferritin levels to < 1000 µg/l. Currently, two iron chelators are available in the US, deferoxamine for intravenous use and deferasirox for oral use. These options now provide potentially useful drugs for treating this iron overload problem. A third chelating agent is available in Europe, deferiprone for oral use, but not available in the US. Clinical trials in the MDS are ongoing with iron chelating agents to address the question of whether iron chelation alters the natural history of patients with MDS who are transfusion dependent. Reversal of some of the consequences of iron overload in MDS by iron chelation therapy have been shown. Both the MDS Foundation and the National Comprehensive Cancer Network MDS Guidelines Panel have recommended that chelation therapy be considered to decrease iron overload in selected MDS patients. Evidence also suggests a potential value exists to iron chelation in patients who will undergo a stem cell transplant. Although deferasirox is generally well tolerated (other than episodes of gastrointestinal distress and kidney dysfunction in some patients), recently a safety warning by the FDA and Novartis was added to deferasirox treatment guidelines. Following postmarketing use of deferasirox, rare cases of acute kidney failure or liver failure occurred, some resulting in death. Due to this, patients should be closely monitored on deferasirox therapy prior to the start of therapy and regularly thereafter.

PROGNOSIS

The outlook in MDS is variable, with about 30% of patients progressing to refractory AML. The median survival rate varies from years to months, depending on type. Stem-cell transplantation offers possible cure,

with survival rates of 50% at 3 years, although older patients do poorly. Indicators of a good prognosis: Younger age; normal or moderately reduced neutrophil or platelet counts; low blast counts in the bone marrow (< 20%) and no blasts in the blood; no Auer rods; ringed sideroblasts; normal or mixed karyotypes without complex chromosome abnormalities; and *in vitro* marrow culture with a nonleukemic growth pattern

Indicators of a poor prognosis: Advanced age; severe neutropenia or thrombocytopenia; high blast count in the bone marrow (20-29%) or blasts in the blood; Auer rods; absence of ringed sideroblasts; abnormal localization or immature granulocyte precursors in bone marrow section; completely or mostly abnormal karyotypes, or complex marrow chromosome abnormalities and *in vitro* bone marrow culture with a leukemic growth pattern

Karyotype prognostic factors:

- Good: normal, -Y, del(5q), del(20q)
- Intermediate or variable: +8, other single or double anomalies
- Poor: complex (>3 chromosomal aberrations); chromosome 7 anomalies.

The IPSS is the most commonly used tool in MDS to predict long-term outcome. Cytogenetic abnormalities can be detected by conventional cytogenetics, a FISH panel for MDS, or virtual karyotype.

Genetic Markers

Although not yet formally incorporated in the generally accepted classification systems, molecular profiling of myelodysplastic syndrome genomes has increased the understanding of prognostic molecular factors for this disease. For example, in low-risk MDS, *IDH1* and *IDH2* mutations are associated with significantly worsened survival.

EPIDEMIOLOGY

The exact number of people with MDS is not known because it can go undiagnosed and no tracking of the syndrome is mandated. Some estimates are on the order of 10,000 to 20,000 new cases each year in the United States alone. The number of new cases each year is probably increasing as the age of the population increases, and some authors propose that the number of new cases in those over 70 may be as high as 15 per 100,000 per year. The typical age at diagnosis of MDS is between 60 and 75 years; a few people are younger than 50, and diagnoses are rare in children. Males are slightly more commonly affected than females.

HISTORY

Since the early 20th century, some people with acute myelogenous leukemia were begun to be recognized to have a preceding period of anemia and abnormal blood cell production. These conditions were lumped together with other diseases under the term "refractory anemia". The first description of "preleukemia" as a specific entity was published in 1953 by Block *et al.* The early identification, characterization and classification of this disorder were problematical, and the syndrome went by many names until the 1976 FAB classification was published and popularized the term MDS.

SHWACHMAN–DIAMOND SYNDROME

Shwachman–Diamond syndrome (SDS), or Shwachman–Bodian–Diamond syndrome, is a rare congenital disorder characterized by exocrine pancreatic insufficiency, bone marrow dysfunction, skeletal abnormalities and short stature. After cystic fibrosis (CF), it is the second most common cause of exocrine pancreatic insufficiency in children.

SIGNS AND SYMPTOMS

The syndrome shows a wide range of abnormalities and symptoms. The main characteristics of the syndrome are exocrine pancreatic dysfunction, hematologic abnormalities and growth retardation. Only the first two of these are included in the clinical diagnostic criteria.

- Hematologic abnormalities: neutropenia may be intermittent or persistent and is the most common hematological finding. Low neutrophil counts leave patients at risk of developing severe recurrent infections that may be life-threatening. Anemia (low red blood cell counts) and thrombocytopenia (low platelet counts) may also occur. Bone marrow is typically hypocellular, with maturation arrest in the myeloid lineages that give rise to neutrophils, macrophages, platelets and red blood cells. Patients may also develop progressive marrow failure or transform to acute myelogenous leukemia.
- Exocrine pancreatic dysfunction: Pancreatic exocrine insufficiency arises due to a lack of acinar cells that produce digestive enzymes. These are extensively depleted and replaced by fat. A lack of pancreatic digestive enzymes leaves patients unable to digest and absorb fat. However, pancreatic status may improve with age in some patients.
- Growth retardation: More than 50% of patients are below the third percentile for height, and short stature appears to be unrelated to nutritional status. Other skeletal abnormalities include metaphyseal dysostosis (45% of patients), thoracic dystrophy (rib cage abnormalities in 46% of patients) and costochondral thickening (shortened ribs with flared ends in 32% of patients). Skeletal problems are one of the most variable components of SDS, with 50% affected siblings from the same family discordant for clinical presentation or type of abnormality. Despite this, a careful review of radiographs from 15 patients indicated that all of them had at least one skeletal anomaly, though many were subclinical.
- Other features include metaphysial dysostosis, mild hepatic dysfunction, increased frequency of infections.

GENETICS

Shwachman–Diamond syndrome is characterized by an autosomal recessive mode of inheritance. The gene that is mutated in this syndrome, *SBDS*, lies on the long arm of chromosome 7 at cytogenetic position 7q11. It is composed of five exons and has an associated mRNA transcript that is 1.6 kilobase pairs in length. The *SBDS* gene resides in a block of genomic sequence that is locally duplicated on the chromosome. The second copy contains a non-functional version of the *SBDS* gene that is 97% identical to the original gene, but has accumulated inactivating mutations over time.

It is considered to be a pseudogene. In a study of 158 SDS families, 75% of disease-associated mutations appeared to be the result of gene conversion, while 89% of patients harboured at least one such mutation. Gene conversion occurs when the intact *SBDS* gene and its pseudogene copy aberrantly recombine at meiosis, leading to an incorporation of pseudogene-like sequences into the 'good copy' of the *SBDS* gene, thereby inactivating it. Two gene conversion mutations predominate; one is a splice site mutation affecting the 5' splice site of intron two, while the second is an exon two nonsense mutation.

MECHANISMS

The *SBDS* gene is expressed in all tissues and encodes a protein of 250 amino acid residues. The function of this protein is not known and it has no primary sequence similarity to any other protein or structural domain that would indicate a possible function. The atomic structure of an archaeal ortholog of the human protein has been determined by x-ray crystallography and indicates a novel three-dimensional fold in the most N-terminal of the three structural domains and many of the known human disease associated mutations and truncations occur

within this structural domain. There is however, a great deal of indirect evidence to suggest that the SBDS protein may be involved in an aspect of cellular RNA metabolism or ribosome assembly or function.

The wide occurrence of the gene in all archaea and eukaryotes supports a role for this protein in a very fundamental and evolutionarily conserved aspect of cellular biology. A specific function for SBDS in RNA metabolism or ribosome assembly or function is supported by its localization to the nucleolus, the nuclear subdomain where these processes occur. At present, it is not obvious how disruption of a basic cellular process causes the tissue- and organ-specific manifestations seen in SDS. However, unusual and combinations of tissues and organs are also affected in Diamond–Blackfan anemia, X-linked dyskeratosis congenita, and cartilage–hair hypoplasia—three diseases that may also be linked to defective ribosome function.

DIAGNOSIS

Initially, the clinical presentation of SDS may appear similar to cystic fibrosis. However, CF can be excluded with a normal chloride in sweat test but faecal elastase as a marker of pancreatic function will be reduced. The variation, intermittent nature, and potential for long-term improvement of some clinical features make this syndrome difficult to diagnose. SDS may present with either malabsorption, or hematological problems. Rarely, SDS may present with skeletal defects, including severe rib cage abnormalities that lead to difficulty in breathing. Diagnosis is generally based on evidence of exocrine pancreatic dysfunction and neutropenia. Skeletal abnormalities and short stature are characteristics that can be used to support the diagnosis. The gene responsible for the disease has been identified and genetic testing is now available. Though useful in diagnostics, a genetic test does not surmount the need for careful clinical assessment and monitoring of all patients.

MANAGEMENT

Pancreatic exocrine insufficiency may be treated through pancreatic enzyme supplementation, while severe skeletal abnormalities may require surgical intervention. Neutropenia may be treated with granulocyte-colony stimulating factor (GCSF) to boost peripheral neutrophil counts. However, there is ongoing and unresolved concern that this drug could contribute to the development of leukemia. Signs of progressive marrow failure may warrant bone marrow transplantation (BMT). This has been used successfully to treat hematological aspects of disease. However, SDS patients have an elevated occurrence of BMT-related adverse events, including graft-versus-host disease (GVHD) and toxicity relating to the pre-transplant conditioning regimen. In the long run, study of the gene that is mutated in SDS should improve understanding of the molecular basis of disease. This, in turn, may lead to novel therapeutic strategies, including gene therapy and other gene- or protein-based approaches.

EPIDEMIOLOGY

It is thought to have an estimated incidence of 1 in 75,000 people.

HISTORY

The disease was first described as a coherent clinical entity in May 1964 by Bodian, Sheldon, and Lightwood. It was subsequently described by Shwachman, Diamond, Oski, and Khaw in November of the same year. In 2001, linkage analysis in SDS families indicated that affected gene mapped to a large region of human chromosome seven. In 2002, this interval was refined to a region on the long arm of the chromosome next to the centromere. In 2003 mutations in the *SBDS* gene (Shwachman–Bodian–Diamond syndrome) were found to be associated with disease.

Eponym

Shwachman–Diamond syndrome, less commonly known as Shwachman–Bodian–Diamond syndrome, is named for Harry Shwachman (1910 – September 12, 1986), an American physician, Martin Bodian (1912 – May 12, 1994), a British ophthalmologist who worked in New York City, and Louis Klein Diamond (May 11, 1902 – June 14, 1999), an American pediatrician.

TAR SYNDROME

TAR Syndrome (thrombocytopenia with absent radius) is a rare genetic disorder that is characterized by the absence of the radius bone in the forearm and a dramatically reduced platelet count.

Fig. TAR syndrome.

PRESENTATION

- Symptoms of thrombocytopenia, or a lowered platelet count, leads to bruising and potentially life-threatening hemorrhage.
- Absence of the radius bone in the forearm with preservation of the thumb.

Other common links between people with TAR seem to include anemia, heart problems, kidney problems, knee joint problems, frequently lactose intolerance. Different cases with leukemia in patients with TAR are described in.

GENETICS

A 2007 research article identified a region of chromosome 1, 1q21.1, containing 11 genes (including HFE2, LIX1L, PIAS3, ANKRD35, ITGA10, RBM8A, PEX11B, POLR3GL, TXNIP, and GNRR2), that is heterozygously deleted in thirty of thirty patients with TAR. This deletion was also found in 32% of unaffected family members, indicating that the condition requires an additional modifier. This modifier was discovered in 2012. A study identified two separate single-nucleotide polymorphism (SNP) in *RBM8A*. These abnormalities resulting in reduced Y14 production that were responsible for all but two of the cases studied, one a 5'UTR SNP with a frequency of 3.05% and the other an intronic SNP with a frequency of 0.42% in 7504 healthy

individuals of the Cambridge BioResource. The microdeletion was not found in 5919 controls of the Wellcome Trust Case Control Consortium.

TREATMENT

Treatments range from platelet transfusions to surgery aimed at either centralizing the hand over the ulna to improve functionality of the hand or aimed at 'normalizing' the appearance of the arm, which is much shorter and 'clubbed.' There is some controversy surrounding the role of surgery. The infant mortality rate has been curbed by new technology, including platelet transfusions, which can even be performed in utero. The critical period is the first and sometimes second year of life. For most people with TAR, platelet counts improve as they grow out of childhood.

EPIDEMIOLOGY

The incidence is 0.42 per 100,000 live births.

HISTORY

In 1929 Greenwald and Sherman described the first patient with TAR Syndrome. 40 years later Hall collected 40 cases and introduced the name "Thrombocytopenia with absent radius". In 1988 Hedberg published an article with 100 cases. The bigenetic background was described in 2007 and 2012.

4

Bleeding Disorders

HAEMOPHILIA

Haemophilia, also spelled hemophilia, is a mostly inherited genetic disorder that impairs the body's ability to make blood clots, a process needed to stop bleeding. This results in people bleeding longer after an injury, easy bruising, and an increased risk of bleeding inside joints or the brain. Those with a mild case of the disease may have symptoms only after an accident or during surgery. Bleeding into a joint can result in permanent damage while bleeding in the brain can result in long term headaches, seizures, or a decreased level of consciousness.

There are two main types of haemophilia: haemophilia A, which occurs due to not enough clotting factor VIII, and haemophilia B, which occurs due to not enough clotting factor IX. They are typically inherited from one's parents through an X chromosome with a nonfunctional gene. Rarely a new mutation may occur during early development or haemophilia may develop later in life due to antibodies forming against a clotting factor. Other types include haemophilia C, which occurs due to not enough factor XI, and parahaemophilia, which occurs due to not enough factor V. Acquired haemophilia is associated with cancers, autoimmune disorders, and pregnancy. Diagnosis is by testing the blood for its ability to clot and its levels of clotting factors.

Fig. Haemophilia.

Prevention may occur by removing an egg, fertilizing it, and testing the embryo before transferring it to the uterus. Treatment is by replacing the missing blood clotting factors. This may be done on a regular basis or during bleeding episodes. Replacement may take place at home or in hospital. The clotting factors are made either from

human blood or by recombinant methods. Up to 20% of people develop antibodies to the clotting factors which makes treatment more difficult. The medication desmopressin may be used in those with mild haemophilia A. Studies of gene therapy are in early human trials. Haemophilia A affects about 1 in 5,000–10,000, while haemophilia B affects about 1 in 40,000, males at birth. As haemophilia A and B are both X-linked recessive disorders, females are rarely severely affected. Some females with a nonfunctional gene on one of the X chromosomes may be mildly symptomatic. Haemophilia C occurs equally in both sexes and is mostly found in Ashkenazi Jews. In the 1800s haemophilia was common within the royal families of Europe. The difference between haemophilia A and B was determined in 1952.

SIGNS AND SYMPTOMS

Characteristic symptoms vary with severity. In general symptoms are internal or external bleeding episodes, which are called "bleeds". People with more severe haemophilia suffer more severe and more frequent bleeds, while people with mild haemophilia usually suffer more minor symptoms except after surgery or serious trauma. In cases of moderate haemophilia symptoms are variable which manifest along a spectrum between severe and mild forms. In both haemophilia A and B, there is spontaneous bleeding but a normal bleeding time, normal prothrombin time, normal thrombin time, but prolonged partial thromboplastin time. 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 cases of severe and moderate haemophilia. Complications may arise from the disease itself 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 (haemophilic arthropathy), 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.

Haemophilic arthropathy is characterized by chronic proliferative synovitis and cartilage destruction. If an intra-articular bleed is not drained early, it may cause apoptosis of chondrocytes and affect the synthesis of proteoglycans. The hypertrophied and fragile synovial lining while attempting to eliminate excessive blood may be more likely to easily rebleed, leading to a vicious cycle of hemarthrosis-synovitis-hemarthrosis. In addition, iron

deposition in the synovium may induce an inflammatory response activating the immune system and stimulating angiogenesis, resulting in cartilage and bone destruction.

GENETICS

Females possess two X-chromosomes, males have one X and one Y-chromosome. Since the mutations causing the disease are X-linked recessive, a female 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 the male 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 the disorder will develop. 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. This is a recessive trait and can be passed on if cases are more severe with carrier. 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 son, either the female is a carrier for the blood disorder 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, people 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 can be diagnosed before, during or after birth if there is a family history of the condition. Several options are available to parents. If there is no family history of haemophilia, it is usually only diagnosed when a child begins to walk or crawl. They may experience joint bleeds or easy bruising. Mild haemophilia may only be discovered later, usually after an injury or a dental or surgical procedure.

Before Pregnancy

Genetic testing and counselling are available to help determine the risk of passing the condition onto a child. This may involve testing a sample of tissue or blood to look for signs of the genetic mutation that causes haemophilia.

During Pregnancy

A pregnant woman with a history of haemophilia in her family can test for the haemophilia gene. Such tests include:

- Chorionic villus sampling (CVS) – a small sample of the placenta is removed from the womb and tested for the haemophilia gene, usually during weeks 11-14 of pregnancy
- Amniocentesis – a sample of amniotic fluid is taken for testing, usually during weeks 15-20 of pregnancy.

There's a small risk of these procedures causing problems such as miscarriage or premature labour, so the woman may discuss this with the doctor in charge of her care.

After Birth

If haemophilia is suspected after a child has been born, a blood test can usually confirm the diagnosis. Blood from the umbilical cord can be tested at birth if there's a family history of haemophilia. A blood test will also be able to identify whether a child has haemophilia A or B, and how severe it is.

Classification

There are several types of haemophilia: haemophilia A, haemophilia B, haemophilia C, *parahaemophilia*, and *acquired haemophilia A*. Haemophilia A, is a recessive X-linked genetic disorder resulting in a deficiency of functional clotting Factor VIII. Haemophilia B, is also a recessive X-linked genetic disorder involving a lack of functional clotting Factor IX. Haemophilia C, is an autosomal genetic disorder involving a lack of functional clotting Factor XI. Haemophilia C is not completely recessive, as heterozygous individuals also show increased bleeding. The type of haemophilia known as *parahaemophilia* is a mild and rare form and is due to a deficiency in factor V. This type can be inherited or acquired. A non-genetic form of haemophilia is caused by autoantibodies against factor VIII and so is known as *acquired haemophilia A*. Acquired haemophilia can be associated with cancers, autoimmune disorders and following childbirth.

MANAGEMENT

There is no long-term cure. Treatment is by replacing the missing blood clotting factors.

Clotting Factors

Clotting factors are usually not needed in mild haemophilia. In moderate haemophilia clotting factors are typically only needed when bleeding occurs or to prevent bleeding with certain events. In severe haemophilia

preventive use is often recommended two or three times a week and may continue for life. Rapid treatment of bleeding episodes decreases damage to the body. Factor VIII is used in haemophilia A and factor IX in haemophilia B. Factor replacement can be either isolated from human blood serum, recombinant, or a combination of the two. Some people 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 person becomes refractory to replacement coagulation factor as a result of circulating inhibitors, this may be partially overcome with recombinant human factor VII. In early 2008, the US Food and Drug Administration (FDA) approved anti-haemophilic factor, genetically engineered from the genes of Chinese hamster ovary cells.

Since 1993 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. Clotting factors are either given preventively or on-demand. Preventive use 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 (or episodic) treatment involves treating bleeding episodes once they arise. In 2007, a trial 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.

Other

Desmopressin (DDAVP) may be used in those with mild haemophilia A. Tranexamic acid or epsilon aminocaproic acid may be given along with clotting factors to prevent breakdown of clots. Pain medicines, steroids, and physical therapy may be used to reduce pain and swelling in an affected joint.

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, medicines 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.

PROGNOSIS

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 people 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.

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

Scientific Discovery

The first medical professional to describe the disease was Abulcasis. 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, 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, NH in 1720. The idea that affected males could pass the trait onto their unaffected daughters was not described until 1813 when John F. Hay, published an account in The New England Journal of Medicine.

In 1924, a Finnish doctor discovered a hereditary bleeding disorder similar to haemophilia localised in the Åland Islands, 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

Haemophilia has featured prominently in European royalty and thus is sometimes known as 'the royal disease'. Queen Victoria passed the mutation for haemophilia B to her son Leopold and, through two of her daughters, Alice and Beatrice, to various royals across the continent, including the royal families of Spain, Germany, and Russia. In Russia, Tsarevich Alexei, the son and heir of Tsar Nicholas II, famously suffered from haemophilia, which he had

gotten from his mother, Empress Alexandra, one of Queen Victoria's granddaughters. The haemophilia of Alexei would result in the rise to prominence of the Russian mystic Grigori Rasputin, at the imperial court. It was claimed that Rasputin was successful at treating Tsarevich Alexei'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 Tsarevich 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 in which 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

Up until late-1985 many people with haemophilia received clotting factor products that posed a risk of HIV and hepatitis C infection, the plasma used to create the products was not screened or tested, neither had most of the products been subject to any form of viral inactivation. Tens of thousands worldwide were infected as a result of contaminated factor products including more than 10,000 people in the United States, 3,500 British, 1,400 Japanese, 700 Canadians, 250 Irish, and 115 Iraqis. Infection via the tainted factor products had mostly stopped by 1986 by which time viral inactivation methods had largely been put into place, although some products were shown to still be dangerous in 1987.

RESEARCH

Gene Therapy

In those with severe haemophilia, gene therapy may reduce symptoms to those that a mild or moderate person with haemophilia might have. The best results have been found in haemophilia B. In 2016 early stage human research was ongoing with a few sites recruiting participants. In 2017 a gene therapy trial on nine people with haemophilia A reported that high doses did better than low doses. It is not currently an accepted treatment for haemophilia.

THROMBOCYTOPENIA

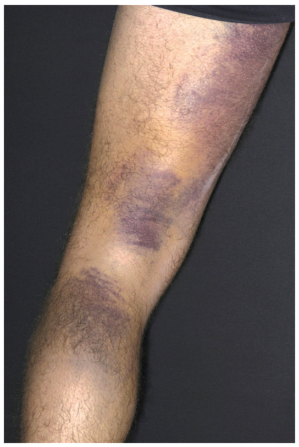


Fig. Thrombocytopenia.

Thrombocytopenia is a condition characterized by abnormally low levels of thrombocytes, also known as platelets, in the blood. A normal human platelet count ranges from 150,000 to 450,000 platelets per microliter of blood. These limits are determined by the 2.5th lower and upper percentile, so values outside this range do not necessarily indicate disease. One common definition of thrombocytopenia requiring emergency treatment is a platelet count below 50,000 per microliter.

SIGNS AND SYMPTOMS

Thrombocytopenia usually has no symptoms and is picked up on a routine full blood count (or complete blood count). Some individuals with thrombocytopenia may experience external bleeding such as nosebleeds, and/or bleeding gums. Some women may have heavier or longer periods or breakthrough bleeding. Bruising, particularly purpura in the forearms and petechiae in the feet, legs, and mucous membranes, may be caused by spontaneous bleeding under the skin. Eliciting a full medical history is vital to ensure the low platelet count is not secondary to another disorder. It is also important to ensure that the other blood cell types, such as red blood cells and white blood cells, are not also suppressed. Painless, round and pinpoint (1 to 3 mm in diameter) petechiae usually appear and fade, and sometimes group to form ecchymoses. Larger than petechiae, ecchymoses are purple, blue or yellow-green areas of skin that vary in size and shape. They can occur anywhere on the body.

A person with this disease may also complain of malaise, fatigue and general weakness (with or without accompanying blood loss). Acquired thrombocytopenia may be associated with a history of drug use. Inspection typically reveals evidence of bleeding (petechiae or ecchymoses), along with slow, continuous bleeding from any injuries or wounds. Adults may have large, blood-filled bullae in the mouth. If the person's platelet count is between 30,000 and 50,000/mm, bruising with minor trauma may be expected; if it is between 15,000 and 30,000/mm, spontaneous bruising will be seen (mostly on the arms and legs).

CAUSES

Thrombocytopenia can be inherited or acquired.

Decreased Production

Abnormally low platelet production may be caused by:

- Dehydration, Vitamin B_{12} or folic acid deficiency
- Leukemia or myelodysplastic syndrome or aplastic anemia
- Decreased production of thrombopoietin by the liver in liver failure
- Sepsis, systemic viral or bacterial infection
- Leptospirosis
- Hereditary syndromes
  - Congenital amegakaryocytic thrombocytopenia
  - Thrombocytopenia absent radius syndrome
  - Fanconi anemia
  - Bernard-Soulier syndrome (associated with large platelets)
  - May-Hegglin anomaly
  - Grey platelet syndrome
  - Alport syndrome
  - Wiskott–Aldrich syndrome.

Increased Destruction

Abnormally high rates of platelet destruction may be due to immune or non-immune conditions, including:

- Immune thrombocytopenic purpura
- Thrombotic thrombocytopenic purpura
- Hemolytic-uremic syndrome
- Disseminated intravascular coagulation
- Paroxysmal nocturnal hemoglobinuria
- Antiphospholipid syndrome
- Systemic lupus erythematosus
- Post-transfusion purpura
- Neonatal alloimmune thrombocytopenia
- Hypersplenism
- Dengue fever
- Gaucher's disease
- Zika virus.

Medication-induced

The following medications can induce thrombocytopenia through direct myelosuppression.

- Valproic acid
- Methotrexate
- Carboplatin
- Interferon
- Isotretinoin
- Panobinostat
- H_2 blockers and proton-pump inhibitors.

Other Causes

- Lab error, possibly due to the anticoagulant EDTA in CBC specimen tubes; a *citrated* platelet count is a useful follow-up study
- Snakebite
- Niacin toxicity
- Lyme disease
- Thrombocytapheresis (also called Plateletpheresis).

DIAGNOSIS

Laboratory tests for thrombocytopenia might include full blood count, liver enzymes, kidney function, vitamin B_{12} levels, folic acid levels, erythrocyte sedimentation rate, and peripheral blood smear. If the cause for the low platelet count remains unclear, a bone marrow biopsy is usually recommended to differentiate cases of decreased platelet production from cases of peripheral platelet destruction. Thrombocytopenia in hospitalized alcoholics may be caused by spleen enlargement, folate deficiency, and, most frequently, the direct toxic effect of alcohol on production, survival time, and function of platelets. Platelet count begins to rise after 2 to 5 days' abstinence from alcohol. The condition is generally benign, and clinically significant hemorrhage is rare. In severe thrombocytopenia, a bone marrow study can determine the number, size and maturity of the megakaryocytes.

This information may identify ineffective platelet production as the cause of thrombocytopenia and rule out a malignant disease process at the same time.

TREATMENT

Treatment is guided by the severity and specific cause of the disease. Treatment focuses on eliminating the underlying problem, whether that means discontinuing drugs suspected to cause it or treating underlying sepsis. Diagnosis and treatment of serious thrombocytopenia is usually directed by a hematologist. Corticosteroids may be used to increase platelet production. Lithium carbonate or folate may also be used to stimulate platelet production in the bone marrow.

Thrombotic Thrombocytopenic Purpura

Treatment of thrombotic thrombocytopenic purpura (TTP) is a medical emergency, since the associated hemolytic anemia and platelet activation can lead to renal failure and changes in the level of consciousness. Treatment of TTP was revolutionized in the 1980s with the application of plasmapheresis. According to the Furlan-Tsai hypothesis, this treatment works by removing antibodies against the von Willebrand factor-cleaving protease ADAMTS-13. The plasmapheresis procedure also adds active ADAMTS-13 protease proteins to the patient, restoring a normal level of von Willebrand factor multimers. Patients with persistent antibodies against ADAMTS-13 do not always manifest TTP, and these antibodies alone are not sufficient to explain how plasmapheresis treats TTP.

Idiopathic Thrombocytopenic Purpura

Many cases of ITP can be left untreated, and spontaneous remission (especially in children) is not uncommon. However, counts of under 50,000 are usually monitored with regular blood tests, and those with counts of under 10,000 are usually treated, as the risk of serious spontaneous bleeding is high with such a low platelet count. Any patient experiencing severe bleeding symptoms is also usually treated. The threshold for treating ITP has decreased since the 1990s; hematologists recognize that patients rarely spontaneously bleed with platelet counts greater than 10,000, although there are documented exceptions to this observation. Thrombopoetin analogues have been tested extensively for the treatment of ITP. These agents had previously shown promise but had been found to stimulate antibodies against endogenous thrombopoietin or lead to thrombosis. Romiplostim (trade name Nplate, formerly AMG 531) was found to be safe and effective for the treatment of ITP in refractory patients, especially those who relapsed following splenectomy.

Heparin-induced Thrombocytopenia

Discontinuation of heparin is critical in a case of heparin-induced thrombocytopenia (HIT). Beyond that, however, clinicians generally treat to avoid thrombosis. Treatment may include a direct thrombin inhibitor, such as lepirudin or argatroban. Other blood thinners sometimes used in this setting include bivalirudin and fondaparinux. Platelet transfusions are not routinely used to treat HIT because thrombosis, not bleeding, is the primary problem. Warfarin is not recommended until platelets have normalized.

Congenital Amegakaryocytic Thrombocytopenia

Bone marrow/stem cell transplants are the only known cures for this genetic disease. Frequent platelet transfusions are required to keep the patient from bleeding to death before the transplant can be performed, although this is not always the case.

NEONATAL THROMBOCYTOPENIA

Thrombocytopenia affects a few percent of newborns, and its prevalence in neonatal intensive care units (NICU) is high. Normally, it is mild and resolves without consequences. Most cases affect preterm birth infants and result from placental insufficiency and/or fetal hypoxia. Other causes, such as alloimmunity, genetics, autoimmunity, and infection, are less frequent.

Thrombocytopenia that starts after the first 72 hours since birth is often the result of underlying sepsis or necrotizing enterocolitis (NEC). In the case of infection, PCR tests may be useful for rapid pathogen identification and detection of antibiotic resistance genes. Possible pathogens include viruses (*e.g.,* Cytomegalovirus (CMV), rubella virus, HIV), bacteria (*e.g., Staphylococcus sp.*, *Enterococcus sp.*, *Streptococcus agalactiae* (GBS), *Listeria monocytogenes*, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*), fungi (*e.g., Candida sp.*), and *Toxoplasma gondii*. The severity of thrombocytopenia may be correlated with pathogen type; some research indicates that the most severe cases are related to fungal or gram-negative bacterial infection. The pathogen may be transmitted during or before birth, by breast feeding, or during transfusion. Interleukin-11 is being investigated as a drug for managing thrombocytopenia, especially in cases of sepsis or necrotizing enterocolitis (NEC).

ACQUIRED PLATELET FUNCTION DEFECT

Idiopathic thrombocytopenic purpura (ITP)

Immune thrombocytopenia (ITP) is a type of thrombocytopenic purpura defined as isolated low platelet count (thrombocytopenia) with normal bone marrow and the absence of other causes of thrombocytopenia. It causes a characteristic purpuric rash and an increased tendency to bleed. Two distinct clinical syndromes manifest as an acute condition in children and a chronic condition in adults. The acute form often follows an infection and has a spontaneous resolution within two months. Chronic immune thrombocytopenia persists longer than six months with a specific cause being unknown. ITP is an autoimmune disease with antibodies detectable against several platelet surface antigens. ITP is diagnosed by a low platelet count in a complete blood count (a common blood test). However, since the diagnosis depends on the exclusion of other causes of a low platelet count, additional investigations (such as a bone marrow biopsy) may be necessary in some cases. In mild cases, only careful observation may be required but very low counts or significant bleeding may prompt treatment with corticosteroids, intravenous immunoglobulin, anti-D immunoglobulin, or immunosuppressive drugs. *Refractory ITP* (not responsive to conventional treatment) may require splenectomy, the surgical removal of the spleen. Platelet transfusions may be used in severe bleeding together with a very low count. Sometimes the body may compensate by making abnormally large platelets.

Signs and Symptoms

Signs include the spontaneous formation of bruises (purpura) and petechiae (tiny bruises), especially on the extremities, bleeding from the nostrils and/or gums, and menorrhagia (excessive menstrual bleeding), any of which may occur if the platelet count is below 20,000 per ìl. A very low count (<10,000 per µl) may result in the spontaneous formation of hematomas (blood masses) in the mouth or on other mucous membranes. Bleeding time from minor lacerations or abrasions is usually prolonged. Serious and possibly fatal complications due to extremely low counts (<5,000 per ìl) include subarachnoid or intracerebral hemorrhage (bleeding inside the skull or brain), lower gastrointestinal bleeding or other internal bleeding. An ITP patient with an extremely low count is vulnerable to internal bleeding caused by blunt abdominal trauma, as might be experienced in a motor vehicle crash. These complications are not likely when the platelet count is above 20,000 per µl.

Pathogenesis

In approximately 60 percent of cases, antibodies against platelets can be detected. Most often these antibodies are against platelet membrane glycoproteins IIb-IIIa or Ib-IX, and are of the immunoglobulin G (IgG) type. The Harrington–Hollingsworth experiment, established the immune pathogenesis of ITP. The coating of platelets with IgG renders them susceptible to opsonization and phagocytosis by splenic macrophages, as well by Kupffer cells in the liver. The IgG autoantibodies are also thought to damage megakaryocytes, the precursor cells to platelets, although this is believed to contribute only slightly to the decrease in platelet numbers. Recent research now indicates that impaired production of the glycoprotein hormone thrombopoietin, which is the stimulant for platelet production, may be a contributing factor to the reduction in circulating platelets. This observation has led to the development of a class of ITP-targeted drugs referred to as thrombopoietin receptor agonists. The stimulus for auto-antibody production in ITP is probably abnormal T cell activity. Preliminary findings suggest that these T cells can be influenced by drugs that target B cells, such as rituximab.

Diagnosis

The diagnosis of ITP is a process of exclusion. First, it has to be determined that there are no blood abnormalities other than a low platelet count, and no physical signs other than bleeding. Then, secondary causes (5–10 percent of suspected ITP cases) should be excluded. Such secondary causes include leukemia, medications (*e.g.,* quinine, heparin), lupus erythematosus, cirrhosis, HIV, hepatitis C, congenital causes, antiphospholipid syndrome, von Willebrand factor deficiency, onyalai and others. In approximately one percent of cases, autoimmune hemolytic anemia and ITP coexist, a condition referred to as Evans syndrome, a condition that points to CLL as a possible cause. Despite the destruction of platelets by splenic macrophages, the spleen is normally not enlarged. In fact, an enlarged spleen should lead to a search for other possible causes for the thrombocytopenia. Bleeding time is usually prolonged in ITP patients. However, the use of bleeding time in diagnosis is discouraged by the American Society of Hematology practice guidelines and a normal bleeding time does not exclude a platelet disorder. Bone marrow examination may be performed on patients over the age of 60 and those who do not respond to treatment, or when the diagnosis is in doubt. On examination of the marrow, an increase in the production of megakaryocytes may be observed and may help in establishing a diagnosis of ITP. An analysis for anti-platelet antibodies is a matter of clinician's preference, as there is disagreement on whether the 80 percent specificity of this test is sufficient to be clinically useful.

Treatment

With rare exceptions, there is usually no need to treat based on platelet counts. Many older recommendations suggested a certain platelet count threshold (usually somewhere below 20.0/μl) as an indication for hospitalization or treatment. Current guidelines recommend treatment only in cases of significant bleeding. Treatment recommendations sometimes differ for adult and pediatric ITP.

Steroids

Initial treatment usually consists of the administration of corticosteroids, a group of medications that suppress the immune system. The dose and mode of administration is determined by platelet count and whether there is active bleeding: in urgent situations, infusions of dexamethasone or methylprednisolone may be used, while oral prednisone or prednisolone may suffice in less severe cases. Once the platelet count has improved, the dose of steroid is gradually reduced while the possibility of relapse is monitored. 60–90 percent will experience a relapse during dose reduction or cessation. Long-term steroids are avoided if possible because of potential side-effects that include osteoporosis, diabetes and cataracts.

Anti-D

Another option, suitable for Rh-positive patients with functional spleens is intravenous administration of Rho(D) immune globulin [Human; Anti-D]. The mechanism of action of anti-D is not fully understood. However, following administration, anti-D-coated red blood cell complexes saturate Fcγ receptor sites on macrophages, resulting in preferential destruction of red blood cells (RBCs), therefore sparing antibody-coated platelets. There are two anti-D products indicated for use in patients with ITP: WinRho SDF and Rhophylac. The most common adverse reactions are headache (15%), nausea/vomiting (12%) chills (<2%) and fever (1%).

Steroid-sparing Agents

There is increasing use of immunosuppressants such as mycophenolate mofetil and azathioprine because of their effectiveness. In chronic refractory cases, where immune pathogenesis has been confirmed, the off-label use of the *vinca* alkaloid and chemotherapy agent vincristine may be attempted. However, vincristine has significant side effects and its use in treating ITP must be approached with caution, especially in children.

Intravenous Immunoglobulin

Intravenous immunoglobulin (IVIg) may be infused in some cases in order to decrease the rate at which macrophages consume antibody-tagged platelets. However, while sometimes effective, it is costly and produces improvement that generally lasts less than a month. Nevertheless, in the case of an ITP patient already scheduled for surgery who has a dangerously low platelet count and has experienced a poor response to other treatments, IVIg can rapidly increase platelet counts, and can also help reduce the risk of major bleeding by transiently increasing platelet counts.

Thrombopoietin Receptor Agonists

Thrombopoietin receptor agonists are pharmaceutical agents that stimulate platelet production in the bone marrow. In this, they differ from the previously discussed agents that act by attempting to curtail platelet destruction.

Two such products are currently available:

1. Romiplostim (trade name Nplate) is a thrombopoiesis stimulating Fc-peptide fusion protein (peptibody) that is administered by subcutaneous injection. Designated an orphan drug in 2003 under United States law, clinical trials demonstrated romiplostim to be effective in treating chronic ITP, especially in relapsed post-splenectomy patients. Romiplostim was approved by the United States Food and Drug Administration (FDA) for long-term treatment of adult chronic ITP on August 22, 2008.
2. Eltrombopag (trade name Promacta in the USA, Revolade in the EU) is an orally-administered agent with an effect similar to that of romiplostim. It too has been demonstrated to increase platelet counts and decrease bleeding in a dose-dependent manner. Developed by GlaxoSmithKline and also designated an orphan drug by the FDA, Promacta was approved by the FDA on November 20, 2008.

Side effects of thrombopoietin receptor agonists include headache, joint or muscle pain, dizziness, nausea or vomiting, and an increased risk of blood clots.

Surgery

Splenectomy (removal of the spleen) may be considered in patients who are either unresponsive to steroid treatment, have frequent relapses, or cannot be tapered off steroids after a few months. Platelets which have been bound by antibodies are taken up by macrophages in the spleen (which have Fc receptors), and so removal

of the spleen reduces platelet destruction. The procedure is potentially risky in ITP cases due to the increased possibility of significant bleeding during surgery. Durable remission following splenectomy is achieved in 60 to 65 percent of ITP cases, less so in older subjects. The use of splenectomy to treat ITP has diminished since the development of steroid therapy and other pharmaceutical remedies.

Platelet Transfusion

Platelet transfusion alone is normally not recommended except in an emergency, and is usually unsuccessful in producing a long-term platelet count increase. This is because the underlying autoimmune mechanism that is destroying the patient's platelets will also destroy donor platelets, and so platelet transfusions are *not* considered a long-term treatment option.

H. Pylori *Eradication*

In adults, particularly those living in areas with a high prevalence of *Helicobacter pylori* (which normally inhabits the stomach wall and has been associated with peptic ulcers), identification and treatment of this infection has been shown to improve platelet counts in a third of patients. In a fifth, the platelet count normalized completely; this response rate is similar to that found in treatment with rituximab, which is more expensive and less safe. In children, this approach is not supported by evidence, except in high prevalence areas. Urea breath testing and stool antigen testing perform better than serology-based tests; moreover, serology may be false-positive after treatment with IVIG.

Other Agents

- Dapsone (also called diphenylsulfone, DDS, or avlosulfon) is an anti-infective sulfone drug. Dapsone may also be helpful in treating lupus, rheumatoid arthritis, and as a second-line treatment for ITP. The mechanism by which dapsone assists in ITP is unclear but an increased platelet count is seen in 40–60 percent of recipients.
- The off-label use of rituximab, a chimeric monoclonal antibody against the B cell surface antigen CD20, may sometimes be an effective alternative to splenectomy. However, significant side-effects can occur, and randomized controlled trials are inconclusive.

Epidemiology

A normal platelet count is considered to be in the range of 150,000–450,000 per microlitre (μl) of blood for most healthy individuals. Hence one may be considered thrombocytopenic below that range, although the threshold for a diagnosis of ITP is not tied to any specific number. The incidence of ITP is estimated at 50–100 new cases per million per year, with children accounting for half of that amount. At least 70 percent of childhood cases will end up in remission within six months, even without treatment. Moreover, a third of the remaining chronic cases will usually remit during follow-up observation, and another third will end up with only mild thrombocytopenia (defined as a platelet count above 50,000). A number of immune related genes and polymorphisms have been identified as influencing predisposition to ITP, with FCGR3a-V158 allele and KIRDS2/DL2 increasing susceptibility and KIR2DS5 shown to be protective.

ITP is usually chronic in adults and the probability of durable remission is 20–40 percent. The male to female ratio in the adult group varies from 1:1.2 to 1.7 in most age ranges (childhood cases are roughly equal for both genders) and the median age of adults at the diagnosis is 56–60. The ratio between male and female adult cases tends to widen with age. In the United States, the adult chronic population is thought to be

approximately 60,000—with women outnumbering men approximately 2 to 1, which has resulted in ITP being designated an orphan disease. The mortality rate due to chronic ITP varies but tends to be higher relative to the general population for any age range. In a study conducted in Great Britain, it was noted that ITP causes an approximately 60 percent higher rate of mortality compared to gender- and age-matched subjects without ITP. This increased risk of death with ITP is largely concentrated in the middle-aged and elderly. Ninety-six percent of reported ITP-related deaths were individuals 45 years or older. No significant difference was noted in the rate of survival between males and females.

Pregnancy

Anti-platelet autoantibodies in a pregnant woman with ITP will attack the patient's own platelets and will also cross the placenta and react against fetal platelets. Therefore, ITP is a significant cause of fetal and neonatal immune thrombocytopenia. Approximately 10% of newborns affected by ITP will have platelet counts <50,000/uL and 1% to 2% will have a risk of intracerebral hemorrhage comparable to infants with neonatal alloimmune thrombocytopenia (NAIT).

No lab test can reliably predict if neonatal thrombocytopenia will occur. The risk of neonatal thrombocytopenia is increased with:

- Mothers with a history of splenectomy for ITP
- Mothers who had a previous infant affected with ITP
- Gestational (maternal) platelet count less than 100,000/uL.

It is recommended that pregnant women with thrombocytopenia or a previous diagnosis of ITP should be tested for serum antiplatelet antibodies. A woman with symptomatic thrombocytopenia and an identifiable antiplatelet antibody should be started on therapy for their ITP which may include steroids or IVIG. Fetal blood analysis to determine the platelet count is not generally performed as ITP-induced thrombocytopenia in the fetus is generally less severe than NAIT. Platelet transfusions may be performed in newborns, depending on the degree of thrombocytopenia. It is recommended that neonates be followed with serial platelet counts for the first few days after birth.

History

After initial reports by the Portuguese physician Amato Lusitano in 1556 and Lazarus de la Rivière (physician to the King of France) in 1658, it was the German physician and poet Paul Gottlieb Werlhof who in 1735 wrote the most complete initial report of the purpura of ITP. Platelets were unknown at the time. The name "Werlhof's disease" was used more widely before the current descriptive name became more popular. Platelets were described in the early 19th century, and in the 1880s several investigators linked the purpura with abnormalities in the platelet count. The first report of a successful therapy for ITP was in 1916, when a young Polish medical student, Paul Kaznelson, described a female patient's response to a splenectomy. Splenectomy remained a first-line remedy until the introduction of steroid therapy in the 1950s.

CHRONIC MYELOGENOUS LEUKEMIA

Chronic myelogenous leukemia (CML), also known as chronic myeloid leukemia, is a cancer of the white blood cells. It is a form of leukemia characterized by the increased and unregulated growth of predominantly myeloid cells in the bone marrow and the accumulation of these cells in the blood. CML is a clonal bone marrow stem cell disorder in which a proliferation of mature granulocytes (neutrophils, eosinophils and basophils) and their precursors is found. It is a type of myeloproliferative neoplasm associated with a characteristic chromosomal translocation called the Philadelphia chromosome. CML is largely treated with targeted drugs

called tyrosine-kinase inhibitors (TKIs) which have led to dramatic improved long-term survival rates since 2001. These drugs have revolutionized treatment of this disease and allow most patients to have a good quality of life when compared to the former chemotherapy drugs. In Western countries, CML accounts for 15–25% of all adult leukemias and 14% of leukemias overall (including the pediatric population, where CML is less common).

Signs and Symptoms

The way CML presents depends on the stage of the disease at diagnosis as it has been known to skip stages in some cases. Most patients (~90%) are diagnosed during the chronic stage which is most often asymptomatic. In these cases it may be diagnosed incidentally with an elevated white blood cell count on a routine laboratory test. It can also present with symptoms indicative of hepatosplenomegaly and the resulting upper quadrant pain this causes. The enlarged spleen may put pressure on the stomach causing a loss of appetite and resulting weight loss. It may also present with mild fever and night sweats due to an elevated basal level of metabolism. Some (<10%) are diagnosed during the accelerated stage which most often presents bleeding, petechiae and ecchymosis. In these patients fevers are most commonly the result of opportunistic infections. Some patients are initially diagnosed in the blast phase in which the symptoms are most likely fever, bone pain and an increase in bone marrow fibrosis.

Cause

In most cases no obvious cause for CML can be isolated.

Risk Factors

CML is more common in males than in females (male to female ratio of 1.4:1) and appears more commonly in the elderly with a median age at diagnosis of 65 years. Exposure to ionising radiation appears to be a risk factor, based on a 50 fold higher incidence of CML in Hiroshima and Nagasaki nuclear bombing survivors. The rate of CML in these individuals seems to peak about 10 years after the exposure.

Pathophysiology

CML was the first cancer to be linked to a clear genetic abnormality, the chromosomal translocation known as the Philadelphia chromosome. This chromosomal abnormality is so named because it was first discovered and described in 1960 by two scientists from Philadelphia, Pennsylvania, USA: Peter Nowell of the University of Pennsylvania and David Hungerford of Fox Chase Cancer Center. In this translocation, parts of two chromosomes (the 9th and 22nd) switch places. As a result, part of the BCR ("breakpoint cluster region") gene from chromosome 22 is fused with the ABL gene on chromosome 9. This abnormal "fusion" gene generates a protein of p210 or sometimes p185 weight (p210 is short for 210 kDa protein, a shorthand used for characterizing proteins based solely on size). Because abl carries a domain that can add phosphate groups to tyrosine residues (a tyrosine kinase), the *bcr-abl* fusion gene product is also a tyrosine kinase.

The fused BCR-ABL protein interacts with the interleukin 3beta(c) receptor subunit. The BCR-ABL transcript is continuously active and does not require activation by other cellular messaging proteins. In turn, BCR-ABL activates a cascade of proteins that control the cell cycle, speeding up cell division. Moreover, the BCR-ABL protein inhibits DNA repair, causing genomic instability and making the cell more susceptible to developing further genetic abnormalities. The action of the BCR-ABL protein is the pathophysiologic cause of chronic myelogenous leukemia. With improved understanding of the nature of the BCR-ABL protein and its action as a tyrosine kinase, targeted therapies (the first of which was imatinib) that specifically inhibit the activity of the

BCR-ABL protein have been developed. These tyrosine kinase inhibitors can induce complete remissions in CML, confirming the central importance of bcr-abl as the cause of CML.

Diagnosis

CML is often suspected on the basis of a complete blood count, which shows increased granulocytes of all types, typically including mature myeloid cells. Basophils and eosinophils are almost universally increased; this feature may help differentiate CML from a leukemoid reaction. A bone marrow biopsy is often performed as part of the evaluation for CML, and CML is diagnosed by cytogenetics that detects the translocation t(9;22)(q34;q11.2) which involves the ABL1 gene in chromosome 9 and the BCR gene in chromosome 22. As a result of this translocation, the chromosome looks smaller than its homologue chromosome, and this appearance is known as the Philadelphia chromosome chromosomal abnormality. Thus, this abnormality can be detected by routine cytogenetics, and the involved genes BCR-ABL1 can be detected by fluorescent in situ hybridization, as well as by PCR.

Controversy exists over so-called *Ph-negative* CML, or cases of suspected CML in which the Philadelphia chromosome cannot be detected. Many such patients in fact have complex chromosomal abnormalities that mask the (9;22) translocation, or have evidence of the translocation by FISH or RT-PCR in spite of normal routine karyotyping. The small subset of patients without detectable molecular evidence of bcr-abl fusion may be better classified as having an undifferentiated myelodysplastic/myeloproliferative disorder, as their clinical course tends to be different from patients with CML. CML must be distinguished from a leukemoid reaction, which can have a similar appearance on a blood smear.

Classification

CML is often divided into three phases based on clinical characteristics and laboratory findings. In the absence of intervention, CML typically begins in the *chronic* phase, and over the course of several years progresses to an *accelerated* phase and ultimately to a *blast crisis*. Blast crisis is the terminal phase of CML and clinically behaves like an acute leukemia. Drug treatment will usually stop this progression if started early. One of the drivers of the progression from chronic phase through acceleration and blast crisis is the acquisition of new chromosomal abnormalities (in addition to the Philadelphia chromosome). Some patients may already be in the accelerated phase or blast crisis by the time they are diagnosed.

Chronic Phase

Approximately 85% of patients with CML are in the chronic phase at the time of diagnosis. During this phase, patients are usually asymptomatic or have only mild symptoms of fatigue, left side pain, joint and/or hip pain, or abdominal fullness. The duration of chronic phase is variable and depends on how early the disease was diagnosed as well as the therapies used. In the absence of treatment, the disease progresses to an accelerated phase. Precise patient staging based on clinical markers and personal genomic profile will likely prove beneficial in the assessment of disease history with respect to progression risk.

Accelerated Phase

Criteria for diagnosing transition into the accelerated phase are somewhat variable; the most widely used criteria are those put forward by investigators at M.D. Anderson Cancer Center, by Sokal et al., and the World Health Organization.

The WHO criteria are perhaps most widely used, and define the accelerated phase by any of the following:

- 10–19% myeloblasts in the blood or bone marrow
- >20% basophils in the blood or bone marrow
- Platelet count <100,000, unrelated to therapy
- Platelet count >1,000,000, unresponsive to therapy
- Cytogenetic evolution with new abnormalities in addition to the Philadelphia chromosome
- Increasing splenomegaly or white blood cell count, unresponsive to therapy.

The patient is considered to be in the accelerated phase if any of the above are present. The accelerated phase is significant because it signals that the disease is progressing and transformation to blast crisis is imminent. Drug treatment often becomes less effective in the advanced stages.

Blast Crisis

Blast crisis is the final phase in the evolution of CML, and behaves like an acute leukemia, with rapid progression and short survival. Blast crisis is diagnosed if any of the following are present in a patient with CML:

- >20% myeloblasts or lymphoblasts in the blood or bone marrow
- Large clusters of blasts in the bone marrow on biopsy
- Development of a chloroma (solid focus of leukemia outside the bone marrow).

Treatment

The only curative treatment for CML is a bone marrow transplant or an allogeneic stem cell transplant. Other than this there are four major mainstays of treatment in CML: treatment with tyrosine kinase inhibitors, myelosuppressive or leukopheresis therapy (to counteract the leukocytosis during early treatment), splenectomy and interferon alfa-2b treatment. Due to the high median age of patients with CML it is relatively rare for CML to be seen in pregnant women, despite this, however, chronic myelogenous leukemia can be treated with relative safety at any time during pregnancy with Interferon-alpha hormones.

Chronic Phase

In the past, antimetabolites (*e.g.,* cytarabine, hydroxyurea), alkylating agents, interferon alfa 2b, and steroids were used as treatments of CML in the chronic phase, but since the 2000s have been replaced by Bcr-Abl tyrosine-kinase inhibitors drugs that specifically target BCR-ABL, the constitutively activated tyrosine kinase fusion protein caused by the Philadelphia chromosome translocation. Despite the move to replacing cytotoxic antineoplastics (standard anticancer drugs) with tyrosine kinase inhibitors sometimes hydroxyurea is still used to counteract the high leukocyte counts encountered during treatment with tyrosine kinase inhibitors like imatinib; in these situations it may be the preferred myelosuppressive agent due to its relative lack of leukemogenic effects and hence the relative lack of potential for secondary hematologic malignancies to result from treatment. IRIS, an international study that compared interferon/cytarabine combination and the first of these new drugs imatinib, with long-term follow up, demonstrated the clear superiority of tyrosine-kinase-targeted inhibition over existing treatments.

Imatinib

The first of this new class of drugs was imatinib mesylate (marketed as Gleevec or Glivec), approved by the U.S. Food and Drug Administration (FDA) in 2001. Imatinib was found to inhibit the progression of CML in the

majority of patients (65–75%) sufficiently to achieve regrowth of their normal bone marrow stem cell population (a cytogenetic response) with stable proportions of maturing white blood cells. Because some leukemic cells (as evaluated by RT-PCR) persist in nearly all patients, the treatment has to be continued indefinitely. Since the advent of imatinib, CML has become the first cancer in which a standard medical treatment may give to the patient a normal life expectancy.

Dasatinib, Nilotinib, Radotinib and Bosutinib

To overcome imatinib resistance and to increase responsiveness to TK inhibitors, four novel agents were later developed. The first, dasatinib, blocks several further oncogenic proteins, in addition to more potent inhibition of the BCR-ABL protein, and was initially approved in 2007 by the US FDA to treat CML in patients who were either resistant to or intolerant of imatinib. A second new TK inhibitor, nilotinib, was also approved by the FDA for the same indication. In 2010, nilotinib and dasatinib were also approved for first-line therapy, making three drugs in this class available for treatment of newly diagnosed CML. In 2012, Radotinib joined the class of novel agents in the inhibition of the BCR-ABL protein and was approved in South Korea for patients resistant to or intolerant of imatinib. Bosutinib received US FDA and EU European Medicines Agency approval on September 4, 2012 and 27 March 2013 respectively for the treatment of adult patients with Philadelphia chromosome-positive (Ph+) chronic myelogenous leukemia (CML) with resistance, or intolerance to prior therapy.

Treatment-resistant CML

While capable of producing significantly improved responses compared with the action of imatinib, neither dasatinib nor nilotinib could overcome drug resistance caused by one particular mutation found to occur in the structure of BCR-ABL known as the T315I mutation (i.e. where the 315th amino acid is mutated from a threonine residue to an isoleucine residue). Two approaches were developed to the treatment of CML as a result:

In 2007, Chemgenex released results of an open-label Phase 2/3 study (CGX-635-CML-202) that investigated the use of a non BCR-ABL targeted agent omacetaxine, administered subcutaneously (under the skin) in patients who had failed with imatinib and exhibited T315I kinase domain mutation. This is a study which is ongoing through 2014. In September 2012, the FDA approved omacetaxine for the treatment of CML in the case of resistance to other chemotherapeutic agents. Independently, ARIAD pharmaceuticals, adapting the chemical structures from first and second-generation TK inhibitors, arrived at a new pan-BCR-ABL inhibitor which showed (for the first time) efficacy against T315I, as well as all other known mutations of the oncoprotein. The drug, ponatinib, gained FDA approval in December 2012 for treatment of patients with resistant or intolerant CML. Just as with second generation TK inhibitors, early approval is being sought to extend the use of ponatinib to newly diagnosed CML also.

Vaccination

In 2005, encouraging but mixed results of vaccination were reported with the *BCR/abl* p210 fusion protein in patients with stable disease, with GM-CSF as an adjuvant.

Prognosis

Before the advent of tyrosine kinase inhibitors, the median survival time for CML patients had been about 3–5 years from time of diagnosis. With the use of tyrosine kinase inhibitors, survival rates have improved

dramatically. A 2006 followup of 553 patients using imatinib (Gleevec) found an overall survival rate of 89% after five years. A 2011 followup of 832 patients using imatinib who achieved a stable cytogenetic response found an overall survival rate of 95.2% after 8 years, which is similar to the rate in the general population. Fewer than 1% of patients died because of leukemia progression.

MULTIPLE MYELOMA

Multiple myeloma is a blood cancer that starts in the plasma cells in the bone marrow. Bone marrow is the soft, spongy tissue found inside most bones. It helps make blood cells. Plasma cells help your body fight infection by producing proteins called antibodies. With multiple myeloma, plasma cells grow out of control in the bone marrow and form tumors in the areas of solid bone. The growth of these bone tumors weakens the solid bones. It also makes it harder for the bone marrow to make healthy blood cells and platelets.

Causes

The cause of multiple myeloma is unknown. Past treatment with radiation therapy increases the risk for this type of cancer. Multiple myeloma mainly affects older adults.

Symptoms

Multiple myeloma most commonly causes:

- Low red blood cell count (anemia), which can lead to fatigue and shortness of breath
- Low white blood cell count, which makes you more likely to get infections
- Low platelet count, which can lead to abnormal bleeding

As the cancer cells grow in the bone marrow, you may have bone pain, most often in the ribs or back.

The cancer cells can weaken bones. As a result:

- You may develop broken bones (bone fractures) just from doing normal activities.
- If cancer grows in the spine bones, it can press on the nerves. This can lead to numbness or weakness of the arms or legs.

Exams and Tests

The health care provider will perform a physical exam and ask about your symptoms.

Blood tests can help diagnose this disease. These tests include:

- Albumin level
- Calcium level
- Total protein level
- Kidney function
- Complete blood count (CBC)
- Immunofixation
- Quantitative nephelometry.

Bone x-rays may show fractures or hollowed out areas of bone. If your provider suspects this type of cancer, a bone marrow biopsy will be performed. Bone density testing may show bone loss. If tests show that you have multiple myeloma, more tests will be done to see how far the cancer has spread. This is called staging. Staging helps guide treatment and follow-up.

Treatment

People who have mild disease or in whom the diagnosis is not certain are usually closely monitored. Some people have a form of multiple myeloma that grows slowly (smoldering myeloma), which takes years to cause symptoms. Various types of medicines are used to treat multiple myeloma. They are most often given to prevent complications such as bone fractures and kidney damage. Radiation therapy may be used to relieve bone pain or to shrink a tumor that is pushing on the spinal cord.

A bone marrow transplant may be recommended:

- An autologous bone marrow or stem cell transplantation is performed using a person's own stem cells.
- An allogeneic transplant uses someone else's stem cells. This treatment has serious risks, but may offer the chance of a cure.

You and your provider may need to manage other concerns during your treatment, including:

- Having chemotherapy at home
- Managing your pets
- Bleeding problems
- Dry mouth
- Eating enough calories
- Safe eating during cancer treatment.

Support Groups

You can ease the stress of illness by joining a cancer support group. Sharing with others who have common experiences and problems can help you not feel alone.

Outlook (Prognosis)

Outlook depends on the person's age and the stage of disease. In some cases, the disease progresses very rapidly. In other cases, it takes years for symptoms to appear. In general, multiple myeloma is treatable, but only in rare cases can it be cured.

Possible Complications

Kidney failure is a frequent complication. Others may include:

- Bone fractures
- High level of calcium in the blood, which can be very dangerous
- Increased chances for infection, especially in the lungs
- Anemia
- Weakness or loss of movement due to tumor pressing on spinal cord.

When to Contact a Medical Professional

Call your provider if you have multiple myeloma and you develop an infection, or numbness, loss of movement, or loss of sensation.

Alternative Names

Plasma cell dyscrasia; Plasma cell myeloma; Malignant plasmacytoma; Plasmacytoma of bone; Myeloma - multiple

Patient Instructions

- Bone marrow transplant - discharge.

MYELOFIBROSIS

Myelofibrosis, also known as osteomyelofibrosis, is a relatively rare bone marrow cancer. It is currently classified as a myeloproliferative neoplasm, in which the proliferation of an abnormal clone of hematopoietic stem cells in the bone marrow and other sites results in fibrosis, or the replacement of the marrow with scar tissue. The term *myelofibrosis* alone usually refers to primary myelofibrosis (PMF), also known as chronic idiopathic myelofibrosis (cIMF); the terms idiopathic and primary mean that in these cases the disease is of unknown or spontaneous origin. This is in contrast with myelofibrosis that develops secondary to polycythemia vera or essential thrombocythaemia. Myelofibrosis is a form of myeloid metaplasia, which refers to a change in cell type in the blood-forming tissue of the bone marrow, and often the two terms are used synonymously. The terms agnogenic myeloid metaplasia and myelofibrosis with myeloid metaplasia (MMM) are also used to refer to primary myelofibrosis.

Signs and Symptoms

The primary sign of myelofibrosis is reactive bone marrow fibrosis, but it is often accompanied by:

- Abdominal fullness related to an enlarged spleen (splenomegaly).
- Bone pain
- Bruising and easy bleeding due to inadequate numbers of platelets
- Cachexia (loss of appetite, weight loss, and fatigue)
- Enlargement of both the liver and spleen
- Fatigue
- Gout and high uric acid levels
- Increased susceptibility to infection, such as pneumonia
- Pallor and shortness of breath due to anemia
- In rarer cases, a raised red blood cell volume
- Cutaneous myelofibrosis is a rare skin condition characterized by dermal and subcutaneous nodules.

Causes

There is an association between mutations to the JAK2, CALR, or MPL gene and myelofibrosis. Approximately 90% of those with myelofibrosis have one of these mutations and 10% carry none of these mutations. These mutations are not specific to myelofibrosis, and are linked to other myeloproliferative neoplasms, specifically polycythemia vera and essential thrombocythemia.

The V617F mutation to the JAK2 protein is found in approximately half of individuals with primary myelofibrosis. The V617F mutation is a change of valine to phenylalanine at the 617 position. Janus kinases (JAKs) are non-receptor tyrosine kinases essential for the activation of signaling that is mediated by cytokine receptors lacking catalytic activity. These include receptors for erythropoietin, thrombopoietin, most interleukins and interferon. JAK2 mutations are significant because JAK2 plays a role in controlling production of blood cells from hematopoietic stem cells. The V617F mutation appears to make hematopoietic cells more sensitive to growth factors that need JAK2 for signal transduction, which include erythropoietin and thrombopoietin. The MPL gene codes for a protein that acts as a receptor for thrombopoietin. A mutation in that gene, known as a W515 mutation, leads to the production of an abnormal thrombopoietin receptor protein, which results in the

overproduction of abnormal megakaryocytes. The abnormal megakaryocytes stimulate other cells, the fibroblasts, to produce collagen in the bone marrow.

Mechanism

Myelofibrosis is a clonal neoplastic disorder of hematopoiesis, the formation of blood cellular components. It is one of the myeloproliferative disorders, diseases of the bone marrow in which excess cells are produced at some stage. Production of cytokines such as fibroblast growth factor by the abnormal hematopoietic cell clone (particularly by megakaryocytes) leads to replacement of the hematopoietic tissue of the bone marrow by connective tissue via collagen fibrosis. The decrease in hematopoietic tissue impairs the patient's ability to generate new blood cells, resulting in progressive pancytopenia, a shortage of all blood cell types. However, the proliferation of fibroblasts and deposition of collagen is a secondary phenomenon, and the fibroblasts themselves are not part of the abnormal cell clone.

In primary myelofibrosis, progressive scarring, or fibrosis, of the bone marrow occurs, for the reasons outlined above. The result is extramedullary hematopoiesis, *i.e.,* blood cell formation occurring in sites other than the bone marrow, as the haemopoetic cells are forced to migrate to other areas, particularly the liver and spleen. This causes an enlargement of these organs. In the liver, the abnormal size is called hepatomegaly. Enlargement of the spleen is called splenomegaly, which also contributes to causing pancytopenia, particularly thrombocytopenia and anemia. Another complication of extramedullary hematopoiesis is poikilocytosis, or the presence of abnormally shaped red blood cells. Myelofibrosis can be a late complication of other myeloproliferative disorders, such as polycythemia vera, and less commonly, essential thrombocythaemia. In these cases, myelofibrosis occurs as a result of somatic evolution of the abnormal hematopoietic stem cell clone that caused the original disorder. In some cases, the development of myelofibrosis following these disorders may be accelerated by the oral chemotherapy drug hydroxyurea. The cause and risk factors for primary myelofibrosis are unknown.

Sites of Hematopoiesis

The principal site of extramedullary hematopoiesis in myelofibrosis is the spleen, which is usually markedly enlarged, sometimes weighing as much as 4000 g. As a result of massive enlargement of the spleen, multiple subcapsular infarcts often occur in the spleen, meaning that due to interrupted oxygen supply to the spleen partial or complete tissue death happens. On the cellular level, the spleen contains red blood cell precursors, granulocyte precursors and megakaryocytes, with the megakaryocytes prominent in their number and in their bizarre shapes. Megakaryocytes are believed to be involved in causing the secondary fibrosis seen in this condition, as discussed under "Mechanism" above. Sometimes unusual activity of the red blood cells, white blood cells, or platelets is seen. The liver is often moderately enlarged, with foci of extramedullary hematopoiesis. Microscopically, lymph nodes also contain foci of hematopoiesis, but these are insufficient to cause enlargement. There are also reports of hematopoiesis taking place in the lungs. These cases are associated with hypertension in the pulmonary arteries. The bone marrow in a typical case is hypercellular and diffusely fibrotic. Both early and late in disease, megakaryocytes are often prominent and are usually dysplastic.

Diagnosis

Epidemiologically, the disorder usually develops slowly and is mainly observed in people over the age of 50. It may also develop as a side-effect of treatment with some drugs that target hematological disorders, such as polycythemia vera or chronic myelogenous leukemia. Diagnosis of myelofibrosis is made on the basis of bone marrow biopsy. A physical exam of the abdomen may reveal enlargement of the spleen, the liver, or both.

Blood tests are also used in diagnosis. Primary myelofibrosis can begin with a blood picture similar to that found in polycythemia vera or chronic myelogenous leukemia. Most people with myelofibrosis have moderate to severe anemia. Eventually thrombocytopenia, a decrease of blood platelets develops. When viewed through a microscope, a blood smear will appear markedly abnormal, with presentation of pancytopenia, which is a reduction in the number of all blood cell types: Red blood cells, white blood cells, and platelets. Red blood cells may show abnormalities including bizarre shapes, such as teardrop-shaped cells, and nucleated red blood cell precursors may appear in the blood smear. (Normally, mature red blood cells in adults do not have a cell nucleus, and the presence of nucleated red blood cells suggests that immature cells are being released into the bloodstream in response to a very high demand for the bone marrow to produce new red blood cells.) Immature white cells are also seen in blood samples, and basophil counts are increased. When late in the disease progression an attempt is made to take a sample of bone marrow by aspiration, it may result in a dry tap, meaning that where the needle can normally suck out a sample of semi-liquid bone marrow, it produces no sample because the marrow has been replaced with collagen fibres. A bone marrow biopsy will reveal collagen fibrosis, replacing the marrow that would normally occupy the space.

Treatment

The one known curative treatment is allogeneic stem cell transplantation, but this approach involves significant risks. Other treatment options are largely supportive, and do not alter the course of the disorder (with the possible exception of ruxolitinib, as discussed below). These options may include regular folic acid, allopurinol or blood transfusions. Dexamethasone, alpha-interferon and hydroxyurea (also known as hydroxycarbamide) may play a role. Lenalidomide and thalidomide may be used in its treatment, though peripheral neuropathy is a common troublesome side-effect. Frequent blood transfusions may also be required. If the patient is diabetic and is taking a sulfonylurea, this should be stopped periodically to rule out drug-induced thrombocytopenia.

Splenectomy is sometimes considered as a treatment option for patients with myelofibrosis in whom massive splenomegaly is contributing to anaemia because of hypersplenism, particularly if they have a heavy requirement for blood transfusions. However, splenectomy in the presence of massive splenomegaly is a high-risk procedure, with a mortality risk as high as 3% in some studies. In November 2011, the FDA approved ruxolitinib (Jakafi) as a treatment for intermediate or high-risk myelofibrosis. Ruxolitinib serves as an inhibitor of JAK 1 and 2. The *New England Journal of Medicine* (NEJM) published results from two Phase III studies of ruxolitinib. These data showed that the treatment significantly reduced spleen volume, improved symptoms of myelofibrosis, and was associated with much improved overall survival rates compared to placebo.

History

Myelofibrosis was first described in 1879 by Gustav Heuck. Older terms include "myelofibrosis with myeloid metaplasia" and "agnogenic myeloid metaplasia". The World Health Organization utilized the name "chronic idiopathic myelofibrosis", while the International Working Group on Myelofibrosis Research and Treatment calls the disease "primary myelofibrosis". In 2008 WHO has adopted the name of "primary myelofibrosis." Eponyms for the disease are Heuck-Assmann disease or Assmann's Disease, for Herbert Assmann, who published a description under the term "osteosclerosis" in 1907. It was characterised as a myeloproliferative condition in 1951 by William Dameshek. The Leukemia and Lymphoma Society describes myelofibrosis as a rare type of blood cancer, manifesting as a type of chronic leukemia.

POLYCYTHEMIA VERA

Polycythemia vera is an uncommon neoplasm in which the bone marrow makes too many red blood cells. It may also result in the overproduction of white blood cells and platelets. Most of the health concerns associated

with polycythemia vera are caused by the blood being thicker as a result of the increased red blood cells. It is more common in the elderly and may be symptomatic or asymptomatic. Common signs and symptoms include itching (pruritus), and severe burning pain in the hands or feet that is usually accompanied by a reddish or bluish coloration of the skin. Patients with polycythemia vera are more likely to have gouty arthritis. Treatment consists primarily of phlebotomy.

Signs and Symptoms

People with polycythemia vera can be asymptomatic. A classic symptom of polycythemia vera is pruritus or itching, particularly after exposure to warm water (such as when taking a bath), which may be due to abnormal histamine release or prostaglandin production. Such itching is present in approximately 40% of patients with polycythemia vera. Gouty arthritis may be present in up to 20% of patients. Peptic ulcer disease is also common in patients with polycythemia vera; most likely due to increased histamine from mast cells, but may be related to an increased susceptibility to infection with the ulcer-causing bacterium *H. pylori*. Another possible mechanism for the development for peptic ulcer is increased histamine release and gastric hyperacidity related with polycythemia vera. A classic symptom of polycythemia vera (and the related myeloproliferative disease essential thrombocythemia) is erythromelalgia. This is a burning pain in the hands or feet, usually accompanied by a reddish or bluish coloration of the skin. Erythromelalgia is caused by an increased platelet count or increased platelet "stickiness" (aggregation), resulting in the formation of tiny blood clots in the vessels of the extremity; it responds rapidly to treatment with aspirin. Patients with polycythemia vera are prone to the development of blood clots (thrombosis). A major thrombotic complication (*e.g.,* heart attack, stroke, deep venous thrombosis, or Budd-Chiari syndrome) may sometimes be the first symptom or indication that a person has polycythemia vera. Headaches, lack of concentration and fatigue are common symptoms that occur in patients with polycythemia vera as well.

Pathophysiology

Polycythemia vera (PCV), being a primary polycythemia, is caused by neoplastic proliferation and maturation of erythroid, megakaryocytic and granulocytic elements to produce what is referred to as panmyelosis. In contrast to secondary polycythemias, PCV is associated with a low serum level of the hormone erythropoietin (EPO). Instead, PCV cells often carry activating mutation in the tyrosine kinase (JAK2) gene, which acts in signaling pathways of the EPO-receptor, making those cells proliferate independent from EPO.

Diagnosis

Physical exam findings are non-specific, but may include enlarged liver or spleen, plethora, or gouty nodules. The diagnosis is often suspected on the basis of laboratory tests. Common findings include an elevated hemoglobin level and hematocrit, reflecting the increased number of red blood cells; the platelet count or white blood cell count may also be increased. The erythrocyte sedimentation rate (ESR) is decreased due to a decrease in zeta potential. Because polycythemia vera results from an essential decrease in erythrocyte production, patients have a low erythropoietin (EPO) level.

In primary polycythemia, there may be 8 to 9 million and occasionally 11 million erythrocytes per cubic millimeter of blood (a normal range for adults is 4-6), and the hematocrit may be as high as 70 to 80%. In addition, the total blood volume sometimes increases to as much as twice normal. The entire vascular system can become markedly engorged with blood, and circulation times for blood throughout the body can increase up to twice the normal value. The increased numbers of erythrocytes can cause the viscosity of the blood to increase as much as five times normal. Capillaries can become plugged by the very viscous blood, and the flow of blood through the vessels tends to be extremely sluggish.

As a consequence of the above, people with untreated polycythemia vera are at a risk of various thrombotic events (deep venous thrombosis, pulmonary embolism), heart attack and stroke, and have a substantial risk of Budd-Chiari syndrome (hepatic vein thrombosis), or myelofibrosis. The condition is considered chronic; no cure exists. Symptomatic treatment can normalize the blood count and most patients can live a normal life for years. The disease appears more common in Jews of European extraction than in most non-Jewish populations. Some familial forms of polycythemia vera are noted, but the mode of inheritance is not clear. A mutation in the JAK2 kinase (V617F) is strongly associated with polycythemia vera. *JAK2* is a member of the Janus kinase family and makes the erythroid precursors hypersensitive to erythropoietin (EPO). This mutation may be helpful in making a diagnosis or as a target for future therapy.

Following history and examination, the British Committee for Standards in Haematology (BCSH) recommend the following tests are performed:

- Full blood count/film (raised haematocrit; neutrophils, basophils, platelets raised in half of patients)
- JAK2 mutation
- Serum ferritin
- Renal and liver function tests.

If the JAK2 mutation is negative and there is no obvious secondary causes the BCSH suggest the following tests:

- Red cell mass
- Arterial oxygen saturation
- Abdominal ultrasound
- Serum erythropoietin level
- Bone marrow aspirate and trephine
- Cytogenetic analysis
- Erythroid burst-forming unit (BFU-E) culture.

Other features that may be seen in polycythemia vera include a low ESR and a raised leukocyte alkaline phosphatase. The diagnostic criteria for polycythemia vera have recently been updated by the BCSH. This replaces the previous Polycythemia Vera Study Group criteria.

Criteria	Notes
A1	High erythrocyte volume fraction (EVF or haematocrit) (>0.52 in men, >0.48 in women) OR raised red cell mass (>25% above predicted)
A2	Mutation in JAK2

JAK2-negative polycythemia vera - diagnosis requires A1 + A2 + A3 + either another A or two B criteria:

Criteria	Notes
A1	Raised red cell mass (>25% above predicted) OR haematocrit >0.60 in men, >0.56 in women
A2	Absence of mutation in JAK2
A3	No cause of secondary erythrocytosis
A4	Palpable splenomegaly
A5	Presence of an acquired genetic abnormality (excluding BCR-ABL) in the haematopoietic cells
B1	Thrombocytosis (platelet count >450 * 109/l)
B2	Neutrophil leucocytosis (neutrophil count > 10 * 109/l in non-smokers; > 12.5*109/l in smokers)
B3	Radiological evidence of splenomegaly
B4	Endogenous erythroid colonies or low serum erythropoietin

Treatment

Untreated, polycythemia vera can be fatal. Research has found that the "1.5-3 years of median survival in the absence of therapy has been extended to at least 10-20 years because of new therapeutic tools." As the condition cannot be cured, treatment focuses on treating symptoms and reducing thrombotic complications by reducing

the erythrocyte levels. Phlebotomy is one form of treatment, which often may be combined with other therapies. The removal of blood from the body induces iron deficiency, thereby decreasing the haemoglobin/hematocrit level, and reducing the risk of blood clots. Phlebotomy is typically performed to bring their hematocrit (red blood cell percentage) down below 45 for men or 42 for women. It has been observed that phlebotomy also improves cognitive impairment. Low dose aspirin (75–81 mg daily) is often prescribed. Research has shown that aspirin reduces the risk for various thrombotic complications.

Chemotherapy for polycythemia may be used, either for maintenance, or when the rate of bloodlettings required to maintain normal hematocrit is not acceptable, or when there is significant thrombocytosis or intractable pruritus. This is usually with a "cytoreductive agent" (hydroxyurea, also known as hydroxycarbamide). The tendency of some practitioners to avoid chemotherapy if possible, especially in young patients, is a result of research indicating possible increased risk of transformation to acute myelogenous leukemia (AML). While hydroxyurea is considered safer in this aspect, there is still some debate about its long-term safety. In the past, injection of radioactive isotopes (principally phosphorus-32) was used as another means to suppress the bone marrow. Such treatment is now avoided due to a high rate of AML transformation.

Other therapies include interferon injections, and in cases where secondary thrombocytosis (high platelet count) is present, anagrelide may be prescribed. Bone marrow transplants are rarely undertaken in people with polycythemia; since this condition is non-fatal if treated and monitored, the benefits rarely outweigh the risks involved in such a procedure. There are indications that with certain genetic markers, erlotinib may be an additional treatment option for this condition. The JAK2 inhibitor, ruxolitinib, is also used to treat polycythemia.

Epidemiology

Polycythemia vera occurs in all age groups, although the incidence increases with age. One study found the median age at diagnosis to be 60 years, while a Mayo Clinic study in Olmsted County, Minnesota found that the highest incidence was in people aged 70–79 years. The overall incidence in the Minnesota population was 1.9 per 100,000 person-years, and the disease was more common in men than women. A cluster around a toxic site was confirmed in northeast Pennsylvania in 2008.

ESSENTIAL THROMBOCYTHEMIA

Essential thrombocythemia (ET) is a rare chronic blood condition characterised by the overproduction of platelets (thrombocytes) by megakaryocytes in the bone marrow. It may, albeit rarely, develop into acute myeloid leukemia or myelofibrosis. It is one of four myeloproliferative neoplasms (blood disorders that occur when the body makes too many white or red blood cells, or platelets).

Signs and Symptoms

Most people with essential thrombocythemia are without symptoms at the time of diagnosis, which is usually made after noting an elevated platelet level on a routine complete blood count (CBC). The most common symptoms are bleeding (due to dysfunctional platelets), blood clots (*e.g.,* deep vein thrombosis or pulmonary embolism), headache, nausea, vomiting, abdominal pain, visual disturbances, dizziness, fainting, and numbness in the extremities; the most common signs are increased white blood cell count, reduced red blood cell count, and an enlarged spleen.

Cause

In ET, megakaryocytes are more sensitive to growth factors. Platelets derived from the abnormal megakaryocytes are activated, which, along with the elevated platelet count, contributes to the likelihood of

forming blood clots. The increased possibility of bleeding when the platelet count is over 1 million is due to von Willebrand factor (vWF) sequestration by the increased mass of platelets, leaving insufficient vWF for platelet adhesion. A mutation in the JAK2 kinase (V617F) is present in 40–50% of cases and is diagnostic if present. *JAK2* is a member of the Janus kinase family. In 2013, two groups detected calreticulin mutations in a majority of JAK2-negative/MPL-negative patients with essential thrombocythemia and primary myelofibrosis, which makes *CALR* mutations the second most common in myeloproliferative neoplasms. All mutations (insertions or deletions) affected the last exon, generating a reading frame shift of the resulting protein, that creates a novel terminal peptide and causes a loss of endoplasmic reticulum KDEL retention signal.

Diagnosis

The following revised diagnostic criteria for essential thrombocythaemia were proposed in 2005. The diagnosis requires the presence of both A criteria together with B3 to B6, or of criterion A1 together with B1 to B6.

The criteria are as follows:

- A1. Platelet count > 450 × 10/μL for at least 2 months.
- A2. Acquired V617F JAK2 mutation present
- B1. No cause for a reactive thrombocytosis
  - normal inflammatory indices
- B2. No evidence of iron deficiency
  - stainable iron in the bone marrow or normal red cell mean corpuscular volume
- B3. No evidence of polycythemia vera
  - hematocrit < midpoint of normal range or normal red cell mass in presence of normal iron stores
- B4. No evidence of chronic myeloid leukemia
  - But the Philadelphia chromosome may be present in up to 10% of cases. Patients with the Philadelphia chromosome have a potsential for the development of acute leukemia, especially acute lymphocytic leukemia.
- B5. No evidence of myelofibrosis
  - no collagen fibrosis and d" grade 2 reticulin fibrosis (using 0–4 scale)
- B6. No evidence of a myelodysplastic syndrome
  - no significant dysplasia
  - no cytogenetic abnormalities suggestive of myelodysplasia.

Treatment

Indications

Not all those affected will require treatment at presentation. People are usually split up into low and high risk for bleeding/blood clotting groups (based on their age, their medical history, their blood counts and their lifestyles), low risk individuals are usually treated with aspirin, whereas those at high risk are given hydroxycarbamide and/or other treatments that reduce platelet count (such as interferon-α and anagrelide).

Agents

Hydroxycarbamide, interferon-α and anagrelide can lower the platelet count. Low-dose aspirin is used to reduce the risk of blood clot formation unless the platelet count is very high, where there is a risk of bleeding from the disease, and hence this measure would be counter-productive as aspirin-use increases the risk of bleeding. The PT1 study compared hydroxyurea plus aspirin to anagrelide plus aspirin as initial

therapy for ET. Hydroxyurea treated patients had a lower incidence of arterial thrombosis, lower incidence of severe bleeding and lower incidence of transformation to myelofibrosis, but the risk of venous thrombosis was higher with hydroxycarbamide than with anagrelide. It is unknown whether the results are applicable to all ET patients. In people with symptomatic ET and extremely high platelet counts (exceeding 1 million), plateletpheresis can be used to remove platelets from the blood to reduce the risk of thrombosis.

Prognosis

Essential thrombocythemia is sometimes described as a slowly progressive disorder with long asymptomatic periods punctuated by thrombotic or hemorrhagic events. However, well-documented medical regimens can reduce and control the number of platelets, which reduces the risk of these thrombotic or hemorrhagic events. The lifespan of a well controlled ET person is well within the expected range for a person of similar age but without ET. ET is the myeloproliferative neoplasm least likely to progress to acute myeloid leukemia.

Epidemiology

The incidence of ET is 0.6-2.5/100,000 per year, the median age at onset is 65–70 years and it is more frequent in females than in males. The incidence in children is 0.09/100,000 per year.

Pregnancy

Hydroxycarbamide and anagrelide are contraindicated during pregnancy and nursing. Essential thrombocythemia can be linked with a three-fold increase in risk of miscarriage. Throughout pregnancy, close monitoring of the mother and fetus is recommended. Low-dose low molecular weight heparin (*e.g.,* enoxaparin) may be used. For life-threatening complications, the platelet count can be reduced rapidly using plateletpheresis, a procedure that removes platelets from the blood and returns the remainder to the patient.

THROMBOTIC THROMBOCYTOPENIC PURPURA

Thrombotic thrombocytopenic purpura (TTP) is a rare disorder of the blood-coagulation system, causing extensive microscopic clots to form in the small blood vessels throughout the body, resulting in low platelet counts. These small blood clots, called thrombi, can damage many organs including the kidneys, heart, brain, and nervous system. In the era before effective treatment with plasma exchange, the fatality rate was about 95%. With plasma exchange, this has dropped to 10% at six months.

Because the disease generally results from antibodies that activate the immune system to inhibit the ADAMTS13 enzyme, agents that suppress the immune system, such as glucocorticoids, rituximab, cyclophosphamide, vincristine, or ciclosporin, may also be used if a relapse or recurrence follows plasma exchange. Platelets are not transfused unless the patient has a life-threatening bleed, since the transfused platelets would also quickly be consumed by thrombi formation, leading to a minimal increase in circulating platelets. Most cases of TTP arise from autoantibody-mediated inhibition of the enzyme ADAMTS13, a metalloprotease responsible for cleaving large multimers of von Willebrand factor (vWF) into smaller units. The increase in circulating multimers of vWF increases platelet adhesion to areas of endothelial injury, particularly where arterioles and capillaries meet, which in turn results in the formation of small platelet clots called thrombi. As platelets are used up in the formation of thrombi, this then leads to a decrease in the number of overall circulating platelets, which may then cause life-threatening bleeds. The reason why the antibodies form is generally unknown for most patients, though it can be associated with some medications and autoimmune diseases such as HIV and Lupus, as well as pregnancy. A rarer form of TTP, called Upshaw–Schulman syndrome, or "Inherited TTP," results from an autosomal recessive gene

that leads to ADAMTS13 dysfunction from the time of birth, resulting in persisting large vWF multimers, which in turn results in the formation of thrombi (small platelet clots). Red blood cells passing the microscopic clots are subjected to shear stress, which damages their membranes, leading to rupture of red blood cells within blood vessels, which in turn leads to anaemia and schistocyte formation. The presence of these blood clots in the small blood vessels reduces blood flow to organs resulting in cellular injury and end organ damage. Current therapy is based on support and plasmapheresis to reduce circulating antibodies against ADAMTS13 and replenish blood levels of the enzyme.

Signs and Symptoms

The signs and symptoms of TTP may at first be subtle and nonspecific. Many people experience an influenza-like or diarrheal illness before developing TTP. Neurological symptoms are very common and vary greatly in severity. Frequently reported symptoms include feeling very tired, confusion, and headaches. Seizures and symptoms similar to those of a stroke can also be seen. As TTP progresses, blood clots form within small blood vessels (microvasculature), and platelets (clotting cells) are consumed. As a result, bruising, and rarely bleeding can occur. The bruising often takes the form of purpura, while the most common site of bleeding, if it occurs, is from the nose or gums. Larger bruises (ecchymoses) may also develop. The classic presentation of TTP includes a constellation of five medical signs which classically support the clinical diagnosis of TTP, although it is unusual for patients to present with all 5 symptoms.

The pentad includes:

- Fever
- Changes in mental status
- Thrombocytopenia
- Reduced kidney function
- Haemolytic anaemia (microangiopathic hemolytic anemia).

High blood pressure (hypertension) may be found on examination.

Causes

TTP, as with other microangiopathic hemolytic anemias (MAHAs), is caused by spontaneous aggregation of platelets and activation of coagulation in the small blood vessels. Platelets are consumed in the aggregation process and bind vWF. These platelet-vWF complexes form small blood clots which circulate in the blood vessels and cause shearing of red blood cells, resulting in their rupture. The two best understood causes of TTP are due autoimmunity and an inherited deficiency of ADAMTS13 (known as the Upshaw-Schülman syndrome). The majority of the remaining cases are secondary to some other factor.

Autoimmune

TTP of unknown cause was long known as idiopathic TTP but in 1998 the majority of cases were shown to be caused by the inhibition of the enzyme ADAMTS13 by antibodies. The relationship of reduced ADAMTS13 to the pathogenesis of TTP is known as the Furlan-Tsai hypothesis, after the two independent groups of researchers who published their research in the same issue of the *New England Journal of Medicine*. These cases are now classed as an autoimmune disease and are known as autoimmune TTP (not to be confused with immune/idiopathic thrombocytopenic purpura). ADAMTS13 is a metalloproteinase responsible for the breakdown of von Willebrand factor (vWF), a protein that links platelets, blood clots, and the blood vessel wall in the process of blood coagulation. Very large vWF multimers are more prone to lead to coagulation. Hence, without proper cleavage of vWF by ADAMTS13, coagulation occurs at a higher rate, especially in the microvasculature, part of the

blood vessel system where vWF is most active due to high shear stress. In idiopathic TTP, severely decreased (<5% of normal) ADAMTS13 activity can be detected in most (80%) patients, and inhibitors are often found in this subgroup (44–56%).

Genetic

This condition may also be congenital. Such cases may be caused by mutations in the ADAMTS13 gene. This hereditary form of TTP is called the Upshaw–Schulman syndrome. Patients with this inherited ADAMTS13 deficiency have a surprisingly mild phenotype, but develop TTP in clinical situations with increased von Willebrand factor levels, *e.g.,* infection. Reportedly, less than 1% of all TTP cases are due to Upshaw–Schulman syndrome. Patients with this syndrome generally have 5–10% of normal ADAMTS-13 activity.

Secondary

Secondary TTP is diagnosed when the patient's history mentions one of the known features associated with TTP. It comprises about 40% of all cases of TTP.

Predisposing factors are:

- Cancer
- Bone marrow transplantation
- Pregnancy
- Medication use:
  - Antiviral drugs (acyclovir)
  - Certain chemotherapy medications such as gemcitabine and mitomycin C
  - Quinine
  - Oxymorphone
  - Quetiapine
  - Bevacizumab
  - Sunitinib
  - Platelet aggregation inhibitors (ticlopidine, clopidogrel, and prasugrel)
  - Immunosuppressants (ciclosporin, mitomycin, tacrolimus/FK506, interferon-á)
  - Hormone altering drugs (estrogens, contraceptives, hormone replacement therapy)
- HIV-1 infection.

The mechanism of secondary TTP is poorly understood, as ADAMTS13 activity is generally not as depressed as in idiopathic TTP, and inhibitors cannot be detected. Probable etiology may involve, at least in some cases, endothelial damage, although the formation of thrombi resulting in vessel occlusion may not be essential in the pathogenesis of secondary TTP. These factors may also be considered a form of secondary aHUS; patients presenting with these features are, therefore, potential candidates for anticomplement therapy.

Diagnosis

Differential Diagnosis

TTP is characterized by thrombotic microangiopathy (TMA), the formation of blood clots in small blood vessels throughout the body, which can lead to microangiopathic hemolytic anemia and thrombocytopenia. This characteristic is shared by two related syndromes, hemolytic-uremic syndrome (HUS) and atypical hemolytic uremic syndrome (aHUS). Consequently, differential diagnosis of these TMA-causing diseases is essential. In

addition to TMA, one or more of the following symptoms may be present in each of these diseases: neurological symptoms (*e.g.,* confusion, cerebral convulsions seizures,); kidney impairment (*e.g.,* elevated creatinine, decreased estimated glomerular filtration rate [eGFR], abnormal urinalysis); and gastrointestinal (GI) symptoms (*e.g.,* diarrhea nausea/vomiting, abdominal pain, gastroenteritis. Unlike HUS and aHUS, TTP is known to be caused by an acquired defect in the ADAMTS13 protein, so a lab test showing d"5% of normal ADAMTS13 levels is indicative of TTP. ADAMTS13 levels above 5%, coupled with a positive test for shiga-toxin/enterohemorrhagic *E. coli* (EHEC), are more likely indicative of HUS, whereas absence of shiga-toxin/EHEC can confirm a diagnosis of aHUS.

Treatment

Due to the high mortality of untreated TTP, a presumptive diagnosis of TTP is made even when only microangiopathic hemolytic anemia and thrombocytopenia are seen, and therapy is started. Transfusion is contraindicated in thrombotic TTP, as it fuels the coagulopathy. Since the early 1990s, plasmapheresis has become the treatment of choice for TTP. This is an exchange transfusion involving removal of the patient's blood plasma through apheresis and replacement with donor plasma (fresh frozen plasma or cryosupernatant); the procedure must be repeated daily to eliminate the inhibitor and abate the symptoms.

If apheresis is not available, fresh frozen plasma can be infused, but the volume that can be given safely is limited due to the danger of fluid overload. Plasma infusion alone is not as beneficial as plasma exchange. Corticosteroids (prednisone or prednisolone) are usually given. Rituximab, a monoclonal antibody aimed at the CD20 molecule on B lymphocytes, may be used on diagnosis; this is thought to kill the B cells and thereby reduce the production of the inhibitor. A stronger recommendation for rituximab exists where TTP does not respond to corticosteroids and plasmapheresis. Caplacizumab is an alternative option in treating TTP as it has been shown that it induces a faster disease resolution compared with those patient who were on placebo. However, the use of caplacizumab was associated with increase bleeding tendencies in the studied subjects. Most patients with refractory or relapsing TTP receive additional immunosuppressive therapy, *e.g.,* vincristine, cyclophosphamide, splenectomy or a combination of the above.

Children with Upshaw-Schülman syndrome receive prophylactic plasma every two to three weeks; this maintains adequate levels of functioning ADAMTS13. Some tolerate longer intervals between plasma infusions. Additional plasma infusions may be necessary for triggering events, such as surgery; alternatively, the platelet count may be monitored closely around these events with plasma being administered if the count drops. Measurements of blood levels of lactate dehydrogenase, platelets, and schistocytes are used to monitor disease progression or remission. ADAMTS13 activity and inhibitor levels may be measured during follow-up, but in those without symptoms the use of rituximab is not recommended.

Prognosis

The mortality rate is around 95% for untreated cases, but the prognosis is reasonably favourable (80–90% survival) for patients with idiopathic TTP diagnosed and treated early with plasmapheresis.

Epidemiology

The incidence of TTP is about 4-5 cases per million people per year. Idiopathic TTP occurs more often in women and people of African descent, and TTP secondary to autoimmune disorders such as systemic lupus erythematosus occurs more frequently in people of African descent, although other secondary forms do not show this distribution. Pregnant women and women in the *post partum* period accounted for a notable portion (12–31%) of the cases in some studies; TTP affects about one in 25,000 pregnancies.

History

TTP was initially described by Dr. Eli Moschcowitz at the Beth Israel Hospital in New York City in 1925. Moschcowitz ascribed the disease (incorrectly, as now known) to a toxic cause. Moschcowitz noted his patient, a 16-year-old girl, had anemia, petechiae (purpura), microscopic hematuria, and, at autopsy, disseminated microvascular thrombi. In 1966, a review of 16 new cases and 255 previously reported cases led to the formulation of the classical pentad of symptoms and findings (*i.e.,* thrombocytopenia, microangiopathic hemolytic anemia, neurological symptoms, kidney failure, fever); in this series, mortality rates were found to be very high (90%). While a response to blood transfusion had been noted before, a 1978 report and subsequent studies showed blood plasma was highly effective in improving the disease process. In 1991, plasma exchange was reported to provide better response rates compared to plasma infusion. In 1982, the disease had been linked with abnormally large von Willebrand factor multimers. The identification of a deficient protease in people with TTP was made in 1998s. The location of ADAMTS13 within the human genome was identified in 2001.

ACUTE KIDNEY FAILURE

Acute kidney failure is the rapid (less than 2 days) loss of your kidneys' ability to remove waste and help balance fluids and electrolytes in your body.

Causes

There are many possible causes of kidney damage. They include:

- Acute tubular necrosis (ATN)
- Autoimmune kidney disease
- Blood clot from cholesterol (cholesterol emboli)
- Decreased blood flow due to very low blood pressure, which can result from burns, dehydration, hemorrhage, injury, septic shock, serious illness, or surgery
- Disorders that cause clotting within the kidney blood vessels
- Infections that directly injure the kidney, such as acute pyelonephritis or septicemia
- Pregnancy complications, including placenta abruption or placenta previa
- Urinary tract blockage
- Illicit drugs such as cocaine and heroine
- Medicines including non-steroidal anti-inflammatory drugs (NSAIDs), certain antibiotics and blood pressure medicines, intravenous contrast (dye), some cancer and HIV drugs.

Symptoms

Symptoms of acute kidney failure may include any of the following:

- Bloody stools
- Breath odour and metallic taste in the mouth
- Bruising easily
- Changes in mental status or mood
- Decreased appetite
- Decreased sensation, especially in the hands or feet
- Fatigue or slow sluggish movements
- Flank pain (between the ribs and hips)
- Hand tremor
- Heart murmur

- High blood pressure
- Nausea or vomiting, may last for days
- Nosebleeds
- Persistent hiccups
- Prolonged bleeding
- Seizures
- Shortness of breath
- Swelling due to the body keeping in fluid (may be seen in the legs, ankles, and feet)
- Urination changes, such as little or no urine, excessive urination at night, or urination that stops completely.

Exams and Tests

The health care provider will examine you.

Tests to check how well your kidneys are working include:

- BUN
- Creatinine clearance
- Serum creatinine
- Serum potassium
- Urinalysis.

Other blood tests may be done to find the underlying cause of kidney failure. A kidney or abdominal ultrasound is the preferred test for diagnosing a blockage in the urinary tract. X-ray, CT scan, or MRI of the abdomen can also tell if there is a blockage.

Treatment

Once the cause is found, the goal of treatment is to help your kidneys work again and prevent fluid and waste from building up in your body while they heal. Usually, you will have to stay overnight in the hospital for treatment. The amount of liquid you drink will be limited to the amount of urine you can produce. You will be told what you may and may not eat to reduce the buildup of toxins that the kidneys would normally remove. Your diet may need to be high in carbohydrates and low in protein, salt, and potassium.

You may need antibiotics to treat or prevent infection. Water pills (diuretics) may be used to help remove fluid from your body. Medicines will be given through a vein to help control your blood potassium level. You may need dialysis. This is a treatment that does what healthy kidneys normally do — rid the body of harmful wastes, extra salt, and water. Dialysis can save your life if your potassium levels are dangerously high. Dialysis will also be used if:

- Your mental status changes
- You develop pericarditis
- You retain too much fluid
- You cannot remove nitrogen waste products from your body.

Dialysis will most often be short term. In some cases, the kidney damage is so great that dialysis is needed permanently.

When to Contact a Medical Professional

Call your provider if your urine output slows or stops or you have other symptoms of acute kidney failure.

Prevention

To prevent acute kidney failure:

- Health problems such as high blood pressure or diabetes should be well controlled.
- Avoid drugs and medicines that can cause kidney injury.

Alternative Names

Kidney failure; Renal failure; Renal failure - acute; ARF; Kidney injury - acute.

SYMPTOMS

Symptoms may include any of the following:

- Heavy menstrual periods or prolonged bleeding (more than 5 days each period)
- Abnormal vaginal bleeding
- Blood in the urine
- Bleeding under the skin or into the muscles
- Bruising easily or pinpoint red spots on the skin
- Gastrointestinal bleeding resulting in bloody, dark black, or tarry bowel movements; or vomiting blood or material that looks like coffee grounds.
- Nose bleeds.

EXAMS AND TESTS

Tests that may done include:

- Platelet aggregation test
- Platelet count
- PT and PTT.

TREATMENT

Treatment is aimed at fixing the cause of the problem:

- Bone marrow disorders are often treated with platelet transfusions or removing platelets from the blood (platelet pheresis).
- Chemotherapy may be used to treat an underlying condition that is causing the problem.
- Platelet function defects caused by kidney failure are treated with dialysis or medicines.
- Platelet problems caused by a certain medicine are treated by stopping the drug.

OUTLOOK (PROGNOSIS)

Most of the time, treating the cause of the problem corrects the defect.

POSSIBLE COMPLICATIONS

Complications may include:

- Bleeding that does not stop easily
- Anemia (due to excessive bleeding).

WHEN TO CONTACT A MEDICAL PROFESSIONAL

Call your health care provider if:

- You have bleeding and do not know the cause
- Your symptoms get worse
- Your symptoms do not improve after you are treated for an acquired platelet function defect.

PREVENTION

Using medicines as directed can reduce the risk of drug-related acquired platelet function defects. Treating other disorders may also reduce the risk. Some cases cannot be prevented.

ALTERNATIVE NAMES

Acquired qualitative platelet disorders; Acquired disorders of platelet function

CONGENITAL PLATELET FUNCTION DEFECTS

Congenital platelet function defects are conditions that prevent clotting elements in the blood, called platelets, from working as they should. Platelets help the blood clot. Congenital means present from birth.

CAUSES

Congenital platelet function defects are bleeding disorders that cause reduced platelet function.

Most of the time, people with these disorders have a family history of a bleeding disorder, such as:

- Bernard-Soulier syndrome occurs when platelets lack a substance that sticks to the walls of blood vessels. Platelets are typically large and of reduced number. This disorder may cause severe bleeding.
- Glanzmann thrombasthenia is a condition caused by the lack of a protein needed for platelets to clump together. Platelets are typically of normal size and number. This disorder may also cause severe bleeding.
- Platelet storage pool disorder (also called platelet secretion disorder) occurs when substances called granules inside platelets aren't stored or released properly. Granules help platelets function properly. This disorder causes easy bruising or bleeding.

SYMPTOMS

Symptoms may include any of the following:

- Excessive bleeding during and after surgery
- Bleeding gums
- Easy bruising
- Heavy menstrual periods
- Nosebleeds
- Prolonged bleeding with small injuries.

EXAMS AND TESTS

The following tests may be used to diagnose this condition:

- Complete blood count (CBC)

- Partial thromboplastin time (PTT)
- Platelet aggregation test
- Prothrombin time (PT)
- Platelet function analysis
- Flow cytometry.

You may need other tests. Your relatives may need to be tested.

TREATMENT

There is no specific treatment for these disorders. However, your health care provider will likely monitor your condition. *You may also need:*

- To avoid taking aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen and naproxen, because they can worsen bleeding symptoms.
- Platelet transfusions, such as during surgery or dental procedures.

Outlook (Prognosis)

There is no cure for congenital platelet function disorders. Most of the time, treatment can control the bleeding.

POSSIBLE COMPLICATIONS

Complications may include:

- Severe bleeding
- Iron deficiency anemia in menstruating women.

WHEN TO CONTACT A MEDICAL PROFESSIONAL

Call your provider if:

- You have bleeding or bruising and do not know the cause.
- Bleeding does not respond to the usual method of control.

PREVENTION

A blood test can detect the gene responsible for the platelet defect. You may wish to seek genetic counseling if you have a family history of this problem and are considering having children.

ALTERNATIVE NAMES

Platelet storage pool disorder; Glanzmann's thrombasthenia; Bernard-Soulier syndrome; Platelet function defects - congenital.

DISSEMINATED INTRAVASCULAR COAGULATION (DIC)

Disseminated intravascular coagulation (DIC) is a condition in which blood clots form throughout the body blocking small blood vessels. Symptoms may include chest pain, shortness of breath, leg pain, problems speaking, or problems moving parts of the body. As clotting factors and platelets are used up, bleeding may occur. This may include blood in the urine, blood in the stool, or bleeding into the skin. Complications may include organ failure. Relatively common causes include sepsis, surgery, major trauma, cancer, and complications

of pregnancy. Less common causes include snake bites, frostbite, and burns. There are two main types: acute (rapid onset) and chronic (slow onset). Diagnosis is typically based on blood tests. Findings may include low platelets, low fibrinogen, high INR, or high D-dimer. Treatment is mainly directed towards the underlying condition. Other measures may include giving platelets, cryoprecipitate, or fresh frozen plasma. Evidence to support these treatments, however, is poor. Heparin may be useful in the chronic form. About 1% of people admitted to hospital are affected by the condition. In those with sepsis rates are between 20% and 50%. The risk of death among those affected varies from 20 to 50%.

SIGNS AND SYMPTOMS

In DIC, the underlying cause usually leads to symptoms and signs, and DIC is discovered on laboratory testing. The onset of DIC can be sudden, as in endotoxic shock or amniotic fluid embolism, or it may be insidious and chronic, as in cancer. DIC can lead to multiorgan failure and widespread bleeding.

CAUSES

DIC can occur in the following conditions:

- Solid tumors and blood cancers (particularly acute promyelocytic leukemia)
- Obstetric complications: abruptio placentae, pre-eclampsia or eclampsia, amniotic fluid embolism, retained intrauterine fetal demise, septic abortion, post partum haemorrhage
- Massive tissue injury: severe trauma, burns, hyperthermia, rhabdomyolysis, extensive surgery
- Sepsis or severe infection of any kind (infections by nearly all microorganisms can cause DIC, though bacterial infections are the most common): bacterial (Gram-negative and Gram-positive sepsis), viral, fungal, or protozoan infections
- Transfusion reactions (*i.e.,* ABO incompatibility haemolytic reactions)
- Severe allergic or toxic reactions (*i.e.,* snake venom)
- Giant haemangiomas (Kasabach-Merritt syndrome)
- Large aortic aneurysms.

Liver disease, HELLP syndrome, thrombotic thrombocytopenic purpura/Haemolytic uremic syndrome, and malignant hypertension may mimic DIC but do not occur via the same pathways.

PATHOPHYSIOLOGY

Under homeostatic conditions, the body is maintained in a finely tuned balance of coagulation and fibrinolysis. The activation of the coagulation cascade yields thrombin that converts fibrinogen to fibrin; the stable fibrin clot being the final product of hemostasis. The fibrinolytic system then functions to break down fibrinogen and fibrin. Activation of the fibrinolytic system generates plasmin (in the presence of thrombin), which is responsible for the lysis of fibrin clots. The breakdown of fibrinogen and fibrin results in polypeptides called fibrin degradation products (FDPs) or fibrin split products (FSPs). In a state of homeostasis, the presence of plasmin is critical, as it is the central proteolytic enzyme of coagulation and is also necessary for the breakdown of clots, or fibrinolysis.

In DIC, the processes of coagulation and fibrinolysis are dysregulated, and the result is widespread clotting with resultant bleeding. Regardless of the triggering event of DIC, once initiated, the pathophysiology of DIC is similar in all conditions. One critical mediator of DIC is the release of a transmembrane glycoprotein called tissue factor (TF). TF is present on the surface of many cell types (including endothelial cells, macrophages, and monocytes) and is not normally in contact with the general circulation, but is exposed to the circulation after vascular damage. For example, TF is released in response to exposure to cytokines (particularly interleukin 1), tumor necrosis factor, and endotoxin. This plays a major role in the development of DIC in septic conditions.

TF is also abundant in tissues of the lungs, brain, and placenta. This helps to explain why DIC readily develops in patients with extensive trauma. Upon exposure to blood and platelets, TF binds with activated factor VIIa (normally present in trace amounts in the blood), forming the extrinsic tenase complex. This complex further activates factor IX and X to IXa and Xa, respectively, leading to the common coagulation pathway and the subsequent formation of thrombin and fibrin.

The release of endotoxin is the mechanism by which Gram-negative sepsis provokes DIC. In acute promyelocytic leukemia, treatment causes the destruction of leukemic granulocyte precursors, resulting in the release of large amounts of proteolytic enzymes from their storage granules, causing microvascular damage. Other malignancies may enhance the expression of various oncogenes that result in the release of TF and plasminogen activator inhibitor-1 (PAI-1), which prevents fibrinolysis. Excess circulating thrombin results from the excess activation of the coagulation cascade. The excess thrombin cleaves fibrinogen, which ultimately leaves behind multiple fibrin clots in the circulation. These excess clots trap platelets to become larger clots, which leads to microvascular and macrovascular thrombosis. This lodging of clots in the microcirculation, in the large vessels, and in the organs is what leads to the ischemia, impaired organ perfusion, and end-organ damage that occurs with DIC.

Coagulation inhibitors are also consumed in this process. Decreased inhibitor levels will permit more clotting so that a positive feedback loop develops in which increased clotting leads to more clotting. At the same time, thrombocytopenia occurs and this has been attributed to the entrapment and consumption of platelets. Clotting factors are consumed in the development of multiple clots, which contributes to the bleeding seen with DIC. Simultaneously, excess circulating thrombin assists in the conversion of plasminogen to plasmin, resulting in fibrinolysis. The breakdown of clots results in an excess of FDPs, which have powerful anticoagulant properties, contributing to hemorrhage. The excess plasmin also activates the complement and kinin systems. Activation of these systems leads to many of the clinical symptoms that patients experiencing DIC exhibit, such as shock, hypotension, and increased vascular permeability. The acute form of DIC is considered an extreme expression of the intravascular coagulation process with a complete breakdown of the normal homeostatic boundaries. DIC is associated with a poor prognosis and a high mortality rate.

There has been a recent challenge however to the basic assumptions and interpretations of the pathophysiology of DIC. A study of sepsis and DIC in animal models has shown that a highly expressed receptor on the surface of hepatocytes, termed the Ashwell-Morell receptor, is responsible for thrombocytopenia in bacteremia and sepsis due to Streptococcus pneumoniae (SPN) and possibly other pathogens. The thrombocytopenia observed in SPN sepsis was not due to increased consumption of coagulation factors such as platelets, but instead was the result of this receptor's activity enabling hepatocytes to ingest and rapidly clear platelets from circulation.

By removing pro-thrombotic components before they participate in the coagulopathy of DIC, the Ashwell-Morell receptor lessens the severity of DIC, reducing thrombosis and tissue necrosis, and promoting survival. The hemorrhage observed in DIC and among some tissues lacking this receptor may thereby be secondary to increased thrombosis with loss of the mechanical vascular barrier. This discovery has possible significant clinical implications in devising new approaches to reducing the morbidity and mortality of DIC. There is activation of intrinsic as well as extrinsic coagulation pathway, this results in excess thrombus formation in the blood vessels. Due to extensive coagulation there is consumption of coagulation factors which causes bleeding.

DIAGNOSIS

The diagnosis of DIC is not made on a single laboratory value, but rather the constellation of laboratory markers and a consistent history of an illness known to cause DIC. Laboratory markers consistent with DIC include:

- Characteristic history (this is important because severe liver disease can essentially have the same laboratory findings as DIC)

- Prolongation of the prothrombin time (PT) and the activated partial thromboplastin time (aPTT) reflect the underlying consumption and impaired synthesis of the coagulation cascade.
- Fibrinogen level has initially thought to be useful in the diagnosis of DIC but because it is an acute phase reactant, it will be elevated due to the underlying inflammatory condition. Therefore, a normal (or even elevated) level can occur in over 57% of cases. A low level, however, is more consistent with the consumptive process of DIC.
- A rapidly declining platelet count
- High levels of fibrin degradation products, including D-dimer, are found owing to the intense fibrinolytic activity stimulated by the presence of fibrin in the circulation.
- The peripheral blood smear may show fragmented red blood cells (known as schistocytes) due to shear stress from thrombi. However, this finding is neither sensitive nor specific for DIC

A diagnostic algorithm has been proposed by the International Society of Thrombosis and Haemostasis. This algorithm appears to be 91% sensitive and 97% specific for the diagnosis of overt DIC. A score of 5 or higher is compatible with DIC and it is recommended that the score is repeated daily, while a score below 5 is suggestive but not affirmative for DIC and it is recommended that it is repeated only occasionally: It has been recommended that a scoring system be used in the diagnosis and management of DIC in terms of improving outcome.

- Presence of an underlying disorder known to be associated with DIC (no=0, yes=2)
- Global coagulation results,
  - Platelet count (>100k = 0, <100 = 1, <50 = 2)
  - Fibrin degradation products such as D-Dimer (no increase = 0, moderate increase = 2, strong increase = 3)
  - Prolonged prothrombin time (<3 sec = 0, >3 sec = 1, >6 sec = 2)
  - Fibrinogen level (> 1.0g/L = 0; < 1.0g/L = 1).

TREATMENT

Treatment of DIC is centered around treating the underlying condition. Transfusions of platelets or fresh frozen plasma can be considered in cases of significant bleeding, or those with a planned invasive procedure. The target goal of such transfusion depends on the clinical situation. Cryoprecipitate can be considered in those with a low fibrinogen level.

Treatment of thrombosis with anticoagulants such as heparin is rarely used due to the risk of bleeding. Recombinant human activated protein C was previously recommended in those with severe sepsis and DIC, but drotrecogin alfa has been shown to confer no benefit and was withdrawn from the market in 2011. Recombinant factor VII has been proposed as a "last resort" in those with severe hemorrhage due to obstetric or other causes, but conclusions about its use are still insufficient.

PROGNOSIS

Prognosis varies depending on the underlying disorder, and the extent of the intravascular thrombosis (clotting). The prognosis for those with DIC, regardless of cause, is often grim: Between 20% and 50% of patients will die. DIC with sepsis (infection) has a significantly higher rate of death than DIC associated with trauma.

EPIDEMIOLOGY

DIC is observed in approximately 1% of academic hospital admissions. DIC occurs at higher rates in people with bacterial sepsis (83%), severe trauma (31%), and cancer (6.8%).

HYPOPROTHROMBINEMIA

Hypoprothrombinemia is a rare blood disorder in which a deficiency in immunoreactive prothrombin (Factor II), produced in the liver, results in an impaired blood clotting reaction, leading to an increased physiological risk for spontaneous bleeding. This condition can be observed in the gastrointestinal system, cranial vault, and superficial integumentary system, effecting both the male and female population. Prothrombin is a critical protein that is involved in the process of hemostasis, as well as illustrating procoagulant activities. This condition is characterized as an autosomal recessive inheritance congenital coagulation disorder affecting 1 per 2,000,000 of the population, worldwide, but is also attributed as acquired.

MECHANISM

Hypoprothrombinemia is found to present itself as either inherited or acquired, and is a decrease in the synthesis of prothrombin. In the process of inheritance, it marks itself as an autosomal recessive disorder, meaning that both parents must be carriers of the defective gene in order for the disorder to be present in a child. Prothrombin is a glycoprotein that occurs in blood plasma and functions as a precursor to the enzyme, thrombin, which acts to convert fibrinogen into fibrin, therefore, fortifying clots. This clotting process is known as coagulation.

The mechanism specific to prothrombin (factor II) includes the proteolytically cleaving, breakdown of proteins into smaller polypeptides or amino acids, of this coagulation factor in order to form thrombin at the beginning of the cascade, leading to stemming of blood loss. A mutation in factor II would essentially lead to hypoprothrombinemia. The mutation is presented on chromosome 11. Areas where the disease has been shown to present itself at include the liver, since the glycoprotein is stored in this area.

Acquired cases are results from an isolated factor II deficiency. Specific cases include:

1. Vitamin-K Deficiency: In the liver, vitamin K plays an important role in the synthesis of coagulation factor II. Body's capacity in the storage of vitamin K is typically very low. Vitamin K-dependent coagulation factors have a very short half-life, sometimes leading to a deficiency when a depletion of vitamin K occurs. The liver synthesizes inactive precursor proteins in the absence of vitamin K (liver disease). Vitamin K deficiency leads to impaired clotting of the blood and in some cases, causes internal bleeding without an associated injury.
2. Disseminated Intravascular Coagulation (DIC): Involving abnormal, excessive generation of thrombin and fibrin within the blood. Relative to hypoprothrombinemia, due to increased platelet aggregation and coagulation factor consumption involved in the process.
3. Anticoagulants: Warfarin Overdose: Used as a treatment for prevention of blood clots, however, like most drugs, side effects have been shown to increase risk of excessive bleeding by functioning in the disruption of hepatic synthesis of coagulation factors II, VII, IX, and X. Vitamin K is an antagonist to warfarin drug, reversing its activity, causing it to be less effective in the process of blood clotting. Warfarin intake has been shown to interfere with Vitamin-K metabolism.

SYMPTOMS

There are various symptoms that are presented and are typically associated to a specific site that they appear at. Hypoprothrombinemia is characterized by a poor blood clotting function of prothrombin. Some symptoms are presented as severe, while others are mild, meaning that blood clotting is slower than normal. Areas that are usually affected are muscles, joints, and the brain, however, these sites are more uncommon.

The most common symptoms include:

1. Easy bruising

2. Oral mucosal bleeding - Bleeding of the membrane mucus lining inside of the mouth.
3. Soft tissue bleeding.
4. Hemarthrosis - Bleeding in joint spaces.
5. Epistaxis - Acute hemorrhages from areas of the nasal cavity, nostrils, or nasopharynx.
6. Women with this deficiency experience menorrhagia: prolonged, abnormal heavy menstrual bleeding. This is typically a symptom of the disorder when severe blood loss occurs.

Other reported symptoms that are related to the condition:

1. Prolonged periods of bleeding due to surgery, injury, or post birth.
2. Melena - Associated with acute gastrointestinal bleeding, dark black, tarry feces.
3. Hematochezia - Lower gastrointestinal bleeding, passage of fresh, bright red blood through the anus secreted in or with stools. If associated with upper gastrointestinal bleeding, suggestive of a more life-threatening issue.

Type I: Severe hemorrhages are indicators of a more severe prothrombin deficiency that account for muscle hematomas, intracranial bleeding, postoperative bleeding, and umbilical cord hemorrhage, which may also occur depending on the severity, respectively.

Type II: Symptoms are usually more capricious, but can include a variety of the symptoms described previously. Less severe cases of the disorder typically do not involve spontaneous bleeding.

CAUSES AND PREVENTION

Hypoprothrombinemia can be the result of a genetic defect, may be acquired as the result of another disease process, or may be an adverse effect of medication. For example, 5-10% of patients with systemic lupus erythematosus exhibit acquired hypoprothrombinemia due to the presence of autoantibodies which bind to prothrombin and remove it from the bloodstream (lupus anticoagulant-hypoprothrombinemia syndrome). The most common viral pathogen that is involved is Adenovirus, with a prevalence of 50% in postviral cases.

Inheritance:

- Autosomal recessive condition in which both parents must carry the recessive gene in order to pass the disease on to offspring. If both parents have the autosomal recessive condition, the chance of mutation in offspring increases to 100%. An individual will be considered a carrier if one mutant copy of the gene is inherited, and will not illustrate any symptoms. The disease affects both men and women equally, and overall, is a very uncommon inherited or acquired disorder.

Non-inheritance and other factors:

- There are two types of prothrombin deficiencies that occur depending on the mutation:
- Type I (true deficiency), includes a missense or nonsense mutation, essentially decreasing prothrombin production. This is associated with bleeding from birth. Here, plasma levels of prothrombin are typically less than 10% of normal levels.
- Type II, known as dysprothrombinemia, includes a missense mutation at specific Xa factor cleavage sites and serine protease prothrombin regions. Type II deficiency creates a dysfunctional protein with decreased activity and usually normal or low-normal antigen levels. A vitamin K-dependent clotting factor is seldom seen as a contributor to inherited prothrombin deficiencies, but lack of Vitamin K decreases the synthesis of prothrombin in liver cells.
- Acquired underlying causes of this condition include severe liver disease, warfarin overdose, platelet disorders, and disseminated intravascular coagulation (DIC).
- It may also be a rare adverse effect to Rocephin.

DIAGNOSIS

Diagnosis of inherited hypoprothrombinemia, relies heavily on a patient's medical history, family history of bleeding issues, and lab exams performed by a hematologist. A physical examination by a general physician should also be performed in order to determine whether the condition is congenital or acquired, as well as ruling out other possible conditions with similar symptoms. For acquired forms, information must be taken regarding current diseases and medications taken by the patient, if applicable.

Lab tests that are performed to determine diagnosis:

1. Factor Assays: To observe the performance of specific factors (II) to identify missing/poorly performing factors. These lab tests are typically performed first in order to determine the status of the factor.
2. Prothrombin Blood Test: Determines if patient has deficient or low levels of Factor II.
3. Vitamin K1 Test: Performed to evaluate bleeding of unknown causes, nosebleeds, and identified bruising. To accomplish this, a band is wrapped around the patient's arm, 4 inches above the superficial vein site in the elbow pit. The vein is penetrated with the needle and amount of blood required for testing is obtained. Decreased vitamin K levels are suggestive of hypoprothrombinemia. However, this exam is rarely used as a Prothrombin Blood Test is performed beforehand.

TREATMENT AND PROGNOSIS

Treatment is almost always aimed to control hemorrhages, treating underlying causes, and taking preventative steps before performing invasive surgeries. Hypoprothrombinemia can be treated with periodic infusions of purified prothrombin complexes. These are typically used as treatment methods for severe bleeding cases in order to boost clotting ability and increasing levels of vitamin K-dependent coagulation factors.

1. A known treatment for hypoprothrombinemia is menadoxime.
2. Menatetrenone was also listed as a Antihaemorrhagic vitamin.
3. 4-Amino-2-methyl-1-naphthol (Vitamin K5) is another treatment for hypoprothrombinemia.
    1. Vitamin K forms are administered orally or intravenously.
4. Other concentrates include Proplex T, Konyne 80, and Bebulin VH.
   Fresh Frozen Plasma infusion (FFP) is a method used for continuous bleeding episodes, every 3-5 weeks for mention.
    1. Used to treat various conditions related to low blood clotting factors.
    2. Administered by intravenous injection and typically at a 15-20 ml/kg/dose.
    3. Can be used to treat acute bleeding.

Sometimes, underlying causes cannot be controlled or determined, so management of symptoms and bleeding conditions should be priority in treatment. Invasive options, such as surgery or clotting factor infusions, are required if previous methods do not suffice. Surgery is to be avoided, as it causes significant bleeding in patients with hypoprothrombinemia. Prognosis for patients varies and is dependent on severity of the condition and how early the treatment is managed.

1. With proper treatment and care, most people go on to live a normal and healthy life.
2. With more severe cases, a hematologist will need to be seen throughout the patient's life in order to deal with bleeding and continued risks.

RECENT RESEARCH

A 28 month old girl, showed symptoms from 8 months of age and consisted of complaints of painful bruises over lower limbs, and disturbed, painful sleep at night. Family history revealed older brother also suffered similar

problems and died at age of two years possibly due to bleeding - no diagnosis was confirmed. Complete blood count and blood smear was determined as normal. No abnormality in fibrinogen, liver function test, and bleeding time. However, prothrombin levels were less than 1% so patient was transfused with fresh frozen plasma (FFP). Post transfusion methods, patient is now 28 months old and living healthy life. The only treatment that is needed to date is for the painful bruises, which the patient is given FFP every 5-6 weeks.

Twelve day old boy admitted for symptoms consisting of blood stained vomiting and dark colored stool. Upon admission into hospital, patient received vitamin K and FFP transfusion. No family history of similarity in symptoms that were presented. At 40 days old, patient showed symptoms of tonic posturing and constant vomiting. CT scan revealed subdural hemorrhage, and other testing showed low hb levels of 7%, platelets at 3.5 lakhs/cu mm. PT examination was 51 seconds and aPTT at 87 seconds. Prothrombin activity levels were less than 1%. All other exams revealed no abnormalities. Treatment methods included vitamin K and FFP, as well as ventilator support and packed red blood cell transfusion (PRBC). At half a year of age, condition consisted of possible poor neurological outcome secondary to CNS bleeding.

Treatment of very frequent transfusion was needed for patient. Recent study illustrated a patient with 2 weeks of continuous bleeding, with presence of epistaxis, melena, hematuria, and pruritic rash with no previous bleeding history. Vitals were all within normal range, however, presence of ecchymoses was visible in chest, back and upper areas. Lab exams revealed prolonged prothrombin time (PT) of 34.4 and acquired partial thromboplastin time (aPTT) of 81.7, as well as elevated liver function tests. Discontinuation of atorvastatin, caused liver enzymes to go back to normal. Treatment of vitamin K, antibiotics, and fresh frozen plasma (FFP) did not have an impact on coagulopathy. Mixing of PT and aPTT was performed in order to further evaluate coagulopathy and revealed no correction. Factor activity assays were performed to determine the presence of a specific one. Testing revealed that factor II activity could not be quantified. Further studies showed that acquired factor II inhibitor was present without the lupus anticoagulant, with no clear cause associated with the condition. Aimed to control bleeding and getting rid of the inhibitor through directly treating the underlying disease or through immunosuppressive therapy. Corticosteroids and intravenous immunoglobulin improved the PT and aPTT. Did not improve bleeding conditions until treatment of transfusion with activated PCC. Treatment of inhibitor required Rituximab, which was shown to increase factor II levels to 264%. Study shows that when a patient with no history of coagulopathy presents themselves with hemorrhagic diathesis, direct testing of a factor II inhibitor should be performed initially.

FACTOR V

Factor V (pronounced factor five) is a protein of the coagulation system, rarely referred to as proaccelerin or labile factor. In contrast to most other coagulation factors, it is not enzymatically active but functions as a cofactor. Deficiency leads to predisposition for hemorrhage, while some mutations (most notably factor V Leiden) predispose for thrombosis.

GENETICS

The gene for factor V is located on the first chromosome (1q23). It is genomically related to the family of multicopper oxidases, and is homologous to coagulation factor VIII. The gene spans 70 kb, consists of 25 exons, and the resulting protein has a relative molecular mass of approximately 330kDa.

STRUCTURE

Factor V protein consists of six domains: A1-A2-B-A3-C1-C2. The A domains are homologous to the A domains of the copper-binding protein ceruloplasmin, and form a triangular as in that protein. A copper ion is

bound in the A1-A3 interface, and A3 interacts with the plasma. The C domains belong to the phospholipid-binding discoidin domain family, and the C2 domain mediate membrane binding. The B domain C-terminus acts as a cofactor for the anticoagulant protein C activation by protein S. Activation of factor V to factor Va is done by cleavage and release of the B domain, after which the protein no longer assists in activating protein C. The protein is now divided to a heavy chain, consisting of the A1-A2 domains, and a light chain, consisting of the A3-C1-C2 domains. Both form non-covalently a complex in a calcium-dependent manner. This complex is the pro-coagulant factor Va.

PHYSIOLOGY

Factor V synthesis occurs in the liver, principally. The molecule circulates in plasma as a single-chain molecule with a plasma half-life of 12–36 hours. Factor V is able to bind to activated platelets and is activated by thrombin. On activation, factor V is spliced in two chains (heavy and light chain with molecular masses of 110000 and 73000, respectively) which are noncovalently bound to each other by calcium. The thereby activated factor V (now called FVa) is a cofactor of the prothrombinase complex: The activated factor X (FXa) enzyme requires calcium and activated factor V to convert prothrombin to thrombin on the cell surface membrane. Factor Va is degraded by activated protein C, one of the principal physiological inhibitors of coagulation. In the presence of thrombomodulin, thrombin acts to decrease clotting by activating Protein C; therefore, the concentration and action of protein C are important determinants in the negative feedback loop through which thrombin limits its own activation.

ROLE IN DISEASE

Various hereditary disorders of factor V are known. Deficiency is associated with a rare mild form of hemophilia (termed parahemophilia or Owren parahemophilia), the incidence of which is about 1:1,000,000. It inherits in an autosomal recessive fashion. Other mutations of factor V are associated with venous thrombosis. They are the most common hereditary causes for thrombophilia (a tendency to form blood clots). The most common one of these, factor V Leiden, is due to the replacement of an arginine residue with glutamine at amino acid position 506 (R506Q). All prothrombotic factor V mutations (factor V Leiden, factor V Cambridge, factor V Hong Kong) make it resistant to cleavage by activated protein C ("APC resistance"). It therefore remains active and increases the rate of thrombin generation.

HISTORY

Until the discovery of factor V, coagulation was regarded as a product of four factors: calcium (IV) and thrombokinase (III) together acting on prothrombin (II) to produce fibrinogen (I); this model had been outlined by Paul Morawitz in 1905. The suggestion that an additional factor might exist was made by Dr Paul Owren (1905–1990), a Norwegian physician, during his investigations into the bleeding tendency of a lady called Mary (1914–2002). She had suffered from nosebleeds and menorrhagia (excessive menstrual blood loss) for most her life, and was found to have a prolonged prothrombin time, suggesting either vitamin K deficiency or chronic liver disease leading to prothrombin deficiency.

However, neither were the case, and Owren demonstrated this by correcting the abnormality with plasma from which prothrombin had been removed. Using Mary's serum as index, he found that the "missing" factor, which he labeled V (I-IV having been used in Morawitz' model), had particular characteristics. Most investigations were performed during the Second World War, and while Owren published his results in Norway in 1944, he could not publish them internationally until the war was over. They appeared finally in *The Lancet* in 1947. The possibility of an extra coagulation factor was initially resisted on methodological grounds by Drs Armand

Quick and Walter Seegers, both world authorities in coagulation. Confirmatory studies from other groups led to their final approval several years later. Owren initially felt that factor V (labile factor or proaccelerin) activated another factor, which he named VI. VI was the factor that accelerated the conversion from prothrombin to thrombin. It was later discovered that factor V was "converted" (activated) by thrombin itself, and later still that factor VI was simply the activated form of factor V. The complete amino acid sequence of the protein was published in 1987. In 1994 factor V Leiden, resistant to inactivation by protein C, was described; this abnormality is the most common genetic cause for thrombosis.

INTERACTIONS

Factor V has been shown to interact with Protein S.

FACTOR VII DEFICIENCY

Factor VII deficiency is a bleeding disorder characterized by a lack in the production of Factor VII (FVII) (proconvertin), a protein that causes blood to clot in the coagulation cascade. After a trauma factor VII initiates the process of coagulation in conjunction with tissue factor (TF/factor III) in the extrinsic pathway. The condition may be inherited or acquired. It is the most common of the rare congenital coagulation disorders.

SYMPTOMS

Symptoms may differ greatly, as apparently modifiers control to some degree the amount of FVII that is produced. Some affected individuals have few or no symptoms while others may experience life-threatening bleeding. Typically this bleeding disorder manifests itself as a tendency to easy bruising, nose bleeding, heavy and prolonged menstruation, and excessive bleeding after dental or surgical interventions. Newborns may bleed in the head, from the umbilicus, or excessively after circumcision. Other bleeding can be encountered in the gut, in muscles or joints, or the brain. Hematuria may occur. While in congenital disease symptoms may be present at birth or show up later, in patients with acquired FVII deficiency symptoms typically show up in later life. About 3-4% of patients with FVII deficiency may also experience thrombotic episodes.

CAUSES

Inherited or congenital FVII deficiency is passed on by autosomal recessive inheritance. A person needs to inherit a defective gene from both parents. People who have only one defective gene do not exhibit the disease, but can pass the gene on to half their offspring. Different genetic mutations have been described. In persons with the congenital FVII deficiency the condition is lifelong. People with this condition should alert other family members may they also have the condition or carry the gene. In the general population the condition affects about 1 in 300,000 to 500,000 people. However, the prevalence may be higher as not all individuals may express the disease and be diagnosed. In the acquired of FVII deficiency an insufficient amount of factor VII is produced by the liver due to liver disease, vitamin K deficiency, or certain medications (*i.e.,* Coumadin).

DIAGNOSIS

Blood tests are needed to differentiate FVII deficiency from other bleeding disorders. Typical is a discordance between the prolonged prothrombin time (PT) and normal levels for the activated partial thromboplastin time (APTT). FVII levels are <10IU/dl in homozygous individuals, and between 20-60 in heterozygous carriers. The FCVII: C assay supports the diagnosis. The FVII gene (F7) is found on chromosome 13q34. Heterogeneous mutations have been described in FVII deficient patients.

TREATMENT

There are several treatments available for factor VII deficiency; they all replace deficient FVII.

1. Recombinant FVIIa concentrate (rFVIIa) is a recombinant treatment that is highly effective and has no risk of fluid overload or viral disease. It may be the optimal therapy.
2. Plasma derived Factor VII concentrate (pdFVII): This treatment is suitable for surgery but can lead to thrombosis. It is virus attenuated.
3. Prothrombin complex concentrate (PCC) containing factor VII: this treatment is suitable for surgery, but has a risk of thrombosis. It is virus attenuated.
4. Fresh frozen plasma (FFP): This is relatively inexpensive and readily available. While effective this treatment carries a risk of blood-borne viruses and fluid overload.

HISTORY

The condition was first described by Drs. B. Alexander, R. Goldstein, G. Landwehr G, and CD. Cook in 1951.

FACTOR X DEFICIENCY

Factor X deficiency (X as Roman numeral ten) is a bleeding disorder characterized by a lack in the production of factor X (FX), an enzyme protein that causes blood to clot in the coagulation cascade. Produced in the liver FX when activated cleaves prothrombin to generate thrombin in the intrinsic pathway of coagulation. This process is vitamin K dependent and enhanced by activated factor V. The condition may be inherited or, more commonly, acquired.

SYMPTOMS

Symptoms may differ greatly, as apparently modifiers control to some degree the amount of FX that is produced. Some affected individuals have few or no symptoms while others may experience life-threatening bleeding. Typically this bleeding disorder manifests itself as a tendency to easy bruising, nose bleeding, heavy and prolonged menstruation and bleeding during pregnancy and childbirth, and excessive bleeding after dental or surgical interventions. Newborns may bleed in the head, from the umbilicus, or excessively after circumcision. Other bleeding can be encountered in muscles or joints, brain, gut, or urine While in congenital disease symptoms may be present at birth or show up later, in patients with acquired FX deficiency symptoms typically show up in later life.

CAUSES

Inherited or congenital FX deficiency is passed on by autosomal recessive inheritance. A person needs to inherit a defective gene from both parents. People who have only one defective gene usually do not exhibit the disease, but can pass the gene on to half their offspring. Different genetic mutations have been described. In persons with congenital FX deficiency the condition is lifelong. People affected should alert other family members as they may also have the condition or carry the gene. In the general population the condition affects about 1 in 1 million people. However, the prevalence may be higher as not all individuals may express the disease and be diagnosed. In the acquired form of FX deficiency an insufficient amount of factor X is produced by the liver due to liver disease, vitamin K deficiency, buildup of abnormal proteins in organs (amyloidosis) or certain medications (*i.e.,* warfarin). In amyloidosis FX deficiency develops as FX and other coagulation factors are absorbed by amyloid fibrils.

DIAGNOSIS

Blood tests are needed to differentiate FX deficiency from other bleeding disorders. Typical are normal thrombin time, prolonged prothrombin time (PT) and prolonged partial thromboplastin time(PTT).

FX antigen and its coagulant activity can be used to classify the severity of the condition:

1. Type I has low levels of FX antigen and activity.
2. Type II has low coagulant activity but normal or borderline FX antigen levels.

The FX (F10) gene is found on chromosome 13q34. Heterogeneous mutations have been described in FX deficient patients.

TREATMENT

There are several treatments available for bleeding due to factor X deficiency, however a specific FX concentrate is not available (2009).

1. Prothrombin complex concentrate (PCC) supplies FX with a risk of thrombosis.
2. If vitamin K levels are low, vitamin K can be supplied orally or parenterally.

Treatment of FX deficiency in amyloidosis may be more complex and involve surgery (splenectomy) and chemotherapy.

HISTORY

The condition was described independently in the 1950s. Telfer and coworkers described a female patient named Prower in 1956 and Hougie and coworker described a male patient named Stuart in 1957. When experiments showed that serum from these two patients lacked the same factor, these two patients were the first people identified with FX deficiency and the factor was called Stuart-Prower factor, later factor X.

HAEMOPHILIA C

Haemophilia C (also known as plasma thromboplastin antecedent (PTA) deficiency or Rosenthal syndrome) is a mild form of haemophilia affecting both sexes, due to factor XI deficiency. However, it predominantly occurs in Jewish people of Ashkenazi descent. It is the fourth most common coagulation disorder after von Willebrand's disease and haemophilia A and B. In the United States, it is thought to affect 1 in 100,000 of the adult population, making it 10% as common as haemophilia A.

SYMPTOMS

In terms of the signs/symptoms of haemophilia C, unlike individuals with Haemophilia A and B, people affected by it are not ones to bleed spontaneously. In these cases, haemorrhages tend to happen after a major surgery or injury.

However, people affected with haemophilia C might experience symptoms closely related to those of other forms of haemophilia such as the following:

- Oral bleeding.
- Nosebleeds.
- Blood in the urine.
- Post-partum hemorrhage (20% of cases)
- Tonsils(bleeding)

CAUSE

Haemophilia C is caused by a deficiency of coagulation factor XI and is distinguished from haemophilia A and B by the fact it does not lead to bleeding into the joints. Furthermore, it has autosomal recessive inheritance, since the gene for factor XI is located on chromosome 4 (near the prekallikrein gene); and it is not completely recessive,

individuals who are heterozygous also show increased bleeding. Many mutations exist, and the bleeding risk is not always influenced by the severity of the deficiency. Hemophilia C is developed on occasion in individuals with systemic lupus erythematosus, because of inhibitors to the FXI protein.

DIAGNOSIS

The diagnosis of haemophilia C (factor XI deficiency) is centered on prolonged activated partial thromboplastin time. One will find that the factor XI has decreased in the individuals body. In terms of differential diagnosis one must consider: factor VIII deficiency, lupus anticoagulant and heparin contamination.

TREATMENT

In terms of hemophilia C medication cyklokapron is often used for both treatment after an incident of bleeding and as a preventative measure to avoid excessive bleeding during oral surgery. Treatment is usually not necessary, except in relation to operations, leading to many of those having the condition not being aware of it. In these cases, fresh frozen plasma or recombinant factor XI may be used, but only if necessary. The afflicted may often suffer nosebleeds, while females can experience unusual menstrual bleeding which can be avoided by taking birth control such as: IUDs and oral or injected contraceptives to increase coagulation ability by adjusting hormones to levels similar to pregnancy.

GLANZMANN'S THROMBASTHENIA

Glanzmann's thrombasthenia is an abnormality of the platelets. It is an extremely rare coagulopathy (bleeding disorder due to a blood abnormality), in which the platelets contain defective or low levels of glycoprotein IIb/IIIa (GpIIb/IIIa), which is a receptor for fibrinogen. As a result, no fibrinogen bridging of platelets to other platelets can occur, and the bleeding time is significantly prolonged.

SIGNS AND SYMPTOMS

Characteristically, there is increased mucosal bleeding:

- Heavy menstrual bleeding
- Easy bruising
- Nosebleeds
- Bleeding from the gums
- Gastrointestinal bleeding
- Postpartum bleeding
- Increased postoperative bleeding.

The bleeding tendency is variable but may be severe. Bleeding into the joints, particularly spontaneous bleeds, are very rare, in contrast to the hemophilias. Platelet numbers and morphology are normal. Platelet aggregation is normal with ristocetin, but impaired with other agonists such as ADP, thrombin, collagen, or epinephrine.

CAUSE

Glanzmann's thrombasthenia can be inherited in an autosomal recessive manner or acquired as an autoimmune disorder. The bleeding tendency in Glanzmann's thrombasthenia is variable, some individuals having minimal bruising, while others have frequent, severe, potentially fatal hemorrhages. Moreover, platelet $\alpha_{IIb}\beta_3$ levels correlate poorly with hemorrhagic severity, as virtually undetectable $\alpha_{IIb}\beta_3$ levels can correlate with negligible

bleeding symptoms, and 10%–15% levels can correlate with severe bleeding. Unidentified factors other than the platelet defect itself may have important roles.

PATHOPHYSIOLOGY

Glanzmann's thrombasthenia is associated with abnormal integrin $\alpha_{IIb}\beta_3$, formerly known as glycoprotein IIb/IIIa (GpIIb/IIIa), which is an integrin aggregation receptor on platelets. This receptor is activated when the platelet is stimulated by ADP, epinephrine, collagen, or thrombin. GpIIb/IIIa is essential to blood coagulation since the activated receptor has the ability to bind fibrinogen (as well as von Willebrand factor, fibronectin, and vitronectin), which is required for fibrinogen-dependent platelet-platelet interaction (aggregation). In contrast, glycoproteinIb receptors are normal with Glanzmann's thrombasthenia. The role of GpIb is to enable platelet activation by contact with the von Willebrand factor-collagen complex that is exposed when the endothelial blood vessel lining is damaged. GpIb receptors are deficient in a disease known as Bernard–Soulier syndrome. Understanding of the role of GpIIb/IIIa in Glanzmann's thrombasthenia led to the development of GpIIb/IIIa inhibitors, a class of powerful antiplatelet agents.

TREATMENT

Therapy involves both preventive measures and treatment of specific bleeding episodes.

- Dental hygiene lessens gingival bleeding
- Avoidance of antiplatelet agents such as aspirin and other anti-inflammatory drugs (NSAIDs) such as ibuprofen and naproxen, and anticoagulants
- Iron or folate supplementation may be necessary if excessive or prolonged bleeding has caused anemia
- Hepatitis B vaccine
- Antifibrinolytic drugs such as tranexamic acid or å-aminocaproic acid (Amicar)
- Desmopressin (DDAVP) does not normalize the bleeding time in Glanzmann's thrombasthenia but anecdotally improves hemostasis
- Hormonal contraceptives to control excessive menstrual bleeding
- Topical agents such as gelfoam, fibrin sealants, polyethylene glycol polymers, custom dental splints
- Platelet transfusions (only if bleeding is severe; risk of platelet alloimmunization)
- Recombinant factor VIIa, AryoSeven or NovoSeven FDA approved this drug for the treatment of the disease on July 2014.
- Hematopoietic stem cell transplantation (HSCT) for severe recurrent hemorrhages.

EPONYM

It is named after Eduard Glanzmann (1887-1959), the Swiss pediatrician who originally described it.

HAEMOPHILIA A

Haemophilia A (or hemophilia A) is a genetic deficiency in clotting factor VIII, which causes increased bleeding and usually affects males. In the majority of cases it is inherited as an X-linked recessive trait, though there are cases which arise from spontaneous mutations. Factor VIII medication may be used to treat and prevent bleeding in people with haemophilia A.

SIGNS AND SYMPTOMS

In terms of the symptoms of haemophilia A, there are internal or external bleeding episodes. Individuals with more severe haemophilia suffer more severe and more frequent bleeding, while others 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 from a venepuncture or heelprick is another common early sign of haemophilia, these signs may lead to blood tests which indicate haemophilia. In other people, especially those with moderate or mild haemophilia, any trauma will lead to the first serious *bleed*. Haemophilia leads to a severely increased risk of prolonged bleeding from common injuries, or in severe cases bleeding may be spontaneous and without obvious cause. Bleeding may occur anywhere in the body, superficial bleeding such as those caused by abrasions, or shallow lacerations may be prolonged and the scab may easily be broken up due to the lack of fibrin, which may cause re-bleeding.

While superficial bleeding is troublesome, some of the more serious sites of bleeding are:

- Joints
- Muscles
- Digestive tract
- Brain.

Muscle and joint haemorrhages – or haemarthrosis – are indicative of haemophilia, while digestive tract and cerebral haemorrhages are also germane to other coagulation disorders. Though typically not life-threatening, joint bleeding is one of the most serious symptoms of haemophilia. Repeated bleeds into a joint capsule can cause permanent joint damage and disfigurement resulting in chronic arthritis and disability. Joint damage is not a result of blood in the capsule but rather the healing process. When blood in the joint is broken down by enzymes in the body, the bone in that area is also degraded, this exerts a lot of pain upon the person afflicted with the disease.

Complications

One therapeutic conundrum is the development of *inhibitor* antibodies against factor VIII due to frequent infusions. These develop as the body recognises the infused factor VIII as foreign, as the body does not produce its own *copy*. In these individuals, activated factor VII, a precursor to factor VIII in the coagulation cascade, can be infused as a treatment for haemorrhage in individuals with haemophilia and antibodies against replacement factor VIII.

GENETICS

Haemophilia A is inherited as an X-linked recessive trait. It occurs in males and in homozygous females (which is only possible in the daughters of a haemophilic male and a carrier or haemophiliac female). However, mild haemophilia A is known to occur in heterozygous females due to X-inactivation, so it is recommended that levels of factor VIII and IX be measured in all known or potential carriers prior to surgery and in the event of clinically significant bleeding. About 5-10% of people with haemophilia A are affected because they make a dysfunctional version of the factor VIII protein, while the remainder are affected because they produce factor VIII in insufficient amounts (quantitative deficiency). Of those who have severe deficiency (defined as <1% activity of factor VIII), 45-50% have the same mutation, an inversion within the factor VIII gene that results in total elimination of protein production. Since both forms of haemophilia can be caused by a variety of different mutations, initial diagnosis and classification is done by measurement of protein activity rather than by genetic tests, though genetic tests are recommended for testing of family members once a known case of haemophilia is identified. Approximately 30% of patients have no family history; their disease is presumably caused by new mutations.

DIAGNOSIS

The diagnosis of haemophilia A may be suspected as coagulation testing reveals an increased partial

thromboplastin time (PTT) in the context of a normal prothrombin time (PT) and bleeding time. PTT tests are the first blood test done when haemophilia is indicated. However, the diagnosis is made in the presence of very low levels of factor VIII. A family history is frequently present, although not essential. Recently, genetic testing has been made available to determine an individual's risk of attaining or passing on haemophilia. Diagnosis of haemophilia A also includes a severity level, which can range from mild to severe based on the amount of active and functioning factor VIII detected in the blood. Factor VIII levels do not typically change throughout an individual's lifetime. Severe haemophilia A is the most common severity, occurring in the majority of affected people. Individuals with mild haemophilia often experience few or no bleeding episodes except in the case of serious trauma (*i.e.,* tooth extraction, or surgery).

Severity

There are numerous different mutations which can cause haemophilia A, due to differences in changes to the factor VII gene (and the resulting protein). Individuals 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.

Differential Diagnosis

Two of the most common differential diagnoses are haemophilia B which is a deficiency in Factor IX and von Willebrand Disease which is a deficiency in von Willebrand factor (needed for the proper functioning of Factor VIII); haemophilia C is also considered.

TREATMENT

In regards to the treatment of this genetic disorder, most individuals with severe haemophilia require regular supplementation with intravenous recombinant or plasma concentrate Factor VIII. The preventative treatment regime is highly variable and individually determined. In children, an easily accessible intravenous port may have to be inserted to minimise frequent traumatic intravenous cannulation. These devices have made prophylaxis in haemophilia much easier for families because the problems of *finding a vein* for infusion several times a week are eliminated. However, there are risks involved with their use, the most worrisome being that of infection, studies differ but some show an infection rate that is high. These infections can usually be treated with intravenous antibiotics but sometimes the device must be removed, also, there are other studies that show a risk of clots forming at the tip of the catheter, rendering it useless. Some individuals with severe haemophilia, and most with moderate and mild haemophilia, treat only as needed without a regular prophylactic schedule. Mild haemophiliacs often manage their condition with desmopressin, a drug which releases stored factor VIII from blood vessel walls.

Gene Therapy

In December 2017, it was reported that doctors had used a new form of gene therapy to treat haemophilia A.

PROGNOSIS

Two Dutch studies have followed haemophilia patients for a number of years. Both studies found that viral infections were common in haemophiliacs due to the frequent blood transfusions which put them at risk of acquiring blood borne infections, such as HIV, hepatitis B and hepatitis C. In the latest study which followed patients from 1992 to 2001, the male life expectancy was 59 years. If cases with known viral infections were

excluded, the life expectancy was 72, close to that of the general population. 26% of the cases died from AIDS and 22% from hepatitis C. However, these statistics for prognosis are unreliable as there has been marked improvement of infection control and efficacy of anti-retroviral drugs since these studies were done.

EPIDEMIOLOGY

Haemophilia A occurs in approximately 1 in 5,000 males, while the incidence of haemophilia B is 1 in 30,000 in the male population, of these, 85% have haemophilia A and 15% have haemophilia B.

HAEMOPHILIA B

Haemophilia B is a blood clotting disorder causing easy bruising and bleeding due to an inherited mutation of the gene for factor IX, and resulting in a deficiency of factor IX. It is less common than factor VIII deficiency (haemophilia A). Haemophilia B was first recognized as a distinct disease entity in 1952. It is also known by the eponym *Christmas disease*, named after Stephen Christmas, the first patient described with haemophilia B. In addition, the first report of its identification was published in the Christmas edition of the *British Medical Journal*.

SIGNS AND SYMPTOMS

Symptoms include easy bruising, urinary tract bleeding (hematuria), nosebleeds (epistaxis), and bleeding into joints (hemarthrosis).

GENETICS

The factor IX gene is located on the X chromosome (Xq27.1-q27.2). It is an X-linked recessive trait, which explains why only males are affected. In 1990, George Brownlee and Merlin Crossley showed that two sets of genetic mutations were preventing two key proteins from attaching to the DNA of people with a rare and unusual form of haemophilia B – *haemophilia B Leyden* – where sufferers experience episodes of excessive bleeding in childhood but have few bleeding problems after puberty. This lack of protein attachment to the DNA was thereby turning off the gene that produces clotting factor IX, which prevents excessive bleeding.

PATHOPHYSIOLOGY

Factor IX deficiency leads to an increased propensity for haemorrhage, either spontaneously or in response to mild trauma. Factor IX deficiency can cause interference of the coagulation cascade, thereby causing spontaneous hemorrhage when there is trauma. Factor IX when activated activates factor X which helps fibrinogen to fibrin conversion. Factor IX becomes active eventually in coagulation by cofactor factor VIII (specifically IXa). Platelets provide a binding site for both cofactors. This complex (in the coagulation pathway) will eventually activate factor X.

DIAGNOSIS

The diagnosis for hemophilia B can be done via the following tests/methods:

- Coagulation screening test
- Bleeding scores
- Coagulation factor assays.

Differential Diagnosis

The differential diagnosis for this inherited condition is the following: hemophilia A, factor XI deficiency, von Willebrand disease, fibrinogen disorders and Bernard-Soulier syndrome.

TREATMENT

Treatment is given intermittently, when there is significant bleeding. It includes intravenous infusion of factor IX and/or blood transfusions. NSAIDS should be avoided once the diagnosis is made since they can exacerbate a bleeding episode. Any surgical procedure should be done with concomitant tranexamic acid.

HISTORY

In the 1950s and 1960s, with newfound technology and gradual advances in medicine, pharmaceutical scientists found a way to take the factor IX from fresh frozen plasma (FFP) and give it to those with haemophilia B. Though they found a way to treat the disease, the FFP contained only a small amount of factor IX, requiring large amounts of FFP to treat an actual bleeding episode, which resulted in the person requiring hospitalization. By the mid-1960s scientists found a way to get a larger amount of factor IX from FFP. By the late 1960s, pharmaceutical scientists found methods to separate the factor IX from plasma, which allows for neatly packaged bottles of factor IX concentrates. With the rise of factor IX concentrates it became easier for people to get treatment at home. Although these advances in medicine had a significant positive impact on the treatment of haemophilia, there were many complications that came with it. By the early 1980s, scientists discovered that the medicines they had created were transferring blood-borne viruses, such as hepatitis, and HIV, the virus that causes AIDS. With the rise of these deadly viruses, scientists had to find improved methods for screening the blood products they received from donors. In 1982, scientists made a breakthrough in medicine and were able to clone factor IX gene. With this new development it decreased the risk of the many viruses. Although the new factor was created, it wasn't available for haemophilia B patients till 1997.

VON WILLEBRAND DISEASE

Von Willebrand disease (vWD) is the most common hereditary blood-clotting disorder in humans. An acquired form can sometimes result from other medical conditions. It arises from a deficiency in the quality or quantity of von Willebrand factor (vWF), a multimeric protein that is required for platelet adhesion. As well as humans, it is known to affect several breeds of dogs. The three forms of vWD are: hereditary, acquired, and pseudo or platelet type. The three types of hereditary vWD are: vWD type 1, vWD type 2, and vWD type 3. Type 2 contains various subtypes. Platelet type vWD is also an inherited condition. vWD type 1 is the most common type of the disorder which is typically asymptomatic, though mild symptoms such as nosebleeds may occur, and occasionally more severe symptoms. Blood type can affect the presentation and severity of symptoms of vWD. vWD type 2 is the second most common type of the disorder and has mild to moderate symptoms. It is named after the Finnish physician Erik Adolf von Willebrand who first described the condition in 1926.

SIGNS AND SYMPTOMS

The various types of vWD present with varying degrees of bleeding tendency, usually in the form of easy bruising, nosebleeds, and bleeding gums. Women may experience heavy menstrual periods and blood loss during childbirth. Severe internal bleeding and bleeding into joints are uncommon in all but the most severe type, vWD type 3.

GENETICS

The vWF gene is located on the short arm *p* of chromosome 12 (12p13.2). It has 52 exons spanning 178kbp. Types 1 and 2 are inherited as autosomal dominant traits and type 3 is inherited as autosomal recessive.

Occasionally, type 2 also inherits recessively. vWD occurs in approximately 1% of the population and affects men and women equally.

PATHOPHYSIOLOGY

Von Willebrand factor is mainly active in conditions of high blood flow and shear stress. Deficiency of vWF, therefore, shows primarily in organs with extensive small vessels, such as skin, gastrointestinal tract, and uterus. In angiodysplasia, a form of telangiectasia of the colon, shear stress is much higher than in average capillaries, and the risk of bleeding is increased concomitantly. vWF carries Factor VIII. In more severe cases of type 1 vWD, genetic changes are common within the vWF gene and are highly penetrant. In milder cases of type 1 vWD, a complex spectrum of molecular pathology may exist in addition to polymorphisms of the vWF gene alone. The individual's ABO blood group can influence presentation and pathology of vWD. Those individuals with blood group O have a lower mean level than individuals with other blood groups. Unless ABO group–specific vWF:antigen reference ranges are used, normal group O individuals can be diagnosed as type I vWD, and some individuals of blood group AB with a genetic defect of vWF may have the diagnosis overlooked because vWF levels are elevated due to blood group.

DIAGNOSIS

When vWD is suspected, blood plasma of a patient must be investigated for quantitative and qualitative deficiencies of vWF. This is achieved by measuring the amount of vWF in a vWF antigen assay and the functionality of vWF with a glycoprotein (GP)Ib binding assay, a collagen binding assay, or a ristocetin cofactor activity (RiCof) or ristocetin induced platelet agglutination (RIPA) assays. Factor VIII levels are also performed because factor VIII is bound to vWF which protects the factor VIII from rapid breakdown within the blood. Deficiency of vWF can then lead to a reduction in factor VIII levels, which explains the elevation in PTT.

Normal levels do not exclude all forms of vWD, particularly type 2, which may only be revealed by investigating platelet interaction with subendothelium under flow, a highly specialized coagulation study not routinely performed in most medical laboratories. A platelet aggregation assay will show an abnormal response to ristocetin with normal responses to the other agonists used. A platelet function assay may give an abnormal collagen/epinephrine closure time, and in most cases, a normal collagen/ADP time. Type 2N may be considered if factor VIII levels are disproportionately low, but confirmation requires a "factor VIII binding" assay. Additional laboratory tests that help classify sub-types of vWD include von-willebrand multimer analysis, modified ristocetin induced platelet aggregation assay and vWF propeptide to vWF antigen ratio propeptide. In cases of suspected acquired von-Willebrand syndrome, a mixing study study (analysis of patient plasma along with pooled normal plasma/PNP and a mixture of the two tested immediately, at one hour, and at two hours) should be performed. Detection of vWD is complicated by vWF being an acute phase reactant with levels rising in infection, pregnancy, and stress.

Other tests performed in any patient with bleeding problems are a complete blood count-CBC (especially platelet counts), activated partial thromboplastin time-APTT, prothrombin time with International Normalized Ratio-PTINR, thrombin time-TT, and fibrinogen level. Testing for factor IX may also be performed if hemophilia B is suspected. Other coagulation factor assays may be performed depending on the results of a coagulation screen. Patients with von Willebrand disease typically display a normal prothrombin time and a variable prolongation of partial thromboplastin time.

The testing for vWD can be influenced by laboratory procedures. Numerous variables exist in the testing procedure that may affect the validity of the test results and may result in a missed or erroneous diagnosis. The chance of procedural errors are typically greatest during the preanalytical phase (during collecting storage and

transportation of the specimen) especially when the testing is contracted to an outside facility and the specimen is frozen and transported long distances. Diagnostic errors are not uncommon, and the rate of testing proficiency varies amongst laboratories, with error rates ranging from 7 to 22% in some studies to as high as 60% in cases of misclassification of vWD subtype. To increase the probability of a proper diagnosis, testing should be done at a facility with immediate on-site processing in a specialized coagulation laboratory.

Types

The four hereditary types of vWD described are type 1, type 2, type 3, and pseudo- or platelet-type. Most cases are hereditary, but acquired forms of vWD have been described. The International Society on Thrombosis and Haemostasis's classification depends on the definition of qualitative and quantitative defects.

Type 1

Type 1 vWD (60-80% of all vWD cases) is a quantitative defect which is heterozygous for the defective gene. It can arise from failure to secrete vWF into the circulation or from vWF being cleared more quickly than normal. Decreased levels of vWF are detected at 20-50% of normal, *i.e.,* 20-50 IU. Many patients are asymptomatic or may have mild symptoms and not have clearly impaired clotting, which might suggest a bleeding disorder. Often, the discovery of vWD occurs incidentally to other medical procedures requiring a blood work-up.

Most cases of type 1 vWD are never diagnosed due to the asymptomatic or mild presentation of type I and most people usually end up leading a normal life free of complications, with many being unaware that they have the disorder. Trouble may, however, arise in some patients in the form of bleeding following surgery (including dental procedures), noticeable easy bruising, or menorrhagia (heavy menstrual periods). The minority of cases of type 1 may present with severe hemorrhagic symptoms.

Type 2

Type 2 vWD (15-30% of cases) is a qualitative defect and the bleeding tendency can vary between individuals. Four subtypes exist: 2A, 2B, 2M, and 2N. These subtypes depend on the presence and behaviour of the underlying multimers.

Type 2A

The vWF is quantitatively normal but qualitatively defective. The ability of the defective von Willebrand factors to coalesce and form large vWF multimers is impaired, resulting in decreased quantity of large vWF multimers and low RCoF activity. Only small multimer units are detected in the circulation. Von Willebrand factor antigen (vWF:Ag) assay is low or normal.

Type 2B

This is a "gain of function" defect. The ability of the qualitatively defective vWF to bind to glycoprotein Ib (GPIb) receptor on the platelet membrane is abnormally enhanced, leading to its spontaneous binding to platelets and subsequent rapid clearance of the bound platelets and of the large vWF multimers. Thrombocytopenia may occur. Large vWF multimers are reduced or absent from the circulation. The ristocetin cofactor activity is low when the patient's platelet-poor plasma is assayed against formalin-fixed, normal donor platelets. However, when the assay is performed with the patient's own platelets (platelet-rich plasma), a lower-than-normal amount of ristocetin causes aggregation to occur. This is due to the large vWF multimers remaining bound to the

patient's platelets. Patients with this subtype are unable to use desmopressin as a treatment for bleeding, because it can lead to unwanted platelet aggregation and aggravation of thrombocytopenia.

Type 2M

Type 2M vWD is a qualitative defect of vWF characterized by its decreased ability to bind to GPIb receptor on the platelet membrane and normal capability at multimerization. The vWF antigen levels are normal. The ristocetin cofactor activity is decreased and high molecular weight large vWF multimers are present in the circulation.

Type 2N (Normandy)

This is a deficiency of the binding of vWF to coagulation factor VIII. The vWF antigen test is normal, indicating normal quantity of vWF. The ristocetin cofactor assay is normal. Assay for coagulation factor VIII revealed marked quantitative decrease equivalent to levels seen in hemophilia A. This has led to some vWD type 2N patients being misdiagnosed as having hemophilia A.

Type 3

Type 3 is the most severe form of vWD (homozygous for the defective gene) and is characterized by complete absence of production of vWF. The von Willebrand factor is undetectable in the vWF antigen assay. Since the vWF protects coagulation factor VIII from proteolytic degradation, total absence of vWF leads to extremely low factor VIII level, equivalent to that seen in severe hemophilia A with its clinical manifestations of life-threatening external and internal hemorrhages. The inheritance pattern of vWD type 3 is autosomal recessive, while the inheritance pattern of hemophilia A is X-linked recessive.

Platelet-type

Platelet-type vWD (also known as pseudo-vWD) is an autosomal dominant genetic defect of the platelets. The vWF is qualitatively normal and genetic testing of the von Willebrand gene and vWF protein reveals no mutational alteration. The defect lies in the qualitatively altered GPIb receptor on the platelet membrane which increases its affinity to bind to the vWF. Large platelet aggregates and high molecular weight vWF multimers are removed from the circulation resulting in thrombocytopenia and diminished or absent large vWF multimers. The ristocetin cofactor activity and loss of large vWF multimers are similar to vWD type 2B.

Acquired

Acquired vWD can occur in patients with autoantibodies. In this case, the function of vWF is not inhibited, but the vWF-antibody complex is rapidly cleared from the circulation. A form of vWD occurs in patients with aortic valve stenosis, leading to gastrointestinal bleeding (Heyde's syndrome). This form of acquired vWD may be more prevalent than is presently thought. In 2003, Vincentelli *et al.* noted that patients with acquired vWD and aortic stenosis who underwent valve replacement experienced a correction of their hemostatic abnormalities, but that the hemostatic abnormalities can recur after 6 months when the prosthetic valve is a poor match with the patient. Similarly, acquired vWD contributes to the bleeding tendency in people with an implant of a left ventricular assist device (a pump that pumps blood from the left ventricle of the heart into the aorta). Large multimers of vWF are destroyed by mechanical stress in both conditions. Thrombocytosis is another cause of acquired von Willebrand disease, due to sequestration of vWF via the adhesion of vast numbers of platelets. Acquired vWD has also been described in Wilms' tumour, hypothyroidism, and placental mesenchymal dysplasias.

TREATMENT

For patients with vWD type 1 and vWD type 2A, desmopressin is available as different preparations, recommended for use in cases of minor trauma, or in preparation for dental or minor surgical procedures. Desmopressin stimulates the release of vWF from the Weibel-Palade bodies of endothelial cells, thereby increasing the levels of vWF (as well as coagulant factor VIII) three- to five-fold. Desmopressin is also available as a preparation for intranasal administration (Stimate) and as a preparation for intravenous administration. Recently, the FDA has approved the use of Baxalta's Vonvendi. This is the first recombinant form of vWF. The effectiveness of this treatment is different than desmopressin because it only contains vWF, not vWF with the addition of FVIII. This treatment is only recommended for use by individuals who are 18 years of age or older. Desmopressin is contraindicated in vWD type 2b because of the risk of aggravated thrombocytopenia and thrombotic complications. Desmopressin is probably not effective in vWD type 2M and is rarely effective in vWD type 2N. It is totally ineffective in vWD type 3.

For women with heavy menstrual bleeding, estrogen-containing oral contraceptive medications are effective in reducing the frequency and duration of the menstrual periods. Estrogen and progesterone compounds available for use in the correction of menorrhagia are ethinylestradiol and levonorgestrel (Levona, Nordette, Lutera, Trivora). Administration of ethinylestradiol diminishes the secretion of luteinizing hormone and follicle-stimulating hormone from the pituitary, leading to stabilization of the endometrial surface of the uterus. Desmopressin is a synthetic analog of the natural antidiuretic hormone vasopressin. Its overuse can lead to water retention and dilutional hyponatremia with consequent convulsion.

For patients with vWD scheduled for surgery and cases of vWD disease complicated by clinically significant hemorrhage, human-derived medium purity factor VIII concentrates, which also contain von Willebrand factors, are available for prophylaxis and treatment. Humate P, Alphanate, Wilate and Koate HP are commercially available for prophylaxis and treatment of vWD. Monoclonally purified factor VIII concentrates and recombinant factor VIII concentrates contain insignificant quantity of vWF, so are not clinically useful. Development of alloantibodies occurs in 10-15% of patients receiving human-derived medium-purity factor VIII concentrates and the risk of allergic reactions including anaphylaxis must be considered when administering these preparations. Administration of the latter is also associated with increased risk of venous thromboembolic complications. Blood transfusions are given as needed to correct anemia and hypotension secondary to hypovolemia. Infusion of platelet concentrates is recommended for correction of hemorrhage associated with platelet-type vWD. The antifibrinolytic agents epsilon amino caproic acid and tranexamic acid are useful adjuncts in the management of vWD complicated by clinical hemorrhage. The use topical thrombin JMI and topical Tisseel VH are effective adjuncts for correction of hemorrhage from wounds.

EPIDEMIOLOGY

The prevalence of vWD is about one in 100 individuals. However, the majority of these people do not have symptoms. The prevalence of clinically significant cases is one per 10,000. Because most forms are rather mild, they are detected more often in women, whose bleeding tendency shows during menstruation. It may be more severe or apparent in people with blood type O.

HISTORY

In 1924, a 5-year-old girl who lived on the Finnish Åland Islands was brought to Deaconess Hospital in Berlin, Germany, where she was seen by Dr. Erik von Willebrand. He ultimately assessed 66 members of her family and reported in 1926 that this was a previously undescribed bleeding disorder that differed from hemophilia. Dr von Willebrand recognized the autosomal inheritance pattern, and noted that the bleeding symptoms were

greater in children and in women of childbearing age. Thus, he stated that patients with this syndrome had (1) mucocutaneous bleeding, (2) normal clotting time, (3) autosomal inheritance rather than being linked to the X chromosome, and (4) prolonged bleeding times by the Duke method (ear lobe bleeding time). He subsequently found that blood transfusions were useful not only to correct the anemia, but also to control bleeding. In the 1950s, it became clear that a "plasma factor", antihemophilic factor (FVIII), was decreased in these persons and that Cohn fraction I-0 could correct both the plasma deficiency of FVIII and the prolonged bleeding time. Since this time, the factor causing the long bleeding time was called the "von Willebrand factor" in honor of Dr. Erik von Willebrand. Variant forms of vWF were recognized in the 1970s, and these variations are now recognized as the result of synthesis of an abnormal protein. During the 1980s, molecular and cellular studies distinguished hemophilia A and vWD more precisely. Persons who had vWD had a normal FVIII gene on the X chromosome, and some had an abnormal vWF gene on chromosome 12. Gene sequencing identified many of these persons as having a vWF gene mutation. The genetic causes of milder forms of low vWF are still under investigation, and these forms may not always be caused by an abnormal vWF gene.

5

Thrombosis and Anticoagulation Disorders

THROMBOSIS: AN INTRODUCTION

Thrombosis is the formation of a blood clot inside a blood vessel, obstructing the flow of blood through the circulatory system. When a blood vessel (a vein or an artery) is injured, the body uses platelets (thrombocytes) and fibrin to form a blood clot to prevent blood loss. Even when a blood vessel is not injured, blood clots may form in the body under certain conditions. A clot, or a piece of the clot, that breaks free and begins to travel around the body is known as an embolus. Thrombosis may occur in veins (venous thrombosis) or in arteries. Venous thrombosis leads to congestion of the affected part of the body, while arterial thrombosis (and rarely severe venous thrombosis) affects the blood supply and leads to damage of the tissue supplied by that artery (ischemia and necrosis). A piece of either an arterial or a venous thrombus can break off as an embolus which can travel through the circulation and lodge somewhere else as an embolism. This type of embolism is known as a *thromboembolism*. Complications can arise when a venous thromboembolism (commonly called a VTE) lodges in the lung as a pulmonary embolism. An arterial embolus may travel further down the affected blood vessel where it can lodge as an embolism.

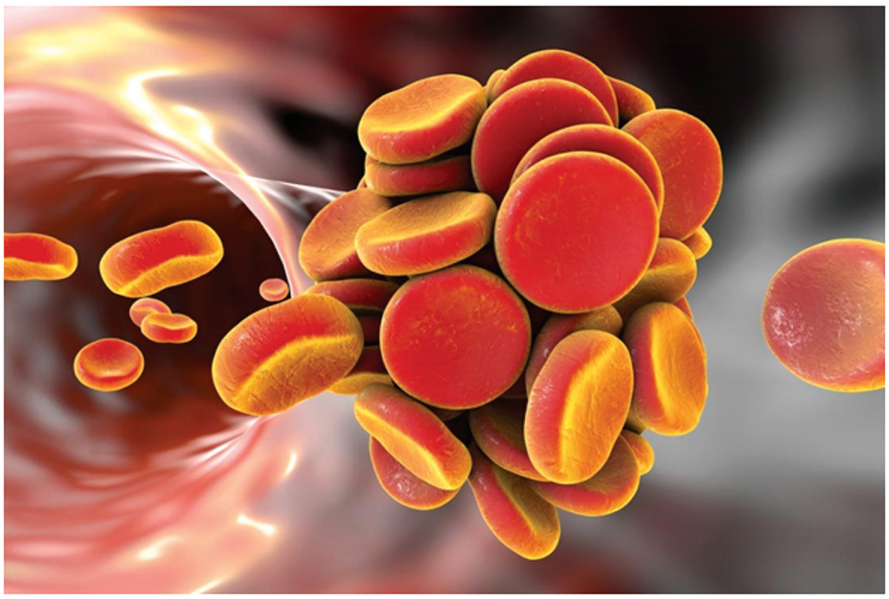


Fig. Thrombosis.

SIGNS AND SYMPTOMS

Venous Thrombosis

A venous thrombus is a blood clot (thrombus) that forms within a vein. *Thrombosis* is a term for a blood clot occurring inside a blood vessel. A common type of venous thrombosis is a deep vein thrombosis (DVT), which is a blood clot in the deep veins of the leg. If the thrombus breaks off (embolizes) and flows towards the lungs, it can become a pulmonary embolism (PE), a blood clot in the lungs. An inflammatory reaction is usually present, mainly in the superficial veins and, for this reason this pathology is called most of the time thrombophlebitis. The inflammatory reaction and the white blood cells play a role in the resolution of venous clots. The initial treatment for venous thromboembolism is typically with either low molecular weight heparin (LMWH) or unfractionated heparin. LMWH appears to have lower rates of side effects; however, both result in similar rates of survival.

Classification

Superficial venous thromboses cause discomfort but generally not serious consequences, as do the deep venous thromboses (DVTs) that form in the deep veins of the legs or in the pelvic veins. Nevertheless, they can progress to the deep veins through the perforator veins or, they can be responsible for a lung embolism mainly if the head of the clot is poorly attached to the vein wall and is situated near the sapheno-femoral junction. When a blood clot breaks loose and travels in the blood, this is called a venous thromboembolism (VTE). The abbreviation DVT/PE refers to a VTE where a deep vein thrombosis (DVT) has moved to the lungs (PE or pulmonary embolism). Since the veins return blood to the heart, if a piece of a blood clot formed in a vein breaks off it can be transported to the right side of the heart, and from there into the lungs. A piece of thrombus that is transported in this way is an *embolus*: the process of forming a thrombus that becomes embolic is called a *thromboembolism*. An embolism that lodges in the lungs is a *pulmonary embolism* (PE). A pulmonary embolism is a very serious condition that can be fatal depending on the dimensions of the embolus. Venous thromboembolism (VTE) refers to both DVTs and PEs. Systemic embolisms of venous origin can occur in patients with an atrial or ventricular septal defect, through which an embolus may pass into the arterial system. Such an event is termed a paradoxical embolism.

Causes

Venous thrombi are caused mainly by a combination of venous stasis and hypercoagulability—but to a lesser extent endothelial damage and activation. The three factors of stasis, hypercoaguability, and alterations in the blood vessel wall represent Virchow's triad, and changes to the vessel wall are the least understood. Various risk factors increase the likelihood of any one individual developing a thrombosis.

Risk Factors

Acquired:

- Older age
- Major surgery and orthopedic surgery
- Cancers, most particularly pancreatic, but not cancers of the lip, oral cavity, and pharynx
- Immobilization, as in orthopedic casts the sitting position, and travel, particularly by air
- Pregnancy and the postpartum period
- Antiphospholipid syndrome (such as lupus anticoagulant)
- Trauma and minor leg injury
- Previous VTE

- Oral contraceptives
- Hormonal replacement therapy, esp. oral
- Central venous catheters
- Inflammatory diseases/some autoimmune diseases
- Nephrotic syndrome
- Obesity
- Infection
- HIV
- myeloprolirative neoplasms including essential thrombocytosis and
- Polycythemia vera
- Chemotherapy
- Heart failure.

Inherited:

- Antithrombin deficiency
- Protein C deficiency
- Protein S deficiency (type I)
- Factor V Leiden
- Prothrombin G20210A
- Dysfibrinogenemia
- Non O-blood type

Mixed:

- Low free protein S
- Activated protein C resistance
- High factor VIII levels
- Hyperhomocysteinemia
- High fibrinogen levels
- High factor IX levels
- High factor XI levels.

The overall absolute risk of venous thrombosis per 100,000 woman years in current use of combined oral contraceptives is approximately 60, compared to 30 in non-users. The risk of thromboembolism varies with different types of birth control pills; Compared with combined oral contraceptives containing levonorgestrel (LNG), and with the same dose of estrogen and duration of use, the rate ratio of deep venous thrombosis for combined oral contraceptives with norethisterone is 0.98, with norgestimate 1.19, with desogestrel (DSG) 1.82, with gestodene 1.86, with drospirenone (DRSP) 1.64, and with cyproterone acetate 1.88. Venous thromboembolism occurs in 100–200 per 100,000 pregnant women every year. Regarding family history, age has substantial effect modification. For individuals with two or more affected siblings, the highest incidence rates is found among those ≥70 years of age (390 per 100,000 in male and 370 per 100,000 in female individuals), whereas the highest incidence ratios compared to those without affected siblings occurred at much younger ages (ratio of 4.3 among male individuals 20 to 29 years of age and 5.5 among female individuals 10 to 19 years of age).

Pathophysiology

In contrast to the understanding for how arterial thromboses occur, as with heart attacks, venous thrombosis formation is not well understood. With arterial thrombosis, blood vessel wall damage is required for thrombosis formation, as it initiates coagulation, but the majority of venous thrombi form without any injured epithelium. Red blood cells and fibrin are the main components of venous thrombi, and the thrombi appear to attach to the blood

vessel wall endothelium, normally a non-thrombogenic surface, with fibrin. Platelets in venous thrombi attach to downstream fibrin, while in arterial thrombi, they compose the core. As a whole, platelets constitute less of venous thrombi when compared to arterial ones. The process is thought to be initiated by tissue factor-affected thrombin production, which leads to fibrin deposition. The valves of veins are a recognized site of VT initiation. Due to the blood flow pattern, the base of the valve sinus is particularly deprived of oxygen (hypoxic). Stasis excacerbates hypoxia, and this state is linked to the activation of white blood cells (leukocytes) and the endothelium. Specifically, the two pathways of hypoxia-inducible factor-1 (HIF-1) and early growth response 1 (EGR-1) are activated by hypoxia, and they contribute to monocyte and endothelial activation. Hypoxia also causes reactive oxygen species (ROS) production that can activate HIF-1, EGR-1, and nuclear factor-κB (NF-κB), which regulates HIF-1 transcription. HIF-1 and EGR-1 pathways lead to monocyte association with endothelial proteins, such as P-selectin, prompting monocytes to release tissue factor filled microvesicles, which presumably initiate fibrin deposition (via thrombin) after binding the endothelial surface.

Prevention

Evidence supports the use of heparin in people following surgery who have a high risk of thrombosis to reduce the risk of DVTs; however, the effect on PEs or overall mortality is not known. In hospitalized non-surgical patients, mortality does not appear to change. It does not appear however to decrease the rate of symptomatic DVTs. Using both heparin and compression stockings appears better than either one alone in reducing the rate of DVT. In hospitalized people who have had a stroke and not had surgery, mechanical measures (compression stockings) resulted in skin damage and no clinical improvement. Data on the effectiveness of compression stockings among hospitalized non-surgical patients without stroke is scarce.

The American College of Physicians (ACP) gave three strong recommendations with moderate quality evidence on VTE prevention in non-surgical patients: that hospitalized patients be assessed for their risk of thromboembolism and bleeding before prophylaxis (prevention); that heparin or a related drug is used if potential benefits are thought to outweigh potential harms; and that graduated compression stockings not be used. As an ACP policy implication, the guideline stated a lack of support for any performance measures that incentivize physicians to apply universal prophylaxis without regard to the risks. Goldhaber recommends that people should be assessed at their hospital discharge for persistent high-risk of venous thrombosis, and that people who adopt a heart-healthy lifestyle might lower their risk of venous thrombosis. In those with cancer who are still walking about yet receiving chemotherapy, LMWH decreases the risk of VTE. Due to potential concerns of bleeding its routine use is not recommended. For people who are having surgery for cancer, it is recommended that they receive anticoagulation therapy (preferably LMWH) in order to prevent a VTE. LMWH is recommended for at least 7–10 days following cancer surgery, and for one month following surgery for people who have a high risk of VTEs. In adults who have had their lower leg casted or placed in a brace for more than a week, LMWH decreased the risk of VTEs. LMWH is recommended for adults not in hospital with an above-knee cast and a below-knee cast, and is safe for this indication. Following the completion of warfarin in those with prior VTE, long term aspirin is beneficial.

Treatment

Evidence-based clinical guidelines were published in 2016 for the treatment of VTE.

Anticoagulation

Recommendations for those without cancer include anticoagulation (stopping further blood clots from forming) with dabigatran, rivaroxaban, apixaban, or edoxaban rather than warfarin or low molecular weight heparin (LMWH). For those with cancer LMWH is recommended. For initial treatment of VTE, fixed doses with

LMWH may be more effective than adjusted doses of unfractionated heparin (UFH) in reducing blood clots. No differences in mortality, prevention of major bleeding, or preventing VTEs from recurring were observed between LMWH and UFH. No differences have been detected in the route of administration of UFH (subcutaneous or intravenous). LMWH is usually administered by a subcutaneous injection, and a persons blood clotting factors do not have to be monitored as closely as with UFH. People with cancer have a higher risk of experiencing reoccurring VTE episodes ("recurrent VTE"), despite taking preventative anticoagulation medication. These people should be given therapeutic doses of LMWH medication, either by switching from another anticoagulant or by taking a higher dose of LMWH. For those with a small pulmonary embolism and few risk factors, no anticoagulation is needed. Anticoagulation is; however, recommended in those who do have risk factors. Thrombolysis is recommended in those with PEs that are causing low blood pressure.

Inferior Vena Cava Filters

Inferior vena cava filters (IVCFs) are not recommended in those who are on anticoagulants. IVCFs may be used in clinical situations where a person has a high risk of experiencing a pulmonary embolism, but cannot be on anticoagulants due to a high risk of bleeding, or they have active bleeding. Retrievable IVCFs are recommended if IVCFs must be used, and a plan should be created to remove the filter when it is no longer needed.

Superficial Venous Thrombosis

Trials suggest that fondaparinux, a factor Xa inhibitor, reduces extension and recurrence of superficial venous thrombosis as well as progression to symptomatic embolism.

Deep Vein Thrombosis

Deep vein thrombosis (DVT), is the formation of a blood clot in a deep vein, most commonly the legs. Symptoms may include pain, swelling, redness, or warmth of the affected area. About half of cases have no symptoms. Complications may include pulmonary embolism, as a result of detachment of a clot which travels to the lungs, and post-thrombotic syndrome. Risk factors include recent surgery, cancer, trauma, lack of movement, obesity, smoking, hormonal birth control, pregnancy and the period following birth, antiphospholipid syndrome, and certain genetic conditions. Genetic factors include deficiencies of antithrombin, protein C, and protein S, and factor V Leiden mutation.

The underlying mechanism typically involves some combination of decreased blood flow rate, increased tendency to clot, and injury to the blood vessel wall. Individuals suspected of having DVT may be assessed using a clinical prediction rule such as the Wells score. A D-dimer test may also be used to assist with excluding the diagnosis or to signal a need for further testing. Diagnosis is most commonly confirmed by ultrasound of the suspected veins. Together, DVT and pulmonary embolism are known as venous thromboembolism (VTE). Anticoagulation (blood thinners) is the standard treatment. Typical medications include low-molecular-weight heparin, warfarin, or a direct oral anticoagulant. Wearing graduated compression stockings may reduce the risk of post-thrombotic syndrome. Prevention may include early and frequent walking, calf exercises, aspirin, anticoagulants, graduated compression stockings, or intermittent pneumatic compression. The rate of DVTs increases from childhood to old age; in adulthood, about one in 1000 adults are affected per year. About 5% of people are affected by a VTE at some point in time.

Signs and Symptoms

Common signs and symptoms of DVT include pain or tenderness, swelling, warmth, redness or discoloration, and distention of surface veins, although about half of those with the condition have no symptoms. Signs and

symptoms alone are not sufficiently sensitive or specific to make a diagnosis, but when considered in conjunction with known risk factors, can help determine the likelihood of DVT. In most suspected cases, DVT is ruled out after evaluation, and symptoms are more often due to other causes, such as cellulitis, Baker's cyst, musculoskeletal injury, or lymphedema. Other differential diagnoses include hematoma, tumors, venous or arterial aneurysms, and connective tissue disorders. Phlegmasia cerulea dolens is a very large and dangerous type of DVT. It is characterized by an acute and almost total venous occlusion of the entire extremity outflow, including the iliac and femoral veins. The leg is usually painful, tinged blue in colour, and swollen, which may result in venous gangrene.

Causes

The three factors of Virchow's triad—venous stasis, hypercoagulability, and changes in the endothelial blood vessel lining (such as physical damage or endothelial activation)—contribute to DVT and are used to explain its formation. Other related causes include activation of immune system components, the state of microparticles in the blood, the concentration of oxygen, and possible platelet activation. Various risk factors contribute to DVT, though many at high risk never develop it.

Acquired risk factors include the strong risk factor of older age, which alters blood composition to favour clotting. Other important acquired risk factors include major surgery and trauma, both of which may increase the risk because of tissue factor from outside the vascular system entering the blood. In orthopedic surgery, venous stasis may be temporarily provoked by a cessation of blood flow as part of the procedure. Cancer can grow in and around veins, causing venous stasis, and can also stimulate increased levels of tissue factor. Pregnancy causes blood to favour clotting, and in the postpartum, placental tearing releases substances that favour clotting. Oral contraceptives and hormonal replacement therapy increase the risk through a variety of mechanisms, including altered blood coagulation protein levels and reduced fibrinolysis.

The disease term venous thromboembolism (VTE) includes the development of either DVT or pulmonary embolism (PE). Genetic factors that increase the risk of VTE include deficiencies of three proteins that normally prevent blood from clotting—protein C, protein S, and antithrombin—in addition to non-O blood type and mutations in the factor V and prothrombin genes. Deficiencies in antithrombin, protein C, and protein S are rare but strong, or moderately strong, risk factors. These three thrombophilia increase the risk of VTE by about 10 times. Factor V Leiden, which makes factor V resistant to inactivation by activated protein C, and the genetic variant prothrombin G20210A, which causes increased prothrombin levels, are predominantly expressed in Caucasians. They moderately increase risk for VTE, by three to eight times for factor V Leiden and two to three times for prothrombin G20210A. Having a non-O blood type roughly doubles VTE risk. Non-O blood type is common in all races, making it an important risk factor. Individuals without O blood type have higher blood levels of von Willebrand factor and factor VIII than those with O blood type, increasing the likelihood of clotting.

Some risk factors influence the location of DVT within the body. In isolated distal DVT, the profile of risk factors appears distinct from proximal DVT. Transient factors, such as surgery and immobilization, appear to dominate, whereas thrombophilias and age do not seem to increase risk. In upper-extremity DVT, the most important risk factor is having a central venous catheter, and thoracic outlet syndrome also increases risk.

Risk Factors

Acquired:

- Older age
- Major surgery and orthopedic surgery
- Cancers, especially of the bone, ovary, brain, pancreas, and lymphomas
- Inactivity and immobilization, as with orthopedic casts, sitting, travel, bed rest, and hospitalization
- Pregnancy and the postpartum period

- Antiphospholipid syndrome
- Trauma, minor leg injury, and lower limb amputation
- Previous VTE
- Combined oral contraceptives
- Hormonal replacement therapy
- Central venous catheters
- Inflammatory diseases/some autoimmune diseases
- Nephrotic syndrome
- Obesity
- Infection
- HIV
- Polycythemia vera
- Chemotherapy
- Intravenous drug use.

Inherited:

- Antithrombin deficiency
- Protein C deficiency
- Protein S deficiency (type I)
- Factor V Leiden
- Prothrombin G20210A
- Dysfibrinogenemia
- Non-O blood type

Mixed:

- Low free protein S
- Activated protein C resistance
- High factor VIII levels
- Hyperhomocysteinemia
- High fibrinogen levels
- High factor IX levels
- High factor XI levels.

Pathophysiology

DVT often develops in the calf veins and "grows" in the direction of venous flow, towards the heart. When DVT does not grow, it can be cleared naturally and dissolved into the blood (fibrinolysis). Veins in the calf or thigh are most commonly affected, including the femoral vein, the popliteal vein, and the iliofemoral vein (as with May–Thurner syndrome). Extensive lower-extremity DVT can reach into the iliac vein of the pelvis or the inferior vena cava. Occasionally the veins of the arm are affected, as after central venous catheter placement and with the rare Paget–Schrötter disease. The mechanism behind arterial thrombosis, such as with heart attacks, is more established than the steps that cause venous thrombosis. With arterial thrombosis, blood vessel wall damage is required, as it initiates coagulation, but clotting in the veins mostly occurs without any such damage. The beginning of venous thrombosis is thought to be caused by tissue factor, which leads to conversion of prothrombin to thrombin, followed by fibrin deposition.

Red blood cells and fibrin are the main components of venous thrombi, and the fibrin appears to attach to the blood vessel wall lining (endothelium), a surface that normally acts to prevent clotting. Platelets and white blood cells are also components. Platelets are not as prominent in venous clots as they are in arterial ones, but they may play a role. Inflammation is associated with VTE, and white blood cells play a role in the formation

and resolution of venous clots. Often, DVT begins in the valves of veins. The blood flow pattern in the valves can cause low oxygen concentrations in the blood (hypoxemia) of a valve sinus. Hypoxemia, which is worsened by venous stasis, activates pathways—ones that include hypoxia-inducible factor-1 and early-growth-response protein 1. Hypoxemia also results in the production of reactive oxygen species, which can activate these pathways, as well as nuclear factor-κB, which regulates hypoxia-inducible factor-1 transcription. Hypoxia-inducible factor-1 and early-growth-response protein 1 contribute to monocyte association with endothelial proteins, such as P-selectin, prompting monocytes to release tissue factor-filled microvesicles, which presumably begin clotting after binding to the endothelial surface. Deep vein thrombosis occurs in the upper extremities in about 4–10% of cases, generally in people with severe underlying diseases, especially cancer.

Diagnosis

DVT diagnosis requires the use of imaging devices such as ultrasound. Clinical assessments, which predict DVT likelihood, can help determine if a D-dimer test is useful. In those not highly likely to have DVT, a normal D-dimer result can rule out a diagnosis.

Classification

Provoked DVTs occur in association with acquired risk factors, such as surgery, oral contraceptives, trauma, immobility, obesity, or cancer; cases without acquired states are called unprovoked or idiopathic. Acute DVT is characterized by pain and swelling and is usually occlusive, which means that it obstructs blood flow, whereas non-occlusive DVT is less symptomatic. The label "chronic" has been applied to symptomatic DVT that persists longer than 10 to 14 days. DVT that has no symptoms, but is found only by screening, is labeled asymptomatic or incidental. DVT in the legs is proximal (or iliofemoral) when above the knee and distal (or calf) when below the knee. DVT below the popliteal vein, a proximal vein behind the knee, is classified as distal and has limited clinical significance compared to proximal DVT. An initial episode of DVT is called incident and any subsequent DVT is termed recurrent. Bilateral DVT refers to clots in both legs while unilateral means that only a single leg is affected.

Probability

In those with suspected DVT, a clinical assessment of probability can be useful to determine which tests to perform. The most studied clinical prediction rule is the Wells score.

Wells score or criteria: (possible score –2 to 9)

1. Active cancer (treatment within last 6 months or palliative): +1 point
2. Calf swelling ≥ 3 cm compared to asymptomatic calf (measured 10 cm below tibial tuberosity): +1 point
3. Swollen unilateral superficial veins (non-varicose, in symptomatic leg): +1 point
4. Unilateral pitting edema (in symptomatic leg): +1 point
5. Previous documented DVT: +1 point
6. Swelling of entire leg: +1 point
7. Localized tenderness along the deep venous system: +1 point
8. Paralysis, paresis, or recent cast immobilization of lower extremities: +1 point
9. Recently bedridden ≥3 days, or major surgery requiring regional or general anesthetic in the past 12 weeks: +1 point
10. Alternative diagnosis at least as likely: –2 points

Those with Wells scores of two or more have a 28% chance of having DVT, those with a lower score have 6% probability. Alternatively, Wells scores can be categorized as high if greater than two, moderate if one or two, and low if less than one, with likelihoods of 53%, 17%, and 5%, respectively.

D-dimer

D-dimers are a fibrin degradation product, and an elevated level can result from plasmin dissolving a clot—or other conditions. Hospitalized patients often have elevated levels for multiple reasons. When individuals are at a high-probability of having DVT, diagnostic imaging is preferred to a D-dimer test. For those with a low or moderate probability of DVT, a D-dimer level might be obtained, which excludes a diagnosis if results are normal. An elevated level requires further investigation with diagnostic imaging to confirm or exclude the diagnosis.

For a suspected first leg DVT in a low-probability situation, the American College of Chest Physicians recommends testing either D-dimer levels with moderate or high sensitivity or compression ultrasound of the proximal veins. These options are suggested over whole-leg ultrasound, and D-dimer testing is the suggested preference overall. The UK National Institute for Health and Care Excellence (NICE) recommends D-dimer testing prior to proximal vein ultrasound. For a suspected first leg DVT in a moderate-probability scenario, a high-sensitivity D-dimer is suggested as a recommended option over ultrasound imaging, with both whole-leg and compression ultrasound possible. The NICE guideline uses a two-point Wells score and does not refer to a moderate probability group.

Imaging

Imaging tests of the veins are used in the diagnosis of DVT, most commonly either proximal compression ultrasound or whole-leg ultrasound. Each technique has drawbacks: a single proximal scan may miss a distal DVT, while whole-leg scanning can lead to distal DVT overtreatment. Doppler ultrasound, CT scan venography, MRI venography, or MRI of the thrombus are also possibilities. Ultrasonography for suspected deep vein thrombosis has a sensitivity of 97% in detecting DVTs of the proximal legs. The gold standard for judging imaging methods is contrast venography, which involves injecting a peripheral vein of the affected limb with a contrast agent and taking X-rays, to reveal whether the venous supply has been obstructed. Because of its cost, invasiveness, availability, and other limitations, this test is rarely performed. In one study, it found a DVT in an additional 20% of patients with pulmonary embolism where an ultrasonography was negative. A fibrinogen uptake test was formerly used to detect deep vein thrombosis.

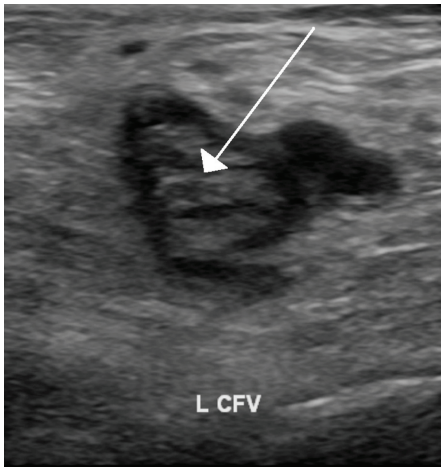


Fig. An ultrasound with a blood clot visible in the left common femoral vein.

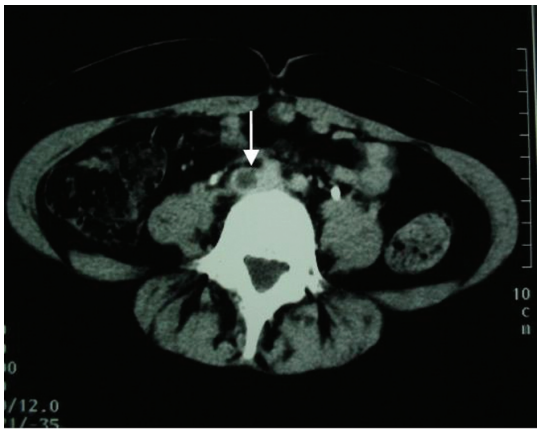


Fig. An abdominal CT scan with a clot in the right common iliac vein.

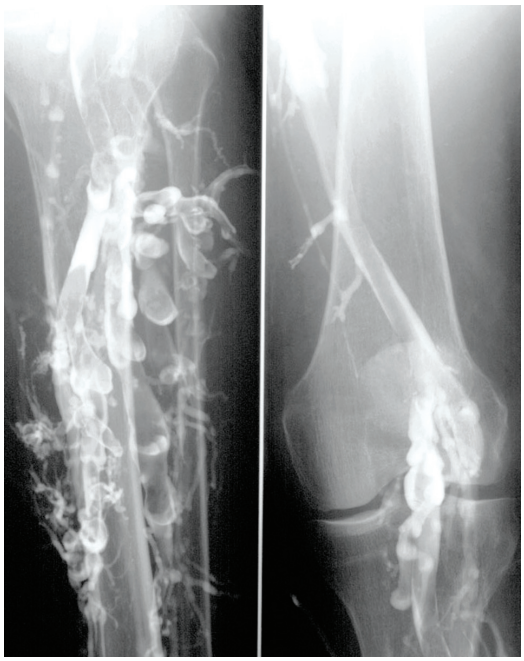


Fig. Venograms of DVT.

Prevention

Depending upon the risk for DVT, different preventive measures are recommended. Walking and calf exercises reduce venous stasis because leg muscle contractions compress the veins and pump blood up towards the heart. In immobile individuals, physical compression methods improve blood flow. Anticoagulation, which increases the risk of bleeding, might be used in high-risk scenarios. The risk of major bleeding with long-term anticoagulation is about 3% per year, and the point where annual VTE risk is thought to warrant long-term anticoagulation is estimated to be between 3 and 9%.

Usually, only when individuals exceed a 9% annual VTE risk is long-term anticoagulation a common consideration. Antithrombin deficiency, a strong or moderately strong risk factor, carries an annual risk of VTE of only 0.8–1.5%; as such, asymptomatic individuals with thrombophilia do not warrant long-term anticoagulation. Aside from anticoagulation, the antiplatelet drug aspirin might be used in some people following orthopedic surgery and in those with a previous VTE. Statins might decrease the risk for people who are otherwise healthy, but the evidence is not clear. Following the completion of warfarin long term aspirin is useful to prevent re occurrence.

Hospital

In 2011, the American College of Physicians (ACP) issued a clinical practice guideline making three strong recommendations based on moderate-quality evidence: That hospitalized patients be assessed for their risk of thromboembolism and bleeding before prophylaxis is started; that heparin or a related drug be used if potential benefits are thought to outweigh potential harms; and that graduated compression stockings not be used. The ACP also drew attention to a lack of support for any performance measures encouraging physicians to apply universal prophylaxis without regard to the risks. A 2014 Cochrane review found that using heparin in medical patients did not change the risk of death or pulmonary embolism. While its use decreased people's risks of DVTs, it also increased people's risks of major bleeding. The review thus recommended the need to balance risks and benefits. The 2012 ACCP guidelines for nonsurgical patients recommend anticoagulation for the acutely ill in cases of elevated risk when neither bleeding nor a high risk of bleeding exists. Mechanical prophylaxis is suggested when risks for bleeding and thrombosis are elevated. For the critically ill, either pharmacological or mechanical prophylaxis is suggested depending upon the risk. Heparin is suggested in outpatients with cancer who have solid tumors and additional risk factors for VTE—listed as "previous venous thrombosis, immobilization, hormonal therapy, angiogenesis inhibitors, thalidomide, and lenalidomide"—and a low risk of bleeding.

After Surgery

Major orthopedic surgery—total hip replacement, total knee replacement, or hip fracture surgery—has a high risk of causing VTE. If prophylaxis is not used after these surgeries, symptomatic VTE has about a 4% chance of developing within 35 days. Options for VTE prevention in people follow nonorthopedic surgery include early walking, mechanical prophylaxis (intermittent pneumatic compression or graduated compression stockings), and drugs (low-molecular-weight heparin and low-dose-unfractionated heparin) depending upon the risk of VTE, risk of major bleeding, and person's preferences. Following major orthopedic surgery, the ACCP recommends treatment with drugs that reduce the risk of clots (such as fondaparinux and aspirin) with low-molecular-weight heparin (LMWH) suggested as a preference. Intermittent pneumatic compression is also an option. Graduated compression stockings are effective after both general and orthopedic surgery.

Pregnancy

The risk of VTE is increased in pregnancy by about five times because of a more hypercoagulable state, a likely adaptation against fatal postpartum hemorrhage. Additionally, pregnant women with genetic risk factors are subject to a roughly three to 30 times increased risk for VTE. Preventative treatments for pregnancy-related VTE in hypercoagulable women were suggested by the ACCP. Homozygous carriers of factor V Leiden or prothrombin G20210A with a family history of VTE were suggested for antepartum LMWH and either LMWH or a vitamin K antagonist (VKA) for the six weeks following childbirth. Those with another thrombophilia and a family history but no previous VTE were suggested for watchful waiting during pregnancy and LMWH or—for those without protein C or S deficiency—a VKA. Homozygous carriers of factor V Leiden or prothrombin G20210A with no personal or family history of VTE were suggested for watchful waiting during pregnancy and LMWH or a VKA for six weeks after childbirth. Those with another thrombophilia but no family or personal history of VTE were suggested for watchful waiting only. Warfarin, a common VKA, can cause harm to the fetus and is not used for VTE prevention during pregnancy.

Travelers

The 2012 ACCP guidelines offered weak recommendations. For at-risk long-haul travelers—those with "previous VTE, recent surgery or trauma, active malignancy, pregnancy, estrogen use, advanced age, limited

mobility, severe obesity, or known thrombophilic disorder"—suggestions included calf exercises, frequent walking, and aisle seating in airplanes to ease walking. The use of graduated compression stockings that fit below the knee and give 15–30 mm Hg of pressure to the ankle was suggested, while aspirin or anticoagulants were not. Compression stockings have sharply reduced the levels of asymptomatic DVT in airline passengers, but the effect on symptomatic VTE is unknown, as none of the individuals studied developed symptomatic VTE.

Treatment

Anticoagulation

Anticoagulation, which prevents further coagulation, but does not act directly on existing clots, is the standard treatment for DVT. Balancing risk vs. benefit is important in determining the duration of anticoagulation, and three months is generally the standard length of treatment. In those with an annual risk of VTE in excess of 9%, as after an unprovoked episode, extended anticoagulation is a possibility. Those who finish VKA treatment after idiopathic VTE with an elevated D-dimer level show an increased risk of recurrent VTE (about 9% vs about 4% for normal results), and this result might be used in clinical decision-making. Thrombophilia test results rarely play a role in the length of treatment. For acute cases in the leg, the ACCP recommended a parenteral anticoagulant (such as LMWH, fondaparinux, or unfractionated heparin) for at least five days and a VKA, the oral anticoagulant, the same day. LMWH and fondaparinux are suggested over unfractionated heparin, but both are retained in those with compromised kidney function, unlike unfractionated heparin. The VKA is generally taken for a minimum of three months to maintain an international normalized ratio of 2.0–3.0, with 2.5 as the target. The benefit of taking a VKA declines as the duration of treatment extends, and the risk of bleeding increases with age.

The ACCP recommended treatment for three months in those with proximal DVT provoked by surgery. A three-month course is also recommended for those with proximal DVT provoked by a transient risk factor, and three months is suggested over lengthened treatment when bleeding risk is low to moderate. Unprovoked DVT patients should have at least three months of anticoagulation and be considered for extended treatment. Those whose first VTE is an unprovoked proximal DVT are suggested for anticoagulation longer than three months unless there is a high risk of bleeding. In that case, three months is sufficient. Those with a second unprovoked VTE are recommended for extended treatment when bleeding risk is low, suggested for extended treatment when bleeding risk is moderate, and suggested for three months of anticoagulation in high-risk scenarios.

Stockings, Walking, and Repeat Imaging

The ACCP recommended initial home treatment instead of hospital treatment for those with acute leg DVT. This applies as long as individuals feel ready for it, and those with severe leg symptoms or comorbidities would not qualify. An appropriate home environment is expected: one that can provide a quick return to the hospital if necessary, support from family or friends, and phone access. In addition to anticoagulation, the ACCP suggested graduated compression stockings—which apply higher pressure (30–40 mm Hg) at the ankles and a lower pressure around the knees—for those with symptomatic DVT. Use should begin as soon as possible after anticoagulation. Evidence however does not support that these stockings reduce the risk of post-thrombotic syndrome nor do they indicate a reduction in recurrent VTE. Use is suggested for two years, though inconvenience and discomfort can reduce compliance. Walking is also suggested for those without severe pain or edema.

Unless a person has medical problems preventing movement, after a person starts anti-coagulation therapy bed rest should not be used to treat acute deep vein thrombosis. There are clinical benefits associated with

walking and no evidence that walking is harmful, but people with DVT are harmed by bed rest except when it is medically necessary. Instead of anticoagulation, a follow-up imaging test (typically ultrasound) about one-week post-diagnosis is an option for those with an acute isolated distal DVT without a high risk for extension; if the clot does not grow, the ACCP does not recommend anticoagulation. This technique can benefit those at a high risk for bleeding. Patients may choose anticoagulation over serial imaging, however, to avoid the inconvenience of another scan if concerns about the risk of bleeding are insignificant. When applied to symptomatic patients with a negative initial ultrasound result, serial testing is inefficient and not cost effective.

IVC Filters, Thrombolysis, and Thrombectomy

Inferior vena cava filters (IVC filters) are used on the presumption that they reduce PE, although their effectiveness and safety profile are not well established. In general, they are only recommended in some high risk scenarios. The ACCP recommended them for those with a contraindication to anticoagulant treatment but not in addition to anticoagulation, unless an individual with an IVC filter but without a risk for bleeding develops acute proximal DVT. In this case, both anticoagulation and an IVC filter are suggested. NICE recommends caval filters in settings where someone with an acute proximal DVT or PE cannot receive anticoagulation, and that the filter is removed when anticoagulation can be safely started. While IVC filters themselves are associated with a long-term risk of DVT, they are not reason enough to maintain extended anticoagulation.

Thrombolysis is the administration of an enzyme (intravenous or directly into the affected vein through a catheter), which acts to enzymatically break up clots. This may reduce the risk of post-thrombotic syndrome by a third, and possibly reduce the risk of leg ulcers, but is associated with an increased risk of bleeding. The ACCP currently suggests anticoagulation rather than thrombolysis, but patients may choose thrombolysis if prevention of post-thrombotic syndrome outweighs concerns over the complexity, bleeding risk, and cost of the procedure. NICE recommends that thrombolysis is considered in those who have had symptoms for less than two weeks, are normally well, have a good life expectancy and a low risk of bleeding. A mechanical thrombectomy device can remove venous clots, although the ACCP considers it an option only when the following conditions apply: "iliofemoral DVT, symptoms for < 7 days (criterion used in the single randomized trial), good functional status, life expectancy of ≥ 1 year, and both resources and expertise are available." Anticoagulation alone is suggested over thrombectomy.

Prognosis

The most frequent complication of proximal DVT is post-thrombotic syndrome, which is caused by a reduction in the return of venous blood to the heart. Some symptoms of post-thrombotic syndrome are pain, edema, paresthesia, and in severe cases, leg ulcers. An estimated 20–50% of those with DVT will develop it, and 5–10% will develop the severe form. PE is the most serious complication of proximal DVT, and the risk of PE is higher when clots are present in the thigh and pelvis. Distal DVT itself is hardly if ever associated with post-thrombotic syndrome or PE. Untreated lower extremity DVT has a 3% PE-related mortality rate, while deaths associated with upper extremity DVT are extremely rare. The presence of a remaining thrombus after a DVT frequently occurs in a minority of people, and it increases the risk of recurrence, though to a lesser extent than an elevated D-dimer. In the 10 years following a DVT, approximately a third of individuals will have a recurrent episode.

Epidemiology

About 1 in 1000 adults per year has DVT, but as of 2011, available data are dominated by North American and European populations. VTE is rare in children, with an incidence of about 1 in 100,000 a year. From childhood to old age, incidence increases by a factor of about 1000, with almost 1% of the elderly experiencing VTE yearly. During pregnancy and after childbirth, acute VTE occurs about once per 1000 deliveries. After

surgery with preventative treatment, VTE develops in about 10 of 1000 people after total or partial knee replacement, and in about 5 of 1000 after total or partial hip replacement. About 300,000–600,000 Americans develop VTE each year, with about 60,000–100,000 deaths attributable to PE. In England, an estimated 25,000 a year die from hospital-related VTE. For unclear reasons, people of Asian descent have a lower VTE risk than whites. In North American and European populations, around 4–8% of people have a thrombophilia, most commonly factor V leiden and prothrombin G20210A. For populations in China, Japan, and Thailand, deficiences in protein S, protein C, and antithrombin predominate. Non-O blood type is present in around 50% of the general population and varies with ethnicity, and it is present in about 70% of those with VTE. Altogether, global data is incomplete.

History

The earliest case of DVT was described by Sushruta in his book Sushruta Samhita around 600–900 BC. Another documented case is thought to have occurred in the 13th century, in the leg of a 20-year-old male. At some point, the increased incidence of DVT in women after childbirth was noticed, and in the late 1700s, a public health recommendation was issued to encourage women to breastfeed as a means to prevent this phenomenon; the DVT was called "milk leg", as it was thought to result from milk building up in the leg. In 1856, German physician and pathologist Rudolf Virchow published what is referred to as Virchow's triad, the three major causes of thrombosis.

The triad provides the theoretical framework for the current explanation of venous thrombosis, although it was focused on the effect of a foreign body in the venous system and the conditions required for clot propagation. Multiple pharmacological therapies for DVT were introduced in the 20th century: oral anticoagulants in the 1940s, subcutaneous LDUH in 1962 and subcutaneous LMWH in 1982. Diagnoses were commonly performed by impedance plethysmography in the 1970s and 1980s, but the use of Doppler ultrasound techniques, with their increased sensitivity and specificity, largely superseded this method.

Economics

Initial DVT costs for an average hospitalized patient in the U.S. are around $7,700–$10,800. VTE follow-up costs at three months, six months, and a year are about $5,000, $10,000, and $33,000 respectively; in Europe, the three and six-month figures are about €1,800 and €3,200. Post-thrombotic syndrome is a significant contributor to DVT follow-up costs. Annual DVT costs in the U.S. are an estimated $5 billion or in excess of $8 billion, and the average annual cost per treated individual is thought to be about $20,000. As an example, if 300,000 symptomatic DVT patients were treated at costs averaging $20,000 annually, that would cost $6 billion a year.

Research Directions

As of 2011, three large randomized controlled trials—the Norwegian CaVent trial, the North American ATTRACT trial, and the Dutch CAVA trial—are studying the effectiveness and safety of catheter-directed thrombolysis. In 2012, two studies found a clinical benefit in taking aspirin to prevent recurrent VTE.

Paget–Schroetter Disease

Paget–Schroetter disease, also known as Paget–von Schrötter disease, is a form of upper extremity deep vein thrombosis (DVT), a medical condition in which blood clots form in the deep veins of the arms. These DVTs typically occur in the axillary or subclavian veins.

Signs and Symptoms

The condition is relatively rare. It usually presents in young and otherwise healthy patients, and also occurs more often in males than females. The syndrome also became known as "effort-induced thrombosis" in the 1960s, as it has been reported to occur after vigourous activity, though it can also occur due to anatomic abnormality such as clavicle impingement or spontaneously. It may develop as a sequela of thoracic outlet syndrome. It is differentiated from secondary causes of upper extremity caused by intravascular catheters. Paget–Schroetter syndrome was described once for a viola player who suddenly increased practice time 10-fold, creating enough repetitive pressure against the brachiocephalic and external jugular veins to cause thrombosis. Symptoms may include sudden onset of pain, warmth, redness, blueness and swelling in the arm. Diagnosis is usually confirmed with an ultrasound. These DVTs have the potential to cause a pulmonary embolism.

Prevention and Treatment

Preventing the development of blood clots in the upper extremities is done by accessing the risk of the development of such clots. The traditional treatment for thrombosis is the same as for a lower extremity DVT, and involves systemic anticoagulation to prevent a pulmonary embolus. Some have also recommended thrombolysis with catheter directed alteplase. If there is thoracic outlet syndrome or other anatomical cause then surgery can be considered to correct the underlying defect.

History

The condition is named after two men. James Paget first proposed the idea of venous thrombosis causing upper extremity pain and swelling, and Leopold von Schrötter later linked the clinical syndrome to thrombosis of the axillary and subclavian veins.

Budd–Chiari Syndrome

Budd–Chiari syndrome is a very rare condition, affecting one in a million adults. The condition is caused by occlusion of the hepatic veins that drain the liver. It presents with the classical triad of abdominal pain, ascites, and liver enlargement. The formation of a blood clot within the hepatic veins can lead to Budd–Chiari syndrome. The syndrome can be fulminant, acute, chronic, or asymptomatic.

Signs and Symptoms

The acute syndrome presents with rapidly progressive severe upper abdominal pain, yellow discoloration of the skin and whites of the eyes, liver enlargement, enlargement of the spleen, fluid accumulation within the peritoneal cavity, elevated liver enzymes, and eventually encephalopathy. The fulminant syndrome presents early with encephalopathy and ascites. Liver cell death and severe lactic acidosis may be present as well. Caudate lobe enlargement is often present. The majority of patients have a slower-onset form of Budd–Chiari syndrome. This can be painless. A system of venous collaterals may form around the occlusion which may be seen on imaging as a "spider's web". Patients may progress to cirrhosis and show the signs of liver failure. On the other hand, incidental finding of a silent, asymptomatic form may not be a cause for concern.

Causes

The cause can be found in more than 80% of patients.

- Primary Budd–Chiari syndrome (75%): thrombosis of the hepatic vein
  - Hepatic vein thrombosis is associated with the following in decreasing order of frequency:

1. Polycythemia vera
2. Pregnancy
3. Postpartum state
4. Use of oral contraceptives
5. Paroxysmal nocturnal hemoglobinuria
6. Hepatocellular carcinoma
7. Lupus anticoagulants

- Secondary Budd–Chiari syndrome (25%): compression of the hepatic vein by an outside structure (*e.g.,* a tumor).

Budd–Chiari syndrome is also seen in tuberculosis, congenital venous webs and occasionally in inferior vena caval stenosis. Often, the patient is known to have a tendency towards thrombosis, although Budd–Chiari syndrome can also be the first symptom of such a tendency. Examples of genetic tendencies include protein C deficiency, protein S deficiency, the Factor V Leiden mutation, hereditary anti-thrombin deficiency and prothrombin mutation G20210A.

An important non-genetic risk factor is the use of estrogen-containing (combined) forms of hormonal contraception. Other risk factors include the antiphospholipid syndrome, aspergillosis, Behçet's disease, dacarbazine, pregnancy, and trauma. Many patients have Budd–Chiari syndrome as a complication of polycythemia vera (myeloproliferative disease of red blood cells). Patients suffering from paroxysmal nocturnal hemoglobinuria (PNH) appear to be especially at risk for Budd–Chiari syndrome, more than other forms of thrombophilia: up to 39% develop venous thromboses and 12% may acquire Budd-Chiari. A related condition is veno-occlusive disease, which occurs in recipients of bone marrow transplants as a complication of their medication. Although its mechanism is similar, it is not considered a form of Budd–Chiari syndrome. Other toxicologic causes of veno-occlusive disease include plant and herbal sources of pyrrolizidine alkaloids such as Borage, Boneset, Coltsfoot, T'u-san-chi, Comfrey, Heliotrope (sunflower seeds), Gordolobo, Germander, and Chaparral.

Pathophysiology

Any obstruction of the venous vasculature of the liver is referred to as Budd–Chiari syndrome, from the venules to the right atrium. This leads to increased portal vein and hepatic sinusoid pressures as the blood flow stagnates. The increased portal pressure causes increased filtration of vascular fluid with the formation of ascites in the abdomen and collateral venous flow through alternative veins leading to esophageal, gastric and rectal varices. Obstruction also causes centrilobular necrosis and peripheral lobule fatty change due to ischemia. If this condition persists chronically what is known as nutmeg liver will develop. Renal failure may occur, perhaps due to the body sensing an "underfill" state and subsequent activation of the renin-angiotensin pathways and excess sodium retention.

Diagnosis

When Budd–Chiari syndrome is suspected, measurements are made of liver enzyme levels and other organ markers (creatinine, urea, electrolytes, LDH). Budd–Chiari syndrome is most commonly diagnosed using ultrasound studies of the abdomen and retrograde angiography. Ultrasound may show obliteration of hepatic veins, thrombosis or stenosis, spiderweb vessels, large collateral vessels, or a hyperechoic cord replacing a normal vein. Computed tomography (CT) or magnetic resonance imaging (MRI) is sometimes employed although these methods are generally not as sensitive. Liver biopsy is nonspecific but sometimes necessary to differentiate between Budd–Chiari syndrome and other causes of hepatomegaly and ascites, such as galactosemia or Reye's syndrome.

Treatment

A minority of patients can be treated medically with sodium restriction, diuretics to control ascites, anticoagulants such as heparin and warfarin, and general symptomatic management. The majority of patients require further intervention. Milder forms of Budd–Chiari may be treated with surgical shunts to divert blood flow around the obstruction or the liver itself. Shunts must be placed early after diagnosis for best results. The TIPS is similar to a surgical shunt: it accomplishes the same goal but has a lower procedure-related mortality—a factor that has led to a growth in its popularity. If all the hepatic veins are blocked, the portal vein can be approached via the intrahepatic part of inferior vena cava, a procedure called DIPS (direct intrahepatic portocaval shunt). Patients with stenosis or vena caval obstruction may benefit from angioplasty. Limited studies on thrombolysis with direct infusion of urokinase and tissue plasminogen activator into the obstructed vein have shown moderate success in treating Budd–Chiari syndrome; however, it is not routinely attempted.

Liver transplantation is an effective treatment for Budd–Chiari. It is generally reserved for patients with fulminant liver failure, failure of shunts or progression of cirrhosis that reduces the life expectancy to 1 year. Long-term survival after transplantation ranges from 69–87%. The most common complications of transplant include rejection, arterial or venous thromboses and bleeding due to anticoagulation. Up to 10% of patients may have a recurrence of Budd–Chiari syndrome after the transplant.

Prognosis

Several studies have attempted to predict the survival of patients with Budd–Chiari syndrome. In general, nearly 2/3 of patients with Budd–Chiari are alive at 10 years. Important negative prognostic indicators include ascites, encephalopathy, elevated Child-Pugh scores, elevated prothrombin time, and altered serum levels of various substances (sodium, creatinine, albumin, and bilirubin). Survival is also highly dependent on the underlying cause of the Budd–Chiari syndrome. For example, a patient with an underlying myeloproliferative disorder may progress to acute leukemia, independently of Budd–Chiari syndrome.

Eponym

It is named after George Budd, a British physician, and Hans Chiari, an Austrian pathologist.

Portal Vein Thrombosis

Portal vein thrombosis is a form of venous thrombosis affecting the hepatic portal vein, which can lead to portal hypertension and reduction in the blood supply to the liver.

Signs and Symptoms

Portal vein thrombosis can cause fever, symptoms of indigestion, and gradually worsening abdominal pain. However, it can also develop without causing symptoms, leading to portal hypertension before it is diagnosed. Other symptoms can develop based on the cause. For example, if portal vein thrombosis develops due to liver cirrhosis, bleeding or other signs of liver disease may be present. If portal vein thrombosis develops due to pylephlebitis, signs of infection such as fever, chills, night sweats may be present.

Causes

Causes can include pancreatitis, cirrhosis, diverticulitis, and cholangiocarcinoma. It is also a known complication of surgical removal of the spleen.

Diagnosis

The diagnosis of portal vein thrombosis is usually made by ultrasound, computed tomography with contrast or magnetic resonance imaging. D-dimer levels in the blood may be elevated as a result of fibrin breakdown.

Treatment

Treatments include anticoagulants, shunts, bypass surgery, and transplants.

Renal Vein Thrombosis

Renal vein thrombosis (RVT) is the formation of a clot in the vein that drains blood from the kidneys, ultimately leading to a reduction in the drainage of one or both kidneys and the possible migration of the clot to other parts of the body. First described by German pathologist Friedrich Daniel von Recklinghausen in 1861, RVT most commonly affects two subpopulations: newly born infants with blood clotting abnormalities or dehydration and adults with nephrotic syndrome. Nephrotic syndrome, a kidney disorder, causes excessive loss of protein in the urine, hypoalbuminemia, hypercholesterolemia and edema, triggering a hypercoagulable state and increasing chances of clot formation.

Other less common causes include hypercoagulable state, cancer, renal transplantation, behcet syndrome, antiphospholipid antibody syndrome or blunt trauma to the back or abdomen. Treatment of RVT mainly focuses on preventing further blood clots in the kidneys and maintaining stable renal function. The use of anticoagulants has become the standard treatment in treating this abnormality. Membranous Glomerulonephritis, the most common cause for nephrotic syndrome in adults, peaks in people ages 40–60 years old and It is twice as likely to occur in men than in women. Since nephrotic syndrome is the most common cause of RVT, people over 40 years old and men are most at risk to develop a renal vein thrombosis.

Mechanism

The mechanism behind RVT is no different from other types of blood clots in other parts of the body. Rudolf Virchow, was the first to describe the physiological mechanism behind venous thrombosis (blood clots) using three related factors, known as Virchow's Triad; damage to the blood vessel (endothelial damage), decrease in blood flow (stasis) and increased coagulability of the blood (thrombophilia or hypercoagulability). it is possible for one of these factors alone to cause a blood clot, but in most cases, a combination or all of these factors induce the formation of a blood clot. Decreased urine output or renal function may be the only observable symptoms caused by a blood clot renal vein. Other less common causes include hypercoagulable state, invasion by renal cell cancer, renal transplantation, behcet syndrome, antiphospholipid antibody syndrome or blunt trauma to the back or abdomen.

Vein Tissue Damage

Damage to the endothelial tissue of the vein can be caused by blunt damage, trauma during venography, a renal transplant, tumors, acute rejection, vasculitis or spontaneous micro-trauma to the endothelium due to homocystinuria. Cystathionine beta synthase deficiency, also known as homocystinuria, is an autosomal recessive inherited disorder in which the body is not able to process certain building blocks of proteins correctly due to a mutation to the *CBS* gene. This mutation causes the amino acid homocystine not to be used properly thus high levels build up in the blood, damaging the endothelial tissue and increasing the likelihood of RVT.

Decreased Blood Flow

The most common cause of RVT in infants is dehydration. Dehydration may be caused by reduction in both volume and circulatory blood volume due to water depleting abnormalities like diarrhea or vomiting. The decrease in blood volume due to dehydration will cause blood flow to be diverted away from the kidneys to other organs, resulting in slower blood flow to the kidneys, increasing chances of a blood clot occurrence. "RVT is known to occur in the absence of clinically obvious shock *e.g.,* following neonatal distress and placement of central venous catheters." RVT can also be induced by post transplant distortion or physical distortion or compression of the renal vein, which depending on the shape distortion can affect the rate of flow through the vein.

Hypercoagulability

Hypercoagulability is an abnormality of the blood that increases the risk of the formation blood clots. Nephrotic syndrome patients have a higher risk of RVT development due to hypercoagulability caused by proteinuria. The increased loss of proteins in the urine caused by nephrotic syndrome results in lower osmotic pressure. Reduced osmotic pressure will trigger the liver to produce more proteins like fibrinogen and beta-thromboglobulin, which promote blood clotting. Other than nephrotic syndrome, there are many other factors that can promote hypercoagulability. Hypercoagulability can be promoted by increased platelet count, enhanced platelet aggregation, increased protein S count, and a decrease in coagulation inhibiters like antithrombin. Hypercoagulability can be inherited and/or acquired. Hyperhomocysteine, a condition known to promote clots, can be caused by a combination of genetic factors and vitamin B_6, vitamin B_{12} and folic acid deficiency. Factor V Leidan and mutations of the prothrombin gene are the two most common genetic causes of hypercoagulability. About 5% of the general population have these heterozygous mutations and in the thrombophilic population, 45–63% have these mutations.

Membranous Glomerulonephritis

The incidence of RVT in people with Nephrotic syndrome ranges from 5% to 65%. Nephrotic syndrome is caused by Membranous Glomerulonephritis, minimal change disease, focal segmental glomerulosclerosis.

Symptoms

Aside from the occasional flank or lower back pain caused by a sudden clot in the major veins to the kidneys, RVT produces few symptoms. Some patients may not display any symptoms while other patients may experience bloody urine, decrease in urine output, edema and worsening proteinuria. Usually the diagnoses of RVT is first made when a nephrotic syndrome patient experiences a pulmonary embolism or a sudden decrease in renal function or renal failure.

These symptoms may vary in duration since a blood clot can resolve itself, but precautions should be taken to prevent the migration of the clot to other parts of the body. The most severe complication of RVT is a pulmonary embolism, caused by a clot, also called a thrombus, that originates from the renal vein or any other vein in the body and migrates to the pulmonary artery. A pulmonary embolism is a serious condition because; it can damage the lungs due to pulmonary hypertension and cause low blood oxygen, damaging other organs in the body. This condition can cause death if left untreated; about 30% percent of patients who have a pulmonary embolism will die, usually within one hour. Infants and young children experiencing dehydration induced RVT, may experience dehydration symptoms (dry mouth, low urine output, loss of skin turgidity) as while as vomiting, nausea and fever, and the usual RVT symptoms like flank pain, blood in the urine, anaemia, edema, enlarged kidneys and kidney failure.

Diagnosis

There are no laboratory tests used to diagnose RVT. Observing the patient's symptoms, medical history and imaging remain the fundamental source for diagnosing RVT. Imaging is used to detect the presence of a blood clot. In an abnormal kidney with RVT, a blood clot is present in the renal vein. In cases where the renal vein is suddenly and/or fully blocked, the kidneys will enlarge, reaching its maximum size within a week. An ultrasound imaging can be used to observe and track the size of the kidneys in RVT patients. Ultrasound is not efficient for use in detecting blood flow in the renal veins and artery. Instead a colour doppler ultrasound may be used to detect renal blood flow. It is most commonly used to detect RVT in patients who have undergone renal transplantation. CT angiography is currently the top choice in diagnosing RVT. It is non-invasive, relatively cheap and fast with high accuracy. CT scanning can be used to detect renal enlargement, renal tumors, blood flow and other renal pathologies. An alternative is magnetic resonance angiography or MRA. It is non-invasive, fast and avoids radiation (unlike a CT scan) but it is relatively expensive. MRA produces detailed images of the renal blood flow, vesicle walls, the kidneys and any surrounding tissue. An inferior venocavography with selective venography can be used to rule out the diagnoses of RVT.

Treatment

Surgery to remove the clot is possible, but rarely performed. In the past, surgical removal of the renal vein clot was the primary treatment but it is very invasive and many complications can occur. In the past decades, treatment has shifted its focus from surgical intervention to medical treatments that include intravenous and oral anticoagulants. The use of anticoagulants may improve renal function in RVT cases by removing the clot in the vein and preventing further clots from occurring. Patients already suffering from nephrotic syndrome may not need to take anticoagulants. In this case, patients should keep an eye out and maintain reduced level of proteinuria by reducing salt and excess protein, and intaking diuretics and statins. Depending on the severity of RVT, patients may be on anticoagulants from a year up to a lifetime. As long as the albumen levels in the bloodstream are below 2.5g/L, it is recommended that RVT patients continue taking anticoagulants. Main anticoagulants that can be used to treat RVT include warfarin and low molecular weight heparin.

Heparin has become very popular, because of its low risk of complications, its availability and because it can easily be administered. Warfarin is known to interact with many other drugs, so careful monitoring is required. If a nephrotic syndrome patient experiences any of the RVT symptoms (flank or back pain, blood in the urine or decreased renal function), he or she should immediately see a doctor to avoid further complications. The main side effect of anticoagulants is the risk of excessive bleeding. Other side effects include: blood in the urine or feces, severe bruising, prolonged nosebleeds (lasting longer than 10 minutes), bleeding gum, blood in your vomit or coughing up blood, unusual headaches, sudden severe back pain, difficulty breathing or chest pain, in women, heavy or increased bleeding during the period, or any other bleeding from the vagina. Warfarin can cause rashes, diarrhea, nausea (feeling sick) or vomiting, and hair loss. Heparin can cause hair loss (alopecia) thrombocytopenia – a sudden drop in the number of platelets in the blood.

It has been reported in a case study of 27 patients with nephrotic syndrome caused RVT, there was a 40% mortality rate, mostly due to hemorrhagic complications and sepsis. In 75% of the remaining surviving patients, the RVT was resolved and renal function returned to normal. It has been concluded that age is not a factor on the survival of RVT patients, although older patient (55 and older) are more likely to develop renal failure. Heparin is crucial in returning normal renal function; in patients that did not take heparin, long term renal damage was observed in 100%. In patients that did take heparin, renal damage was observed in about 33%. By quickly treating, and receiving the correct medications, patients should increase their chances of survival and reduce the risk of the renal vein clot from migrating to another part of the body.

Recent Studies

It is known that diabetes causes changes to factors associated with coagulation and clotting, however not much is known of the risk of thromboembolism, or clots, in diabetic patients. There are some studies that show that diabetes increases the risk of thromboembolism; other studies show that diabetes does not increase the risk of thromboembolism. A study conducted in the Umea University Hospital, in Sweden, observed patients that were hospitalized due to an thromboembolism from 1997 to 1999. The researchers had access to patient information including age, sex, vein thromboembolism diagnosis, diagnostic methods, diabetes type and medical history. This study concluded that there is, in fact, an increased risk of thromboembolism development in diabetic patients, possibly due to factors associated with diabetes or diabetes itself. Diabetic patients are twice as likely to develop a thromboembolism than are non-diabetic patient. The exact mechanism of how diabetes increases the risk of clot formation remains unclear and could possibly be a future direction for study.

From previous studies, it is known that long distance air travel is associated with high risk of venous thrombosis. Long periods of inactivity in a limited amount of space may be a reason for the increased risk of blood clot formation. In addition, bent knees compresses the vein behind the knee (the popliteal vein) and the low humidity, low oxygen, high cabin pressure and consumption of alcohol concentrate the blood. A recent study, published in the British Journal of Haematology in 2014, determined which groups of people, are most at risk for developing a clot during or after a long flight. The study focused on 8755 frequent flying employees from international companies and organizations. It found that travelers who have recently undergone a surgical procedure or who have a malignant disease such as cancer or who are pregnant are most at risk. Preventative measures before flying may be taken in these at-risk groups as a solution.

Patients who have undergone kidney transplant have a high risk of developing RVT (about 0.4% to 6%). RVT is known to account for a large proportion of transplanted kidney failures due to technical problems (damage to the renal vein), clotting disorders, diabetes, consumption of ciclosporin or an unknown problem. Patients who have undergone a kidney transplant are commonly prescribed ciclosporin, an immunosuppressant drug which is known to reduce renal blood flow, increase platelet aggregation in the blood and cause damage to the endothelial tissue of the veins. In a clinical study conducted by the Nuffield Department of Surgery at the Oxford Transplant Centre, UK, transplant patients were given low doses of aspirin, which has a some anti-platelet activity. There is risk of bleeding in transplant patients when using anticoagulants like warfarin and herapin. Low dosage of aspirin was used as an alternative. The study concluded that a routine low-dose of aspirin in kidney transplant patients who are also taking ciclosporin significantly reduces the risk of RVT development.

Cerebral Venous Sinus Thrombosis

Cerebral venous sinus thrombosis (CVST) is the presence of acute thrombosis (a blood clot) in the dural venous sinuses, which drain blood from the brain. Symptoms may include headache, abnormal vision, any of the symptoms of stroke such as weakness of the face and limbs on one side of the body, and seizures. The diagnosis is usually by computed tomography (CT/CAT scan) or magnetic resonance imaging (MRI) employing radiocontrast to demonstrate obstruction of the venous sinuses by thrombus. Treatment is with anticoagulants (medication that suppresses blood clotting), and rarely thrombolysis (enzymatic destruction of the blood clot). Given that there is usually an underlying cause for the disease, tests may be performed to look for these. The disease may be complicated by raised intracranial pressure, which may warrant surgical intervention such as the placement of a shunt.

Signs and Symptoms

Nine in ten people with sinus thrombosis have a headache; this tends to worsen over the period of several days, but may also develop suddenly (thunderclap headache). The headache may be the only symptom of

cerebral venous sinus thrombosis. Many patients have symptoms of stroke: inability to move one or more limbs, weakness on one side of the face or difficulty speaking. This does not necessarily affect one side of the body as in the more common "arterial" stroke. 40% of people have seizures, although it is more common in women who develop sinus thrombosis peripartum (in the period before and after giving birth). These are mostly seizures affecting only one part of the body and unilateral (occurring on one side), but occasionally the seizures are generalised and rarely they lead to status epilepticus (persistent or recurrent seizure activity for a long period of time).

In the elderly, many of the aforementioned symptoms may not occur. Common symptoms in the elderly with this condition are otherwise unexplained changes in mental status and a depressed level of consciousness. The pressure around the brain may rise, causing papilledema (swelling of the optic disc) which may be experienced as visual obscurations. In severely raised intracranial pressure, the level of consciousness is decreased, the blood pressure rises, the heart rate falls and the patient assumes an abnormal posture.

Causes

Cerebral venous sinus thrombosis is more common in particular situations. 85% of patients have at least one of these risk factors:

- Thrombophilia, a tendency to develop blood clots due to abnormalities in coagulation, *e.g.,* factor V Leiden, deficiency of protein C, protein S or antithrombin, or related problems
- Nephrotic syndrome, a kidney problem causing protein loss in the urine
- Chronic inflammatory diseases, such as inflammatory bowel disease, lupus and Behçet's disease
- Pregnancy and puerperium (the period after giving birth)
- Particular blood disorders, especially polycythemia vera and paroxysmal nocturnal hemoglobinuria
- Use of estrogen-containing forms of hormonal contraception
- Meningitis and infections of the ear, nose and throat area such as mastoiditis and sinusitis
- Direct injury to the venous sinuses
- Medical procedures in the head and neck area
- Sickle cell anemia
- Dehydration, primarily in infants and children
- Homocystinuria.

Diagnosis

The diagnosis may be suspected on the basis of the symptoms, for example the combination of headache, signs of raised intracranial pressure and focal neurological abnormalities, or when alternative causes of headache and neurological abnormalities, such as a subarachnoid hemorrhage, have been excluded.

Imaging

There are various neuroimaging investigations that may detect cerebral sinus thrombosis. Cerebral edema and venous infarction may be apparent on any modality, but for the detection of the thrombus itself, the most commonly used tests are computed tomography (CT) and magnetic resonance imaging (MRI), both using various types of radiocontrast to perform a venogram and visualise the veins around the brain. Computed tomography, with radiocontrast in the venous phase (*CT venography* or CTV), has a detection rate that in some regards exceeds that of MRI. The test involves injection into a vein (usually in the arm) of a radioopaque substance, and time is allowed for the bloodstream to carry it to the cerebral veins - at which point the scan is performed. It has a sensitivity of 75-100% (it detects 75-100% of all clots present), and a specificity of 81-100% (it would be incorrectly

positive in 0-19%). In the first two weeks, the "empty delta sign" may be observed (in later stages, this sign may disappear). Magnetic resonance venography employs the same principles, but uses MRI as a scanning modality. MRI has the advantage of being better at detecting damage to the brain itself as a result of the increased pressure on the obstructed veins, but it is not readily available in many hospitals and the interpretation may be difficult. Cerebral angiography may demonstrate smaller clots than CT or MRI, and obstructed veins may give the "corkscrew appearance". This, however, requires puncture of the femoral artery with a sheath and advancing a thin tube through the blood vessels to the brain where radiocontrast is injected before X-ray images are obtained. It is therefore only performed if all other tests give unclear results or when other treatments may be administered during the same procedure.

D-dimer

A 2004 study suggested that the D-dimer blood test, already in use for the diagnosis of other forms of thrombosis, was abnormal (above 500 µg/l) in 34 out of 35 patients with cerebral sinus thrombosis, giving it a sensitivity of 97.1%, a negative predictive value of 99.6%, a specificity of 91.2%, and a positive predictive value of 55.7%. Furthermore, the level of the D-dimer correlated with the extent of the thrombosis. A subsequent study, however, showed that 10% of patients with confirmed thrombosis had a normal D-dimer, and in those who had presented with only a headache 26% had a normal D-dimer. The study concludes that D-dimer is not useful in the situations where it would make the most difference, namely in lower probability cases.

Further Tests

In most patients, the direct cause for the cerebral sinus thrombosis is not readily apparent. Identifying a source of infection is crucial; it is common practice to screen for various forms of thrombophilia (a propensity to form blood clots).

Pathogenesis

The veins of the brain, both the superficial veins and the deep venous system, empty into the dural venous sinuses, which carry blood back to the jugular vein and thence to the heart. In cerebral venous sinus thrombosis, blood clots usually form both in the veins of the brain and the venous sinuses. The thrombosis of the veins themselves causes venous infarction—damage to brain tissue due to a congested and therefore insufficient blood supply. This results in cerebral edema (both *vasogenic* and *cytotoxic* edema), and leads to small petechial haemorrhages that may merge into large haematomas. Thrombosis of the sinuses is the main mechanism behind the increase in intracranial pressure due to decreased resorption of cerebrospinal fluid (CSF). The condition does not lead to hydrocephalus, however, because there is no difference in pressure between various parts of the brain. Any blood clot forms due to an imbalance between coagulation (the formation of the insoluble blood protein fibrin) and fibrinolysis. The three major mechanisms for such an imbalance are enumerated in Virchow's triad: alterations in normal blood flow, injury to the blood vessel wall, and alterations in the constitution of blood (hypercoagulability). Most cases of cerebral venous sinus thrombosis are due to hypercoagulability. It is possible for the clot to break off and migrate (embolise) to the lungs, causing a pulmonary embolism. An analysis of earlier case reports concludes that this occurs in about 10% of cases, but has a very poor prognosis.

Treatment

Various studies have investigated the use of anticoagulation to suppress blood clot formation in cerebral venous sinus thrombosis. Before these trials had been conducted, there had been a concern that small areas of hemorrhage in the brain would bleed further as a result of treatment; the studies showed that this concern was

unfounded. Clinical practice guidelines now recommend heparin or low molecular weight heparin in the initial treatment, followed by warfarin, provided there are no other bleeding risks that would make these treatments unsuitable. Some experts discourage the use of anticoagulation if there is extensive hemorrhage; in that case, they recommend repeating the imaging after 7–10 days. If the hemorrhage has decreased in size, anticoagulants are started, while no anticoagulants are given if there is no reduction.

The duration of warfarin treatment depends on the circumstances and underlying causes of the condition. If the thrombosis developed under temporary circumstances (*e.g.,* pregnancy), three months are regarded as sufficient. If the condition was unprovoked but there are no clear causes or a "mild" form of thrombophilia, 6 to 12 months is advised. If there is a severe underlying thrombosis disorder, warfarin treatment may need to continue indefinitely. Thrombolysis (removal of the blood clot with "clot buster" medication) has been described, either systemically by injection into a vein or directly into the clot during angiography. The 2006 European Federation of Neurological Societies guideline recommends that thrombolysis is only used in patients who deteriorate despite adequate treatment, and other causes of deterioration have been eliminated. It is unclear which drug and which mode of administration is the most effective. Bleeding into the brain and in other sites of the body is a major concern in the use of thrombolysis. American guidelines make no recommendation with regards to thrombolysis, stating that more research is needed. Raised intracranial pressure, if severe or threatening vision, may require therapeutic lumbar puncture (removal of excessive cerebrospinal fluid), medication (acetazolamide), or neurosurgical treatment (optic nerve sheath fenestration or shunting). In certain situations, anticonvulsants may be used to try to prevent seizures. These situations include focal neurological problems (*e.g.,* inability to move a limb) and focal changes of the brain tissue on CT or MRI scan. Evidence to support or refute the use of antiepileptic drugs as a preventive measure, however, is lacking.

Prognosis

In 2004 the first adequately large scale study on the natural history and long-term prognosis of this condition was reported; this showed that at 16 months follow-up 57.1% of patients had full recovery, 29.5%/2.9%/2.2% had respectively minor/moderate/severe symptoms or impairments, and 8.3% had died. Severe impairment or death were more likely in those aged over 37 years, male, affected by coma, mental status disorder, intracerebral hemorrhage, thrombosis of the deep cerebral venous system, central nervous system infection and cancer. A subsequent systematic review of nineteen studies in 2006 showed that mortality is about 5.6% during hospitalisation and 9.4% in total, while of the survivors 88% make a total or near-total recovery. After several months, two thirds of the cases has resolution ("recanalisation") of the clot. The rate of recurrence was low (2.8%). In children with CVST the risk of death is high. Poor outcome is more likely if a child with CVST develops seizures or has evidence of venous infarction on imaging.

Epidemiology

Cerebral venous sinus thrombosis is rare, with an estimated 3-4 cases per million annual incidence in adults. While it may occur in all age groups, it is most common in the third decade. 75% are female. Given that older studies show no difference in incidence between men and women, it has been suggested that the use of oral contraceptives in women is behind the disparity between the sexes. A 1995 report from Saudi Arabia found a doubled incidence at 7 cases per 100,000; this was attributed to the fact that Behçet's disease, which increases risk of CVST, is more common in the Middle East. A 1973 report found that CVST could be found on autopsy (examination of the body after death) in nine percent of all people. Many of these were elderly and had neurological symptoms in the period leading up to their death, and many suffered from concomitant heart failure. In children, a Canadian study reported in 2001 that CVST occurs in 6.7 per million annually. 43% occur

in the newborn (less than one month old), and a further 10% in the first year of life. Of the newborn, 84% were already ill, mostly from complications after childbirth and dehydration.

History

The first description of thrombosis of the cerebral veins and sinuses is attributed to the French physician Ribes, who in 1825 observed thrombosis of the saggital sinus and cerebral veins in a man who had suffered from seizures and delirium. Until the second half of the 20th century it remained a diagnosis generally made after death. In the 1940s, reports by Dr Charles Symonds and others allowed for the clinical diagnosis of cerebral venous thrombosis, using characteristic signs and symptoms and results of lumbar puncture. Improvements on the diagnosis of cerebral venous sinus thrombosis in life were made with the introduction of venography in 1951, which also aided in the distinction from idiopathic intracranial hypertension, which has similar presenting signs and symptoms in many cases. The British gynecologist Stansfield is credited with the introduction, in 1942, of the just recently introduced anticoagulant heparin in the treatment of CVST in 1942. Clinical trials in the 1990s finally resolved the concern about using anticoagulants in most cases of CVST.

Notable Cases

U.S. Secretary of State Hillary Clinton was hospitalized on December 30, 2012, for anticoagulation treatment of venous thrombosis of the right transverse sinus, which is located at the base of the brain. Clinton's thrombotic episode was discovered on an MRI scan done for follow-up of a cerebral concussion she had suffered 2.5 weeks before after she fell while suffering from gastroenteritis.

Cavernous Sinus Thrombosis

Cavernous sinus thrombosis (CST) is the formation of a blood clot within the cavernous sinus, a cavity at the base of the brain which drains deoxygenated blood from the brain back to the heart. The cause is usually from a spreading infection in the nose, sinuses, ears, or teeth. *Staphylococcus aureus* and *Streptococcus* are often the associated bacteria. Cavernous sinus thrombosis symptoms include: decrease or loss of vision, chemosis, exophthalmos (bulging eyes), headaches, and paralysis of the cranial nerves which course through the cavernous sinus. This infection is life-threatening and requires immediate treatment, which usually includes antibiotics and sometimes surgical drainage.

Signs and Symptoms

The clinical presentation of CST can be varied. Both acute, fulminant disease and indolent, subacute presentations have been reported in the literature. The most common signs of CST are related to anatomical structures affected within the cavernous sinus, notably cranial nerves III-VI, as well as symptoms resulting from impaired venous drainage from the orbit and eye. Classic presentations are abrupt onset of unilateral periorbital edema, headache, photophobia, and bulging of the eye (proptosis).

Other common signs and symptoms include:

- Ptosis, chemosis, cranial nerve palsies (III, IV, V, VI). Sixth nerve palsy is the most common. Sensory deficits of the ophthalmic and maxillary branch of the fifth nerve are common. Periorbital sensory loss and impaired corneal reflex may be noted. Papilledema, retinal hemorrhages, and decreased visual acuity and blindness may occur from venous congestion within the retina. Fever, tachycardia and sepsis may be present. Headache with nuchal rigidity may occur. Pupil may be dilated and sluggishly reactive. Infection can spread to contralateral cavernous sinus within 24–48 hours of initial presentation.

Cause

CST most commonly results from contiguous spread of infection from a nasal furuncle (50%), sphenoidal or ethmoidal sinuses (30%) and dental infections (10%). Less common primary sites of infection include tonsils, soft palate, middle ear, or orbit (orbital cellulitis). The highly anastomotic venous system of the paranasal sinuses allows retrograde spread of infection to the cavernous sinus via the superior and inferior ophthalmic veins. It was previously thought that veins in the area were valveless and that this was the major cause of the retrograde spread, but a recent study has found that the ophthalmic and facial veins are not valveless.

Staphylococcus aureus is the most common infectious microbe, found in 70% of the cases. *Streptococcus* is the second leading cause. Gram-negative rods and anaerobes may also lead to cavernous sinus thrombosis. Rarely, *Aspergillus fumigatus* and mucormycosis cause CST.

Diagnosis

The diagnosis of cavernous sinus thrombosis is made clinically, with imaging studies to confirm the clinical impression. Proptosis, ptosis, chemosis, and cranial nerve palsy beginning in one eye and progressing to the other eye establish the diagnosis. Cavernous sinus thrombosis is a clinical diagnosis with laboratory tests and imaging studies confirming the clinical impression.

Laboratory Tests

CBC, ESR, blood cultures, and sinus cultures help establish and identify an infectious primary source. Lumbar puncture is necessary to rule out meningitis.

Imaging Studies

Sinus films are helpful in the diagnosis of sphenoid sinusitis. Opacification, sclerosis, and air-fluid levels are typical findings. Contrast-enhanced CT scan may reveal underlying sinusitis, thickening of the superior ophthalmic vein, and irregular filling defects within the cavernous sinus; however, findings may be normal early in the disease course. A MRI using flow parameters and an MR venogram are more sensitive than a CT scan, and are the imaging studies of choice to diagnose cavernous sinus thrombosis. Findings may include deformity of the internal carotid artery within the cavernous sinus, and an obvious signal hyperintensity within thrombosed vascular sinuses on all pulse sequences. Cerebral angiography can be performed, but it is invasive and not very sensitive. Orbital venography is difficult to perform, but it is excellent in diagnosing occlusion of the cavernous sinus.

Differential Diagnosis

- Orbital cellulitis
- Internal carotid artery aneurysm
- Stroke
- Migraine headache
- Allergic blepharitis
- Thyroid exophthalmos
- Brain tumor
- Meningitis
- Mucormycosis
- Trauma.

Treatment

Recognizing the primary source of infection (*i.e.,* facial cellulitis, middle ear, and sinus infections) and treating the primary source expeditiously is the best way to prevent cavernous sinus thrombosis.

Antibiotics

Broad-spectrum intravenous antibiotics are used until a definite pathogen is found.

1. Nafcillin 1.5 g IV q4h
2. Cefotaxime 1.5 to 2 g IV q4h
3. Metronidazole 15 mg/kg load followed by 7.5 mg/kg IV q6h.

Vancomycin may be substituted for nafcillin if significant concern exists for infection by methicillin-resistant *Staphylococcus aureus* or resistant *Streptococcus pneumoniae*. Appropriate therapy should take into account the primary source of infection as well as possible associated complications such as brain abscess, meningitis, or subdural empyema. All people with CST are usually treated with prolonged courses (3–4 weeks) of IV antibiotics. If there is evidence of complications such as intracranial suppuration, 6–8 weeks of total therapy may be warranted. All patients should be monitored for signs of complicated infection, continued sepsis, or septic emboli while antibiotic therapy is being administered.

Heparin

Anticoagulation with heparin is controversial. Retrospective studies show conflicting data. This decision should be made with subspecialty consultation. One systematic review concluded that anticoagulation treatment appeared safe and was associated with a potentially important reduction in the risk of death or dependency.

Steroids

Steroid therapy is also controversial in many cases of CST. However, corticosteroids are absolutely indicated in cases of pituitary insufficiency. Corticosteroid use may have a critical role in patients with Addisonian crisis secondary to ischaemia or necrosis of the pituitary that complicates CST.

Surgery

Surgical drainage with sphenoidotomy is indicated if the primary site of infection is thought to be the sphenoidal sinuses.

Prognosis

Cavernous sinus thrombosis has a mortality rate of less than 20% in areas with access to antibiotics. Before antibiotics were available, the mortality was 80–100%. Morbidity rates also dropped from 70% to 22% due to earlier diagnosis and treatment.

Arterial Thrombosis

Arterial thrombosis is the formation of a thrombus within an artery. In most cases, arterial thrombosis follows rupture of atheroma (a fat-rich deposit in the blood vessel wall), and is therefore referred to as *atherothrombosis*. Arterial embolism occurs when clots then migrate downstream, and can affect any organ. Alternatively, arterial occlusion occurs as a consequence of embolism of blood clots originating from the heart (“cardiogenic” emboli).

The most common cause is atrial fibrillation, which causes a blood stasis within the atria with easy thrombus formation, but blood clots can develop inside the heart for other reasons too.

Stroke

A stroke is the rapid decline of brain function due to a disturbance in the supply of blood to the brain. This can be due to ischemia, thrombus, embolus (a lodged particle) or hemorrhage (a bleed). In thrombotic stroke, a thrombus (blood clot) usually forms around atherosclerotic plaques. Since blockage of the artery is gradual, onset of symptomatic thrombotic strokes is slower. Thrombotic stroke can be divided into two categories—large vessel disease and small vessel disease. The former affects vessels such as the internal carotids, vertebral and the circle of Willis. The latter can affect smaller vessels such as the branches of the circle of Willis.

Myocardial Infarction

Myocardial infarction (MI) or heart attack, is caused by ischemia, (restriction in the blood supply), often due to the obstruction of a coronary artery by a thrombus. This restriction gives an insufficient supply of oxygen to the heart muscle which then results in tissue death,(infarction). A lesion is then formed which is the infarct. MI can quickly become fatal if emergency medical treatment is not received promptly. If diagnosed within 12 hours of the initial episode (attack) then thrombolytic therapy is initiated.

Limb Ischemia

An arterial thrombus or embolus can also form in the limbs, which can lead to acute limb ischemia.

CAUSES

Thrombosis prevention is initiated with assessing the risk for its development. Some people have a higher risk of developing thrombosis and its possible development into thromboembolism. Some of these risk factors are related to inflammation. "Virchow's triad" has been suggested to describe the three factors necessary for the formation of thrombosis: stasis of blood, vessel wall injury, and altered blood coagulation. Some risk factors predispose for venous thrombosis while others increase the risk of arterial thrombosis..

Table. Risk factors for thrombosis.

Factor	Notes
Previous episodes of thrombosis	
Vasoconstriction	
Slow or turbulent blood flow	Slow flow is modifiable with exercise
Stroke	
Heart failure	
Sedentary life style	Modifiable
Plaster cast	Transient
Dehydration	Modifiable
Acute respiratory failure	
Dysrhythmias	
Shock	
Obesity	Modifiable
Pregnancy and the post-partum period	
Varicose veins	
Surgery	

Factor	Notes
Trauma	
Estrogen-based oral contraceptive	Discontinuation reduces risk
Hormone replacement therapy	Discontinuation reduces risk
Ovarian hyper-stimulation therapy to treat infertility	
Compression of a vein or artery by abnormality, tumor, hematoma	
Long surgeries	
Pacing wires	
Local vein damage, incompetent valves	
Central venous catheters	
Dialysis catheters	
Repetitive motion injury	
Immobility	Associated with long travel times and
post-surgical - modifiable risk	
Varicose veins	
Spinal cord injury	
Age	
Cancers	
Septicemia	
Polycythemia	
Protein C and/or S deficiency	Congenital; associated with Warfarin necrosis
Antiphospholipid antibody syndrome	Altered coagulation
Factor V Leiden defect	Altered coagulation
Prothrombin G20210A defect	Altered coagulation
Hyperhomocysteinemia	Altered coagulation
Elevated factors II, VIII, IX, XI	Altered coagulation
Antithrombin III deficiency	Altered coagulation
Falls and hip fracture	Related to immobility
Selective estrogen-receptor modulators	
Erythropoiesis-stimulating agents	
Acute medical illness	
Inflammatory bowel disease	
Nephrotic syndrome	
Myeloproliferative disorders	
Paroxysmal nocturnal hemoglo binnuria	
Thrombophilias	
Post-menopausal hormone replacement therapy	Discontinuation reduces risk
Right heart failure	
Venous inflammation/phlebitis	When a thrombus forms, it is thrombophlebitis

MECHANISM

Pathogenesis

The main causes of thrombosis are given in Virchow's triad which lists thrombophilia, endothelial cell injury, and disturbed blood flow.

Hypercoagulability

Hypercoagulability or *thrombophilia*, is caused by, for example, genetic deficiencies or autoimmune disorders. Recent studies indicate that white blood cells play a pivotal role in deep vein thrombosis, mediating numerous pro-thrombotic actions.

Endothelial Cell Injury

Any inflammatory process, such as trauma, surgery or infection, can cause damage to the endothelial lining of the vessel's wall. The main mechanism is exposure of tissue factor to the blood coagulation system. Inflammatory and other stimuli (such as hypercholesterolemia) can lead to changes in gene expression in endothelium producing to a pro-thrombotic state. When this occurs, endothelial cells downregulate substances such as thrombomodulin, which is a key modulator of thrombin activity. The end result is a sustained activation of thrombin and reduced production of protein C and tissue factor inhibitor, which furthers the pro-thrombotic state. Endothelial injury is almost invariably involved in the formation of thrombi in arteries, as high rates of blood flow normally hinder clot formation. In addition, arterial and cardiac clots are normally rich in platelets–which are required for clot formation in areas under high stress due to blood flow.

Disturbed Blood Flow

Causes of disturbed blood flow include stagnation of blood flow past the point of injury, or venous stasis which may occur in heart failure, or after long periods of sedentary behaviour, such as sitting on a long airplane flight. Also, atrial fibrillation, causes stagnant blood in the left atrium (LA), or left atrial appendage (LAA), and can lead to a thromboembolism. Cancers or malignancies such as leukemia may cause increased risk of thrombosis by possible activation of the coagulation system by cancer cells or secretion of procoagulant substances (paraneoplastic syndrome), by external compression on a blood vessel when a solid tumor is present, or (more rarely) extension into the vasculature (for example, renal cell cancers extending into the renal veins). Also, treatments for cancer (radiation, chemotherapy) often cause additional hypercoagulability. There are scores that correlate different aspects of patient data (comorbidities, vital signs, and others) to risk of thrombosis, such as the POMPE-C, which stratifies risk of mortality due to pulmonary embolism in patients with cancer, who typically have higher rates of thrombosis.

Pathophysiology

Natural History

Fibrinolysis is the physiological breakdown of blood clots by enzymes such as plasmin. Organisation: following the thrombotic event, residual vascular thrombus will be re-organised histologically with several possible outcomes. For an occlusive thrombus (defined as thrombosis within a small vessel that leads to complete occlusion), wound healing will reorganise the occlusive thrombus into collagenous scar tissue, where the scar tissue will either permanently obstruct the vessel, or contract down with myofibroblastic activity to unblock the lumen. For a mural thrombus (defined as a thrombus in a large vessel that restricts the blood flow but does not occlude completely), histological reorganisation of the thrombus does not occur via the classic wound healing mechanism. Instead, the platelet-derived growth factor degranulated by the clotted platelets will attract a layer of smooth muscle cells to cover the clot, and this layer of mural smooth muscle will be vascularised by the blood inside the vessel lumen rather than by the vasa vasorum. Ischaemia/infarction: if an arterial thrombus cannot be lysed by the body and it does not embolise, and if the thrombus is large enough to impair or occlude

blood flow in the involved artery, then local ischaemia or infarction will result. A venous thrombus may or may not be ischaemic, since veins distribute deoxygenated blood that is less vital for cellular metabolism. Nevertheless, non-ischaemic venous thrombosis may still be problematic, due to the swelling caused by blockage to venous drainage. In deep vein thrombosis this manifests as pain, redness, and swelling; in retinal vein occlusion this may result in macular oedema and visual acuity impairment, which if severe enough can lead to blindness.

Embolization

A thrombus may become detached and enter circulation as an embolus, finally lodging in and completely obstructing a blood vessel, which unless treated very quickly will lead to tissue necrosis (an infarction) in the area past the occlusion. Venous thrombosis can lead to pulmonary embolism when the migrated embolus becomes lodged in the lung. In people with a "shunt" (a connection between the pulmonary and systemic circulation), either in the heart or in the lung, a venous clot can also end up in the arteries and cause arterial embolism. Arterial embolism can lead to obstruction of blood flow through the blood vessel that is obstructed by it, and lack of oxygen and nutrients (ischemia) of the downstream tissue. The tissue can become irreversibly damaged, a process known as necrosis. This can affect any organ; for instance, arterial embolism of the brain is one of the cause of stroke.

PREVENTION

The use of heparin following surgery is common if there are no issues with bleeding. Generally, a risk-benefit analysis is required, as all anticoagulants lead to an increased risk of bleeding. In people admitted to hospital, thrombosis is a major cause for complications and occasionally death. In the UK, for instance, the Parliamentary Health Select Committee heard in 2005 that the annual rate of death due to thrombosis was 25,000, with at least 50% of these being hospital-acquired. Hence *thromboprophylaxis* (prevention of thrombosis) is increasingly emphasized.

In patients admitted for surgery, graded compression stockings are widely used, and in severe illness, prolonged immobility and in all orthopedic surgery, professional guidelines recommend low molecular weight heparin (LMWH) administration, mechanical calf compression or (if all else is contraindicated and the patient has recently suffered deep vein thrombosis) the insertion of a vena cava filter. In patients with medical rather than surgical illness, LMWH too is known to prevent thrombosis, and in the United Kingdom the Chief Medical Officer has issued guidance to the effect that preventative measures should be used in medical patients, in anticipation of formal guidelines.

TREATMENT

The treatment for thrombosis depends on whether it is in a vein or an artery, the impact on the person, and the risk of complications from treatment.

Anticoagulation

Warfarin and vitamin K antagonists are anticoagulants that can be taken orally to reduce thromboembolic occurrence. Where a more effective response is required, heparin can be given (by injection) concomitantly. As a side effect of any anticoagulant, the risk of bleeding is increased, so the international normalized ratio of blood is monitored. Self-monitoring and self-management are safe options for competent patients, though their practice varies. In Germany, about 20% of patients were self-managed while only 1% of U.S. patients did home self-testing (according to one 2012 study). Other medications such as direct thrombin inhibitors and direct Xa inhibitors are increasingly being used instead of warfarin.

Thrombolysis

Thrombolysis is the pharmacological destruction of blood clots by administering thrombolytic drugs including recombitant tissue plasminogen activator, which enhances the normal destruction of blood clots by the body's enzymes. This carries an increased risk of bleeding so is generally only used for specific situations (such as severe stroke or a massive pulmonary embolism).

Surgery

Arterial thrombosis may require surgery if it causes acute limb ischemia.

Endovascular Treatment

Mechanical clot retrieval and catheter-guided thrombolysis are used in certain situations.

Antiplatelet Agents

Arterial thrombosis is platelet-rich, and inhibition of platelet aggregation with antiplatelet drugs such as aspirin may reduce the risk of recurrence or progression.

PROTEIN C DEFICIENCY

Protein C deficiency is a rare genetic trait that predisposes to thrombotic disease. It was first described in 1981. The disease belongs to a group of genetic disorders known as thrombophilias. Protein C deficiency is associated with an increased incidence of venous thromboembolism (relative risk 8–10), whereas no association with arterial thrombotic disease has been found.

PRESENTATION

Complications

Protein C is vitamin K-dependent. Patients with Protein C deficiency are at an increased risk of developing skin necrosis while on warfarin. Protein C has a short half life (8 hour) compared with other vitamin K-dependent factors and therefore is rapidly depleted with warfarin initiation, resulting in a transient hypercoagulable state.

PATHOPHYSIOLOGY

The main function of protein C is its anticoagulant property as an inhibitor of coagulation factors V and VIII. A deficiency results in a loss of the normal cleaving of Factors Va and VIIIa.

There are two main types of protein C mutations that lead to protein C deficiency:

1. Type I: *Quantitative* defects of protein C (low production or short protein half life)
2. Type II: *Qualitative* defects, in which interaction with other molecules is abnormal. Defects in interaction with thrombomodulin, phospholipids, factors V/VIII and others have been described.

The majority of people with protein C deficiency lack only one copy of the functioning genes, and are therefore heterozygous. Before 1999, only sixteen cases of *homozygous* protein C deficiency had been described (two abnormal copies of the gene, leading to absence of functioning protein C in the bloodstream). This may manifest itself as purpura fulminans in newborn babies.

DIAGNOSTIC TESTING

There are two main types of protein C assays, activity and antigen (immunoassays). Commercially available activity assays are based on chromogenic assays that use activation by snake venom in an activating reagent, or clotting and enzyme-linked immunosorbant assays. Repeated testing for protein C functional activity allows differentiation between transient and congenital deficiency of protein C. Initially, a protein C activity (functional) assay can be performed, and if the result is low, a protein C antigen assay can be considered to determine the deficiency subtype (Type I or Type II). In type I deficiencies, normally functioning protein C molecules are made in reduced quantity.

In type II deficiencies normal amounts of dysfunctional protein C are synthesized. Antigen assays are immunoassays designed to measure the quantity of protein C regardless of its function. Type I deficiencies are therefore characterized by a decrease in both activity and antigen protein C assays whereas type II deficiencies exhibit normal protein C antigen levels with decreased activity levels.

The human protein C gene (PROC) comprises 9 exons, and protein C deficiency has been linked to over 160 mutations to date. Therefore, DNA testing for protein C deficiency is generally not available outside of specialized research laboratories. Manifestation of purpura fulminans as it is usually associated with reduced protein C plasma concentrations of <5 mg IU/dL. The normal concentration of plasma protein C is 70 nM (4 µg/mL) with a half live of approximately 8 hours. Healthy term neonates, however, have lower (and more variable) physiological levels of protein C (ranging between 15-55 IU/dL) than older children or adults, and these concentrations progressively increase throughout the first 6 months of life. Protein C levels may be <10 IU/dL in preterm or twin neonates or those with respiratory distress without manifesting either purpura fulminans or disseminated intravascular coagulation.

TREATMENT

Primary prophylaxis with low-molecular weight heparin, heparin, or warfarin is often considered in known familial cases. Anticoagulant prophylaxis is given to all who develop a venous clot regardless of underlying cause. Studies have demonstrated an increased risk of recurrent venous thromboembolic events in patients with protein C deficiency. Therefore, long-term anticoagulation therapy with warfarin may be considered in these patients. Homozygous protein C defect constitutes a potentially life-threatening disease, and warrants the use of supplemental protein C concentrates. Liver transplant may be considered curative for homozygous protein C deficiency.

EPIDEMIOLOGY

Heterozygous protein C deficiency occurs in 0.14–0.50% of the general population. Based on an estimated carrier rate of 0.2%, a homozygous or compound heterozygous protein C deficiency incidence of 1 per 4 million births could be predicted, although far fewer living patients have been identified. This low prevalence of patients with severe genetic protein C deficiency may be explained by excessive fetal demise, early postnatal deaths before diagnosis, heterogeneity in the cause of low concentrations of protein C among healthy individuals and under-reporting. The incidence of protein C deficiency in individuals who present with clinical symptoms has been reported to be estimated at 1 in 20,000.

PROTEIN S DEFICIENCY

Protein S deficiency is a disorder associated with increased risk of venous thrombosis. Protein S, a vitamin K-dependent physiological anticoagulant, acts as a nonenzymatic cofactor to activate protein C in the degradation of factor Va and factor VIIIa. Decreased (antigen) levels or impaired function of protein S leads to decreased degradation of factor Va and factor VIIIa and an increased propensity to venous thrombosis. Protein S circulates

in human plasma in two forms: approximately 60 percent is bound to complement component C4b β-chain while the remaining 40 percent is free, only free protein S has activated protein C cofactor activity

SIGNS/SYMPTOMS

Among the possible presentation of protein S deficiency are:

- Thrombosis of lower extremities
- Superficial thrombophlebitis
- Redness in affected area
- Purpura fulminans.

CAUSE

In terms of the cause of protein S deficiency it can be in *inherited* via autosomal dominance. A mutation in the PROS1 gene triggers the condition. The cytogenetic location of the gene in question is chromosome 3, specifically 3q11.1 Protein S deficiency can also be *acquired* due to vitamin K deficiency, treatment with warfarin, liver disease, and acute thrombosis (antiphospholipid antibodies may also be a cause as well)

PATHOPHYSIOLOGY

In regards to the mechanism of protein S deficiency, Protein S is principally made in liver cells. Protein S is a cofactor of APC both work to degrade factor V and factor VIII. It has been suggested that Zn2+ might be necessary for Protein S binding to factor Xa. Mutations in this condition change amino acids, which in turn disrupts blood clotting. Functional protein S is lacking, which normally *turns off* clotting proteins, this increases risk of blood clots.

DIAGNOSIS

The diagnosis for deficiency of protein S can be done via reviewing family history of condition and genetic testing, as well as the following:

- Protein S antigen test
- Coagulation test (prothrombin time test)
- Thrombotic disease investigation
- Factor V Leiden test.

Differential Diagnosis

Among the possibilities for differential diagnosis of protein S deficiency are- Antiphospholipid syndrome, disseminated intravascular coagulation and antithrombin deficiency (though this list is not exhaustive)

Types

There are three types of hereditary protein S deficiency:

- *Type I* – decreased protein S activity: decreased *total* protein S levels, as well as decreased free protein S levels.
- *Type II* – decreased in regards to the *cofactor* activity of the protein
- *Type III* – decreased protein S activity: Decreased free protein S levels (normal total protein S levels).

TREATMENT

In terms of treatment for protein S deficiency the following are consistent with the *management* (and administration of) individuals with this condition (the prognosis for *inherited* homozygotes is usually in line with a higher incidence of thrombosis for the affected individual):

- Unfractionated heparin (w/warfarin)
- LMWH/Low molecular weight heparin
- Dabigatran
- Direct Factor Xa Inhibitors
- Graduated compressed stocking
- High degree of prophylaxis.

ANTITHROMBIN III DEFICIENCY

Antithrombin III deficiency (abbreviated ATIII deficiency) is a deficiency of antithrombin III. This deficiency may be inherited or acquired. It is a rare hereditary disorder that generally comes to light when a patient suffers recurrent venous thrombosis and pulmonary embolism, and repetitive intrauterine fetal death (IUFD). Hereditary antithrombin deficiency results hyper state of coagulation which may lead to venous thrombosis. Inheritance is usually autosomal dominant, though a few recessive cases have been noted. The disorder was first described by Egeberg in 1965. The causes of acquired antithrombin deficiency are easier to find than the hereditary deficiency.

This disease is affecting one in thousand people annually. It is type of multifactorial disease where both genetics and environment affect the procoagulant and anticoagulant forces, finally leading the ATIII deficiency. Various mutations in genes, such as deletion or addition of genes, for anticoagulant proteins such as protein C, antithrombin or protein S are one of the risk factors. The deficiency may be caused by adhesion of platelets with immobilised fibrinogen. The patients are treated with anticoagulants or, more rarely, with antithrombin concentrate. In kidney failure, especially nephrotic syndrome, antithrombin is lost in the urine, leading to a higher activity of Factor II and Factor X and in increased tendency to thrombosis.

DIAGNOSIS

Different laboratory diagnosis can be performed for treatments. First, the numerical analysis for antithrombin can be performed. Smaller the number of antithrombin may lead to venous thrombosis and pulmonary embolism. Second, Anticardiolipin antibodies (immunoglobulin G [IgG] and IgM class) can be injected. Third, antigen activity and total tests for Protein C and Protein S can be checked to see if the genes of their proteins have been mutated. Fourth, Prothrombin time (PT) and activated partial thromboplastin time (aPTT) can be calculated to see how fast they take place. Finally, Factor V Leiden test can also be performed in order to check blood clotting and adhesion of platelets. Once a patient develops the congenital antithrombin III deficiency, a sign of anticoagulation can be easily indicated. Image experiments can be studied to evaluate the antithrombin III deficiency. First of all, echocardiography is performed to all patients suffering from antithrombin III deficiency. These patients will be first go through the blood test to find a sign go arterial thrombus, then echocardiography can be tested. Second, doppler ultrasonography is usually performed at the earlier stage than echocardiography to compress. It is used to find the resolution of an acute thrombus. Finally, ventilation-perfusion scanning is test to check for images of pulmonary thrombosis.

MANAGEMENT

Heparin enhances ATIII activity and neutralizes "activated serine protease coagulation factors." Patients with ATIII deficiency requiring anticoagulant therapy with heparin will need higher doses of heparin. ATIII

binds to thrombin and then forms the thrombin-anti thrombin complex or TAT complex. This is a major natural pathway of anticoagulation. This binding of thrombin to AT is greatly enhanced in the presence of heparin. Heparin does not affect vitamin K metabolism, so giving vitamin K1 (Phytonadione) will not reverse the effects of heparin. Heparin is used as "bridging" therapy when initiating a patient on warfarin in a hospital setting. It can be used in DVT prophylaxis and treatment, acute coronary syndromes, and ST-segment elevated MI.

STROKE

A stroke is a medical condition in which poor blood flow to the brain results in cell death. There are two main types of stroke: ischemic, due to lack of blood flow, and hemorrhagic, due to bleeding. They result in part of the brain not functioning properly. Signs and symptoms of a stroke may include an inability to move or feel on one side of the body, problems understanding or speaking, feeling like the world is spinning, or loss of vision to one side. Signs and symptoms often appear soon after the stroke has occurred. If symptoms last less than one or two hours it is known as a transient ischemic attack (TIA) or mini-stroke. A hemorrhagic stroke may also be associated with a severe headache.

The symptoms of a stroke can be permanent. Long-term complications may include pneumonia or loss of bladder control. The main risk factor for stroke is high blood pressure. Other risk factors include tobacco smoking, obesity, high blood cholesterol, diabetes mellitus, previous TIA, and atrial fibrillation. An ischemic stroke is typically caused by blockage of a blood vessel, though there are also less common causes. A hemorrhagic stroke is caused by either bleeding directly into the brain or into the space between the brain's membranes. Bleeding may occur due to a ruptured brain aneurysm. Diagnosis is typically with medical imaging such as a CT scan or magnetic resonance imaging (MRI) scan along with a physical exam. A CT scan is to *rule out* bleeding, not necessarily to *rule out* ischemia, which early on typically does not show up on a CT scan. Other tests such as an electrocardiogram (ECG) and blood tests are done to determine risk factors and rule out other possible causes. Low blood sugar may cause similar symptoms.

Prevention includes decreasing risk factors, as well as possibly aspirin, statins, surgery to open up the arteries to the brain in those with problematic narrowing, and warfarin in those with atrial fibrillation. A stroke or TIA often requires emergency care. An ischemic stroke, if detected within three to four and half hours, may be treatable with a medication that can break down the clot. Aspirin should be used. Some hemorrhagic strokes benefit from surgery.

Treatment to try to recover lost function is called stroke rehabilitation and ideally takes place in a stroke unit; however, these are not available in much of the world. In 2013 approximately 6.9 million people had an ischemic stroke and 3.4 million people had a hemorrhagic stroke. In 2015 there were about 42.4 million people who had previously had a stroke and were still alive. Between 1990 and 2010 the number of strokes which occurred each year decreased by approximately 10% in the developed world and increased by 10% in the developing world. In 2015, stroke was the second most frequent cause of death after coronary artery disease, accounting for 6.3 million deaths (11% of the total). About 3.0 million deaths resulted from ischemic stroke while 3.3 million deaths resulted from hemorrhagic stroke. About half of people who have had a stroke live less than one year. Overall, two thirds of strokes occurred in those over 65 years old.

CLASSIFICATION

Strokes can be classified into two major categories: ischemic and hemorrhagic. Ischemic strokes are caused by interruption of the blood supply to the brain, while hemorrhagic strokes result from the rupture of a blood vessel or an abnormal vascular structure. About 87% of strokes are ischemic, the rest being hemorrhagic. Bleeding can develop inside areas of ischemia, a condition known as "hemorrhagic transformation." It is unknown how many hemorrhagic strokes actually start as ischemic strokes.

Definition

In the 1970s the World Health Organization defined stroke as a "neurological deficit of cerebrovascular cause that persists beyond 24 hours or is interrupted by death within 24 hours", although the word "stroke" is centuries old. This definition was supposed to reflect the reversibility of tissue damage and was devised for the purpose, with the time frame of 24 hours being chosen arbitrarily. The 24-hour limit divides stroke from transient ischemic attack, which is a related syndrome of stroke symptoms that resolve completely within 24 hours. With the availability of treatments which can reduce stroke severity when given early, many now prefer alternative terminology, such as brain attack and acute ischemic cerebrovascular syndrome (modeled after heart attack and acute coronary syndrome, respectively), to reflect the urgency of stroke symptoms and the need to act swiftly.

Ischemic

In an ischemic stroke, blood supply to part of the brain is decreased, leading to dysfunction of the brain tissue in that area. There are four reasons why this might happen:

1. Thrombosis (obstruction of a blood vessel by a blood clot forming locally)
2. Embolism (obstruction due to an embolus from elsewhere in the body),
3. Systemic hypoperfusion (general decrease in blood supply, *e.g.,* in shock)
4. Cerebral venous sinus thrombosis.

Stroke without an obvious explanation is termed "cryptogenic" (of unknown origin); this constitutes 30-40% of all ischemic strokes. There are various classification systems for acute ischemic stroke. The Oxford Community Stroke Project classification (OCSP, also known as the Bamford or Oxford classification) relies primarily on the initial symptoms; based on the extent of the symptoms, the stroke episode is classified as total anterior circulation infarct (TACI), partial anterior circulation infarct (PACI), lacunar infarct (LACI) or posterior circulation infarct (POCI).

These four entities predict the extent of the stroke, the area of the brain that is affected, the underlying cause, and the prognosis. The TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification is based on clinical symptoms as well as results of further investigations; on this basis, a stroke is classified as being due to (1) thrombosis or embolism due to atherosclerosis of a large artery, (2) an embolism originating in the heart, (3) complete blockage of a small blood vessel, (4) other determined cause, (5) undetermined cause (two possible causes, no cause identified, or incomplete investigation). Users of stimulants, such as cocaine and methamphetamine are at a high risk for ischemic strokes.

Hemorrhagic

There are two main types of hemorrhagic stroke:

- Intracerebral hemorrhage, which is basically bleeding within the brain itself (when an artery in the brain bursts, flooding the surrounding tissue with blood), due to either intraparenchymal hemorrhage (bleeding within the brain tissue) or intraventricular hemorrhage (bleeding within the brain's ventricular system).
- Subarachnoid hemorrhage, which is basically bleeding that occurs outside of the brain tissue but still within the skull, and precisely between the arachnoid mater and pia mater (the delicate *innermost* layer of the three layers of the meninges that surround the brain).

The above two main types of hemorrhagic stroke are also two different forms of intracranial hemorrhage, which is the accumulation of blood anywhere within the cranial vault; but the other forms of intracranial hemorrhage, such as epidural hematoma (bleeding between the skull and the dura mater, which is the thick

outermost layer of the meninges that surround the brain) and subdural hematoma (bleeding in the subdural space), are not considered "hemorrhagic strokes".

Hemorrhagic strokes may occur on the background of alterations to the blood vessels in the brain, such as cerebral amyloid angiopathy, cerebral arteriovenous malformation and an intracranial aneurysm, which can cause intraparenchymal or subarachnoid hemorrhage. In addition to neurological impairment, hemorrhagic strokes usually cause specific symptoms (for instance, subarachnoid hemorrhage classically causes a severe headache known as a thunderclap headache) or reveal evidence of a previous head injury.

SIGNS AND SYMPTOMS

Stroke symptoms typically start suddenly, over seconds to minutes, and in most cases do not progress further. The symptoms depend on the area of the brain affected. The more extensive the area of the brain affected, the more functions that are likely to be lost. Some forms of stroke can cause additional symptoms. For example, in intracranial hemorrhage, the affected area may compress other structures. Most forms of stroke are not associated with a headache, apart from subarachnoid hemorrhage and cerebral venous thrombosis and occasionally intracerebral hemorrhage.

Early Recognition

Various systems have been proposed to increase recognition of stroke. Different findings are able to predict the presence or absence of stroke to different degrees. Sudden-onset face weakness, arm drift (*i.e.,* if a person, when asked to raise both arms, involuntarily lets one arm drift downward) and abnormal speech are the findings most likely to lead to the correct identification of a case of stroke increasing the likelihood by 5.5 when at least one of these is present). Similarly, when all three of these are absent, the likelihood of stroke is significantly decreased (– likelihood ratio of 0.39). While these findings are not perfect for diagnosing stroke, the fact that they can be evaluated relatively rapidly and easily make them very valuable in the acute setting. A mnemonic to remember the warning signs of stroke is FAST (facial droop, arm weakness, speech difficulty, and time to call emergency services), as advocated by the Department of Health (United Kingdom) and the Stroke Association, the American Stroke Association, the National Stroke Association (US), the Los Angeles Prehospital Stroke Screen (LAPSS) and the Cincinnati Prehospital Stroke Scale (CPSS). Use of these scales is recommended by professional guidelines. For people referred to the emergency room, early recognition of stroke is deemed important as this can expedite diagnostic tests and treatments. A scoring system called ROSIER (recognition of stroke in the emergency room) is recommended for this purpose; it is based on features from the medical history and physical examination.

Subtypes

If the area of the brain affected contains one of the three prominent central nervous system pathways—the spinothalamic tract, corticospinal tract, and the posterior column–medial lemniscus pathway, symptoms may include:

- Hemiplegia and muscle weakness of the face
- Numbness
- Reduction in sensory or vibratory sensation
- Initial flaccidity (reduced muscle tone), replaced by spasticity (increased muscle tone), excessive reflexes, and obligatory synergies.

In most cases, the symptoms affect only one side of the body (unilateral). Depending on the part of the brain affected, the defect in the brain is *usually* on the opposite side of the body. However, since these pathways also

travel in the spinal cord and any lesion there can also produce these symptoms, the presence of any one of these symptoms does not necessarily indicate a stroke. In addition to the above CNS pathways, the *brainstem* gives rise to most of the twelve cranial nerves.

A brainstem stroke affecting the brainstem and brain, therefore, can produce symptoms relating to deficits in these cranial nerves:

- Altered smell, taste, hearing, or vision (total or partial)
- Drooping of eyelid (ptosis) and weakness of ocular muscles
- Decreased reflexes: Gag, swallow, pupil reactivity to light
- Decreased sensation and muscle weakness of the face
- Balance problems and nystagmus
- Altered breathing and heart rate
- Weakness in sternocleidomastoid muscle with inability to turn head to one side
- Weakness in tongue (inability to stick out the tongue or move it from side to side)

If the *cerebral cortex* is involved, the CNS pathways can again be affected, but also can produce the following symptoms:

- Aphasia (difficulty with verbal expression, auditory comprehension, reading and writing; Broca's or Wernicke's area typically involved)
- Dysarthria (motor speech disorder resulting from neurological injury)
- Apraxia (altered voluntary movements)
- Visual field defect
- Memory deficits (involvement of temporal lobe)
- Hemineglect (involvement of parietal lobe)
- Disorganized thinking, confusion, hypersexual gestures (with involvement of frontal lobe)
- Lack of insight of his or her, usually stroke-related, disability

If the cerebellum is involved, ataxia might be present and this includes:

- Altered walking gait
- Altered movement coordination
- Vertigo and or disequilibrium.

Associated Symptoms

Loss of consciousness, headache, and vomiting usually occur more often in hemorrhagic stroke than in thrombosis because of the increased intracranial pressure from the leaking blood compressing the brain. If symptoms are maximal at onset, the cause is more likely to be a subarachnoid hemorrhage or an embolic stroke.

CAUSES

Thrombotic Stroke

In thrombotic stroke, a thrombus (blood clot) usually forms around atherosclerotic plaques. Since blockage of the artery is gradual, onset of symptomatic thrombotic strokes is slower than that of a hemorrhagic stroke. A thrombus itself (even if it does not completely block the blood vessel) can lead to an embolic stroke if the thrombus breaks off and travels in the bloodstream, at which point it is called an embolus.

Two types of thrombosis can cause stroke:

1. *Large vessel disease* involves the common and internal carotid arteries, the vertebral artery, and the Circle of Willis. Diseases that may form thrombi in the large vessels include (in descending incidence):

atherosclerosis, vasoconstriction (tightening of the artery), aortic, carotid or vertebral artery dissection, various inflammatory diseases of the blood vessel wall (Takayasu arteritis, giant cell arteritis, vasculitis), noninflammatory vasculopathy, Moyamoya disease and fibromuscular dysplasia.

2. *Small vessel disease* involves the smaller arteries inside the brain: branches of the circle of Willis, middle cerebral artery, stem, and arteries arising from the distal vertebral and basilar artery. Diseases that may form thrombi in the small vessels include (in descending incidence): lipohyalinosis (build-up of fatty hyaline matter in the blood vessel as a result of high blood pressure and aging) and fibrinoid degeneration (a stroke involving these vessels is known as a lacunar stroke) and microatheroma (small atherosclerotic plaques).

Sickle-cell anemia, which can cause blood cells to clump up and block blood vessels, can also lead to stroke. A stroke is the second leading cause of death in people under 20 with sickle-cell anemia. Air pollution may also increase stroke risk.

Embolic Stroke

An embolic stroke refers to an arterial embolism (a blockage of an artery) by an embolus, a traveling particle or debris in the arterial bloodstream originating from elsewhere. An embolus is most frequently a thrombus, but it can also be a number of other substances including fat (*e.g.,* from bone marrow in a broken bone), air, cancer cells or clumps of bacteria (usually from infectious endocarditis). Because an embolus arises from elsewhere, local therapy solves the problem only temporarily. Thus, the source of the embolus must be identified. Because the embolic blockage is sudden in onset, symptoms usually are maximal at the start. Also, symptoms may be transient as the embolus is partially resorbed and moves to a different location or dissipates altogether. Emboli most commonly arise from the heart (especially in atrial fibrillation) but may originate from elsewhere in the arterial tree. In paradoxical embolism, a deep vein thrombosis embolizes through an atrial or ventricular septal defect in the heart into the brain.

Causes of stroke related to the heart can be distinguished between high and low-risk:

- High risk: atrial fibrillation and paroxysmal atrial fibrillation, rheumatic disease of the mitral or aortic valve disease, artificial heart valves, known cardiac thrombus of the atrium or ventricle, sick sinus syndrome, sustained atrial flutter, recent myocardial infarction, chronic myocardial infarction together with ejection fraction <28 percent, symptomatic congestive heart failure with ejection fraction <30 percent, dilated cardiomyopathy, Libman-Sacks endocarditis, Marantic endocarditis, infective endocarditis, papillary fibroelastoma, left atrial myxoma and coronary artery bypass graft (CABG) surgery.
- Low risk/potential: calcification of the annulus (ring) of the mitral valve, patent foramen ovale (PFO), atrial septal aneurysm, atrial septal aneurysm *with* patent foramen ovale, left ventricular aneurysm without thrombus, isolated left atrial "smoke" on echocardiography (no mitral stenosis or atrial fibrillation), complex atheroma in the ascending aorta or proximal arch.

Among those who have a complete blockage of one of the carotid arteries, the risk of stroke on that side is about one percent per year. A special form of embolic stroke is the embolic stroke of undetermined source (ESUS). This subset of cryptogenetic stroke is defined as a non-lacunar brain infarct without proximal arterial stenosis or cardioembolic sources. About one out of six ischemic strokes could be classified as ESUS.

Cerebral Hypoperfusion

Cerebral hypoperfusion is the reduction of blood flow to all parts of the brain. The reduction could be to a particular part of the brain depending on the cause. It is most commonly due to heart failure from cardiac arrest or arrhythmias, or from reduced cardiac output as a result of myocardial infarction, pulmonary embolism,

pericardial effusion, or bleeding. Hypoxemia (low blood oxygen content) may precipitate the hypoperfusion. Because the reduction in blood flow is global, all parts of the brain may be affected, especially vulnerable "watershed" areas - border zone regions supplied by the major cerebral arteries. A watershed stroke refers to the condition when the blood supply to these areas is compromised. Blood flow to these areas does not necessarily stop, but instead it may lessen to the point where brain damage can occur.

Venous Thrombosis

Cerebral venous sinus thrombosis leads to stroke due to locally increased venous pressure, which exceeds the pressure generated by the arteries. Infarcts are more likely to undergo hemorrhagic transformation (leaking of blood into the damaged area) than other types of ischemic stroke.

Intracerebral Hemorrhage

It generally occurs in small arteries or arterioles and is commonly due to hypertension, intracranial vascular malformations (including cavernous angiomas or arteriovenous malformations), cerebral amyloid angiopathy, or infarcts into which secondary hemorrhage has occurred. Other potential causes are trauma, bleeding disorders, amyloid angiopathy, illicit drug use (*e.g.,* amphetamines or cocaine). The hematoma enlarges until pressure from surrounding tissue limits its growth, or until it decompresses by emptying into the ventricular system, CSF or the pial surface. A third of intracerebral bleed is into the brain's ventricles. ICH has a mortality rate of 44 percent after 30 days, higher than ischemic stroke or subarachnoid hemorrhage (which technically may also be classified as a type of stroke).

Other

Other causes may include spasm of an artery. This may occur due to cocaine.

Silent Stroke

A silent stroke is a stroke that does not have any outward symptoms, and the patients are typically unaware they have had a stroke. Despite not causing identifiable symptoms, a silent stroke still damages the brain, and places the patient at increased risk for both transient ischemic attack and major stroke in the future. Conversely, those who have had a major stroke are also at risk of having silent strokes. In a broad study in 1998, more than 11 million people were estimated to have experienced a stroke in the United States. Approximately 770,000 of these strokes were symptomatic and 11 million were first-ever silent MRI infarcts or hemorrhages. Silent strokes typically cause lesions which are detected via the use of neuroimaging such as MRI. Silent strokes are estimated to occur at five times the rate of symptomatic strokes. The risk of silent stroke increases with age, but may also affect younger adults and children, especially those with acute anemia.

PATHOPHYSIOLOGY

Ischemic

Ischemic stroke occurs because of a loss of blood supply to part of the brain, initiating the ischemic cascade. Brain tissue ceases to function if deprived of oxygen for more than 60 to 90 seconds, and after approximately three hours will suffer irreversible injury possibly leading to the death of the tissue, *i.e.,* infarction. (This is why fibrinolytics such as alteplase are given only until three hours since the onset of the stroke.) Atherosclerosis may disrupt the blood supply by narrowing the lumen of blood vessels leading to a reduction of blood flow, by

causing the formation of blood clots within the vessel, or by releasing showers of small emboli through the disintegration of atherosclerotic plaques. Embolic infarction occurs when emboli formed elsewhere in the circulatory system, typically in the heart as a consequence of atrial fibrillation, or in the carotid arteries, break off, enter the cerebral circulation, then lodge in and block brain blood vessels. Since blood vessels in the brain are now blocked, the brain becomes low in energy, and thus it resorts into using anaerobic metabolism within the region of brain tissue affected by ischemia. Anaerobic metabolism produces less adenosine triphosphate (ATP) but releases a by-product called lactic acid. Lactic acid is an irritant which could potentially destroy cells since it is an acid and disrupts the normal acid-base balance in the brain. The ischemia area is referred to as the "ischemic penumbra". As oxygen or glucose becomes depleted in ischemic brain tissue, the production of high energy phosphate compounds such as adenosine triphosphate (ATP) fails, leading to failure of energy-dependent processes (such as ion pumping) necessary for tissue cell survival. This sets off a series of interrelated events that result in cellular injury and death.

A major cause of neuronal injury is the release of the excitatory neurotransmitter glutamate. The concentration of glutamate outside the cells of the nervous system is normally kept low by so-called uptake carriers, which are powered by the concentration gradients of ions (mainly Na) across the cell membrane. However, stroke cuts off the supply of oxygen and glucose which powers the ion pumps maintaining these gradients. As a result, the transmembrane ion gradients run down, and glutamate transporters reverse their direction, releasing glutamate into the extracellular space. Glutamate acts on receptors in nerve cells (especially NMDA receptors), producing an influx of calcium which activates enzymes that digest the cells' proteins, lipids, and nuclear material. Calcium influx can also lead to the failure of mitochondria, which can lead further towards energy depletion and may trigger cell death due to programmed cell death. Ischemia also induces production of oxygen free radicals and other reactive oxygen species. These react with and damage a number of cellular and extracellular elements. Damage to the blood vessel lining or endothelium is particularly important. In fact, many antioxidant neuroprotectants such as uric acid and NXY-059 work at the level of the endothelium and not in the brain *per se*. Free radicals also directly initiate elements of the programmed cell death cascade by means of redox signaling.

These processes are the same for any type of ischemic tissue and are referred to collectively as the *ischemic cascade*. However, brain tissue is especially vulnerable to ischemia since it has a little respiratory reserve and is completely dependent on aerobic metabolism, unlike most other organs. In addition to damaging effects on brain cells, ischemia and infarction can result in loss of structural integrity of brain tissue and blood vessels, partly through the release of matrix metalloproteases, which are zinc- and calcium-dependent enzymes that break down collagen, hyaluronic acid, and other elements of connective tissue. Other proteases also contribute to this process. The loss of vascular structural integrity results in a breakdown of the protective blood brain barrier that contributes to cerebral edema, which can cause secondary progression of the brain injury.

Hemorrhagic

Hemorrhagic strokes are classified based on their underlying pathology. Some causes of hemorrhagic stroke are hypertensive hemorrhage, ruptured aneurysm, ruptured AV fistula, transformation of prior ischemic infarction, and drug induced bleeding. They result in tissue injury by causing compression of tissue from an expanding hematoma or hematomas. In addition, the pressure may lead to a loss of blood supply to affected tissue with resulting infarction, and the blood released by brain hemorrhage appears to have direct toxic effects on brain tissue and vasculature. Inflammation contributes to the secondary brain injury after hemorrhage.

DIAGNOSIS

Stroke is diagnosed through several techniques: a neurological examination (such as the NIHSS), CT scans (most often without contrast enhancements) or MRI scans, Doppler ultrasound, and arteriography. The diagnosis

of stroke itself is clinical, with assistance from the imaging techniques. Imaging techniques also assist in determining the subtypes and cause of stroke. There is yet no commonly used blood test for the stroke diagnosis itself, though blood tests may be of help in finding out the likely cause of stroke.

Physical Examination

A physical examination, including taking a medical history of the symptoms and a neurological status, helps giving an evaluation of the location and severity of a stroke. It can give a standard score on *e.g.,* the NIH stroke scale.

Imaging

For diagnosing ischemic (blockage) stroke in the emergency setting:

- CT scans (*without* contrast enhancements)
  Sensitivity = 16% (less than 10% within first 3 hours of symptom onset)
  Specificity = 96%
- MRI scan
  Sensitivity = 83%
  Specificity = 98%

For diagnosing hemorrhagic stroke in the emergency setting:

- CT scans (*without* contrast enhancements)
  Sensitivity = 89%
  Specificity = 100%
- MRI scan
  Sensitivity = 81%
  Specificity = 100%.

For detecting chronic hemorrhages, MRI scan is more sensitive. For the assessment of stable stroke, nuclear medicine scans SPECT and PET/CT may be helpful. SPECT documents cerebral blood flow and PET with FDG isotope the metabolic activity of the neurons. CT scans may not detect an ischemic stroke, especially if it is small, of recent onset, or in the brainstem or cerebellum areas. A CT scan is more to *rule out* certain stroke mimics and detect bleeding.

Underlying Cause

When a stroke has been diagnosed, various other studies may be performed to determine the underlying cause. With the current treatment and diagnosis options available, it is of particular importance to determine whether there is a peripheral source of emboli. Test selection may vary since the cause of stroke varies with age, comorbidity and the clinical presentation.

The following are commonly used techniques:

- An ultrasound/doppler study of the carotid arteries (to detect carotid stenosis) or dissection of the precerebral arteries;
- An electrocardiogram (ECG) and echocardiogram (to identify arrhythmias and resultant clots in the heart which may spread to the brain vessels through the bloodstream);
- A Holter monitor study to identify intermittent abnormal heart rhythms;
- An angiogram of the cerebral vasculature (if a bleed is thought to have originated from an aneurysm or arteriovenous malformation);

- Blood tests to determine if blood cholesterol is high, if there is an abnormal tendency to bleed, and if some rarer processes such as homocystinuria might be involved.

For hemorrhagic strokes, a CT or MRI scan with intravascular contrast may be able to identify abnormalities in the brain arteries (such as aneurysms) or other sources of bleeding, and structural MRI if this shows no cause. If this too does not identify an underlying reason for the bleeding, invasive cerebral angiography could be performed but this requires access to the bloodstream with an intravascular catheter and can cause further strokes as well as complicationes at the insertion site and this investigation is therefore reserved for specific situations. If there are symptoms suggesting that the hemorrhage might have occurred as a result of venous thrombosis, CT or MRI venography can be used to examine the cerebral veins.

Misdiagnosis

Among people with ischemic strokes, misdiagnosed occurs 2 to 26% of the time. A "stroke chameleon" (SC) is stroke which is diagnosed as something else. People not having a stroke may also be misdiagnosed as a stroke. Giving thrombolytics (clot-busting) in such cases causes intracerebral bleeding 1 to 2% of the time, which is less than that of people with strokes. This unnecessary treatment adds to health care costs. Even so, the AHA/ASA guidelines state that starting intravenous tPA in possible mimics is preferred to delaying treatment for additional testing.

Women, African-Americans, Hispanic-Americans, Asian and Pacific Islanders are more often misdiagnosed for a condition other than stroke when in fact having a stroke. In addition, adults under 44 years-of-age are seven times more likely to have a stroke missed than are adults over 75 years-of-age. This is especially the case for younger people with posterior circulation infarcts. Some medical centers have used hyperacute MRI in experimental studies for persons initially thought to have a low likelihood of stroke. And in some of these persons, strokes have been found which were then treated with thrombolytic medication.

PREVENTION

Given the disease burden of strokes, prevention is an important public health concern. Primary prevention is less effective than secondary prevention (as judged by the number needed to treat to prevent one stroke per year). Recent guidelines detail the evidence for primary prevention in stroke. In those who are otherwise healthy, aspirin does not appear beneficial and thus is not recommended. In people who have had a myocardial infarction or those with a high cardiovascular risk, it provides some protection against a first stroke. In those who have previously had a stroke, treatment with medications such as aspirin, clopidogrel, and dipyridamole may be beneficial. The U.S. Preventive Services Task Force (USPSTF) recommends against screening for carotid artery stenosis in those without symptoms.

Risk Factors

The most important modifiable risk factors for stroke are high blood pressure and atrial fibrillation although the size of the effect is small with 833 people have to be treated for 1 year to prevent one stroke. Other modifiable risk factors include high blood cholesterol levels, diabetes mellitus, cigarette smoking (active and passive), heavy alcohol use, drug use, lack of physical activity, obesity, processed red meat consumption, and unhealthy diet. Smoking just one cigarette per day increases the risk more than 30%. Alcohol use could predispose to ischemic stroke, and intracerebral and subarachnoid hemorrhage via multiple mechanisms (for example via hypertension, atrial fibrillation, rebound thrombocytosis and platelet aggregation and clotting disturbances). Drugs, most commonly amphetamines and cocaine, can induce stroke through damage to the blood vessels in the brain and

acute hypertension. Migraine with aura doubles a person's risk for ischemic stroke. Untreated, celiac disease regardless of the presence of symptoms can be an underlying cause of stroke, both in children and adults. High levels of physical activity reduce the risk of stroke by about 26%. There is a lack of high quality studies looking at promotional efforts to improve lifestyle factors. Nonetheless, given the large body of circumstantial evidence, best medical management for stroke includes advice on diet, exercise, smoking and alcohol use. Medication is the most common method of stroke prevention; carotid endarterectomy can be a useful surgical method of preventing stroke.

Blood Pressure

High blood pressure accounts for 35–50% of stroke risk. Blood pressure reduction of 10 mmHg systolic or 5 mmHg diastolic reduces the risk of stroke by ~40%. Lowering blood pressure has been conclusively shown to prevent both ischemic and hemorrhagic strokes. It is equally important in secondary prevention. Even patients older than 80 years and those with isolated systolic hypertension benefit from antihypertensive therapy. The available evidence does not show large differences in stroke prevention between antihypertensive drugs — therefore, other factors such as protection against other forms of cardiovascular disease and cost should be considered. The routine use of beta-blockers following a stroke or TIA has not been shown to result in benefits.

Blood Lipids

High cholesterol levels have been inconsistently associated with (ischemic) stroke. Statins have been shown to reduce the risk of stroke by about 15%. Since earlier meta-analyses of other lipid-lowering drugs did not show a decreased risk, statins might exert their effect through mechanisms other than their lipid-lowering effects.

Diabetes Mellitus

Diabetes mellitus increases the risk of stroke by 2 to 3 times. While intensive blood sugar control has been shown to reduce small blood vessel complications such as kidney damage and damage to the retina of the eye it has not been shown to reduce large blood vessel complications such as stroke.

Anticoagulation Drugs

Oral anticoagulants such as warfarin have been the mainstay of stroke prevention for over 50 years. However, several studies have shown that aspirin and other antiplatelets are highly effective in secondary prevention after a stroke or transient ischemic attack. Low doses of aspirin (for example 75–150 mg) are as effective as high doses but have fewer side effects; the lowest effective dose remains unknown. Thienopyridines (clopidogrel, ticlopidine) might be slightly more effective than aspirin and have a decreased risk of gastrointestinal bleeding, but are more expensive. Clopidogrel has less side effects than ticlopidine.

Dipyridamole can be added to aspirin therapy to provide a small additional benefit, even though headache is a common side effect. Low-dose aspirin is also effective for stroke prevention after having a myocardial infarction. Those with atrial fibrillation have a 5% a year risk of stroke, and this risk is higher in those with valvular atrial fibrillation. Depending on the stroke risk, anticoagulation with medications such as warfarin or aspirin is useful for prevention. Except in people with atrial fibrillation, oral anticoagulants are not advised for stroke prevention —any benefit is offset by bleeding risk. In primary prevention however, antiplatelet drugs did not reduce the risk of ischemic stroke but increased the risk of major bleeding. Further studies are needed to investigate a possible protective effect of aspirin against ischemic stroke in women.

Surgery

Carotid endarterectomy or carotid angioplasty can be used to remove atherosclerotic narrowing of the carotid artery. There is evidence supporting this procedure in selected cases. Endarterectomy for a significant stenosis has been shown to be useful in preventing further strokes in those who have already had one. Carotid artery stenting has not been shown to be equally useful. People are selected for surgery based on age, gender, degree of stenosis, time since symptoms and the person's preferences. Surgery is most efficient when not delayed too long —the risk of recurrent stroke in a patient who has a 50% or greater stenosis is up to 20% after 5 years, but endarterectomy reduces this risk to around 5%. The number of procedures needed to cure one patient was 5 for early surgery (within two weeks after the initial stroke), but 125 if delayed longer than 12 weeks. Screening for carotid artery narrowing has not been shown to be a useful test in the general population. Studies of surgical intervention for carotid artery stenosis without symptoms have shown only a small decrease in the risk of stroke. To be beneficial, the complication rate of the surgery should be kept below 4%. Even then, for 100 surgeries, 5 patients will benefit by avoiding stroke, 3 will develop stroke despite surgery, 3 will develop stroke or die due to the surgery itself, and 89 will remain stroke-free but would also have done so without intervention.

Diet

Nutrition, specifically the Mediterranean-style diet, has the potential for decreasing the risk of having a stroke by more than half. It does not appear that lowering levels of homocysteine with folic acid affects the risk of stroke.

Women

A number of specific recommendations have been made for women including taking aspirin after the 11th week of pregnancy if there is a history of previous chronic high blood pressure and taking blood pressure medications during pregnancy if the blood pressure is greater than 150 mmHg systolic or greater than 100 mmHg diastolic. In those who have previously had preeclampsia other risk factors should be treated more aggressively.

Previous Stroke or TIA

Keeping blood pressure below 140/90 mmHg is recommended. Anticoagulation can prevent recurrent ischemic strokes. Among people with nonvalvular atrial fibrillation, anticoagulation can reduce stroke by 60% while antiplatelet agents can reduce stroke by 20%. However, a recent meta-analysis suggests harm from anticoagulation started early after an embolic stroke. Stroke prevention treatment for atrial fibrillation is determined according to the CHA2DS2–VASc score. The most widely used anticoagulant to prevent thromboembolic stroke in patients with nonvalvular atrial fibrillation is the oral agent warfarin while a number of newer agents including dabigatran are alternatives which do not require prothrombin time monitoring. Anticoagulants, when used following stroke, should not be stopped for dental procedures. If studies show carotid artery stenosis, and the person has a degree of residual function on the affected side, carotid endarterectomy (surgical removal of the stenosis) may decrease the risk of recurrence if performed rapidly after stroke.

MANAGEMENT

Ischemic Stroke

Aspirin reduces the overall risk of recurrence by 13% with greater benefit early on. Definitive therapy within the first few hours is aimed at removing the blockage by breaking the clot down (thrombolysis), or by

removing it mechanically (thrombectomy). The philosophical premise underlying the importance of rapid stroke intervention was summed up as *Time is Brain!* in the early 1990s. Years later, that same idea, that rapid cerebral blood flow restoration results in fewer brain cells dying, has been proved and quantified. Tight blood sugar control in the first few hours does not improve outcomes and may cause harm. High blood pressure is also not typically lowered as this has not been found to be helpful. Cerebrolysin, a mix of pig brain tissue used to treat acute ischemic stroke in many Asian and European countries, does not improve outcomes and may increase the risk of severe adverse events.

Thrombolysis

Thrombolysis, such as with recombinant tissue plasminogen activator (rtPA), in acute ischemic stroke, when given within three hours of symptom onset results in an overall benefit of 10% with respect to living without disability. It does not, however, improve chances of survival. Benefit is greater the earlier it is used. Between three and four and a half hours the effects are less clear. A 2014 review found a 5% increase in the number of people living without disability at three to six months; however, there was a 2% increased risk of death in the short term. After four and a half hours thrombolysis worsens outcomes. These benefits or lack of benefits occurred regardless of the age of the person treated. There is no reliable way to determine who will have an intracranial bleed post-treatment versus who will not. Its use is endorsed by the American Heart Association and the American Academy of Neurology as the recommended treatment for acute stroke within three hours of onset of symptoms as long as there are not other contraindications (such as abnormal lab values, high blood pressure, or recent surgery). This position for tPA is based upon the findings of two studies by one group of investigators which showed that tPA improves the chances for a good neurological outcome. When administered within the first three hours thrombolysis improves functional outcome without affecting mortality. 6.4% of people with large strokes developed substantial brain bleeding as a complication from being given tPA thus part of the reason for increased short term mortality. Additionally, the American Academy of Emergency Medicine states that objective evidence regarding the efficacy, safety, and applicability of tPA for acute ischemic stroke is insufficient to warrant its classification as standard of care. Intra-arterial fibrinolysis, where a catheter is passed up an artery into the brain and the medication is injected at the site of thrombosis, has been found to improve outcomes in people with acute ischemic stroke.

Surgery

Surgical removal of the blood clot causing the ischemic stroke may improve outcomes if done within 7 hours of the start of symptoms in those with an anterior circulation large artery clot. It however does not change the risk of death. Significant complications occur in about 7%. Intravenous thrombolysis is generally used in eligible people even if they are being considered for mechanical thrombectomy. Certain cases may benefit from thrombectomy up to 24 hours after the onset of symptoms. Strokes affecting large portions of the brain can cause significant brain swelling with secondary brain injury in surrounding tissue. This phenomenon is mainly encountered in strokes affecting brain tissue dependent upon the middle cerebral artery for blood supply and is also called "malignant cerebral infarction" because it carries a dismal prognosis. Relief of the pressure may be attempted with medication, but some require hemicraniectomy, the temporary surgical removal of the skull on one side of the head. This decreases the risk of death, although some more people survive with disability who would otherwise have died.

Hemorrhagic Stroke

People with intracerebral hemorrhage require supportive care, including blood pressure control if required. People are monitored for changes in the level of consciousness, and their blood sugar and oxygenation are kept

at optimum levels. Anticoagulants and antithrombotics can make bleeding worse and are generally discontinued (and reversed if possible). A proportion may benefit from neurosurgical intervention to remove the blood and treat the underlying cause, but this depends on the location and the size of the hemorrhage as well as patient-related factors, and ongoing research is being conducted into the question as to which people with intracerebral hemorrhage may benefit. In subarachnoid hemorrhage, early treatment for underlying cerebral aneurysms may reduce the risk of further hemorrhages. Depending on the site of the aneurysm this may be by surgery that involves opening the skull or endovascularly (through the blood vessels).

Stroke Unit

Ideally, people who have had a stroke are admitted to a "stroke unit", a ward or dedicated area in a hospital staffed by nurses and therapists with experience in stroke treatment. It has been shown that people admitted to a stroke unit have a higher chance of surviving than those admitted elsewhere in hospital, even if they are being cared for by doctors without experience in stroke.

Rehabilitation

Stroke rehabilitation is the process by which those with disabling strokes undergo treatment to help them return to normal life as much as possible by regaining and relearning the skills of everyday living. It also aims to help the survivor understand and adapt to difficulties, prevent secondary complications and educate family members to play a supporting role. A rehabilitation team is usually multidisciplinary as it involves staff with different skills working together to help the patient. These include physicians trained in rehabilitation medicine, clinical pharmacists, nursing staff, physiotherapists, occupational therapists, speech and language therapists, and orthotists. Some teams may also include psychologists and social workers, since at least one-third of affected people manifests post stroke depression. Validated instruments such as the Barthel scale may be used to assess the likelihood of a stroke patient being able to manage at home with or without support subsequent to discharge from a hospital.

Good nursing care is fundamental in maintaining skin care, feeding, hydration, positioning, and monitoring vital signs such as temperature, pulse, and blood pressure. Stroke rehabilitation begins almost immediately. For most people with stroke, physical therapy (PT), occupational therapy (OT) and speech-language pathology (SLP) are the cornerstones of the rehabilitation process. Often, assistive technology such as wheelchairs, walkers and canes may be beneficial. Many mobility problems can be improved by the use of ankle foot orthoses. PT and OT have overlapping areas of expertise; however, PT focuses on joint range of motion and strength by performing exercises and relearning functional tasks such as bed mobility, transferring, walking and other gross motor functions. Physiotherapists can also work with patients to improve awareness and use of the hemiplegic side. Rehabilitation involves working on the ability to produce strong movements or the ability to perform tasks using normal patterns. Emphasis is often concentrated on functional tasks and patient's goals. One example physiotherapists employ to promote motor learning involves constraint-induced movement therapy. Through continuous practice the patient relearns to use and adapt the hemiplegic limb during functional activities to create lasting permanent changes. OT is involved in training to help relearn everyday activities known as the activities of daily living (ADLs) such as eating, drinking, dressing, bathing, cooking, reading and writing, and toileting. Speech and language therapy is appropriate for people with the speech production disorders: dysarthria and apraxia of speech, aphasia, cognitive-communication impairments, and problems with swallowing.

Patients may have particular problems, such as dysphagia, which can cause swallowed material to pass into the lungs and cause aspiration pneumonia. The condition may improve with time, but in the interim, a nasogastric tube may be inserted, enabling liquid food to be given directly into the stomach. If swallowing is still deemed unsafe, then a percutaneous endoscopic gastrostomy (PEG) tube is passed and this can remain indefinitely.

Treatment of spasticity related to stroke often involves early mobilizations, commonly performed by a physiotherapist, combined with elongation of spastic muscles and sustained stretching through various positionings. Gaining initial improvement in range of motion is often achieved through rhythmic rotational patterns associated with the affected limb. After full range has been achieved by the therapist, the limb should be positioned in the lengthened positions to prevent against further contractures, skin breakdown, and disuse of the limb with the use of splints or other tools to stabilize the joint. Cold in the form of ice wraps or ice packs have been proven to briefly reduce spasticity by temporarily dampening neural firing rates. Electrical stimulation to the antagonist muscles or vibrations has also been used with some success.

Stroke rehabilitation should be started as quickly as possible and can last anywhere from a few days to over a year. Most return of function is seen in the first few months, and then improvement falls off with the "window" considered officially by U.S. state rehabilitation units and others to be closed after six months, with little chance of further improvement. However, patients have been known to continue to improve for years, regaining and strengthening abilities like writing, walking, running, and talking. Daily rehabilitation exercises should continue to be part of the stroke patient's routine. Complete recovery is unusual but not impossible and most patients will improve to some extent: proper diet and exercise are known to help the brain to recover. Some current and future therapy methods include the use of virtual reality and video games for rehabilitation. These forms of rehabilitation offer potential for motivating patients to perform specific therapy tasks that many other forms do not. Many clinics and hospitals are adopting the use of these off-the-shelf devices for exercise, social interaction, and rehabilitation because they are affordable, accessible and can be used within the clinic and home. Mirror therapy is associated with improved motor function of the upper extremity in patients with stroke. Other non-invasive rehabilitation methods used to augment physical therapy of motor function in stroke patients include transcranial magnetic stimulation and transcranial direct-current stimulation. and robotic therapies.

A stroke can also reduce people's general fitness. Reduced fitness can reduce capacity for rehabilitation as well as general health. Physical exercises as part of a rehabilitation programme following a stroke appear safe. Cardiorespiratory fitness training that involves walking in rehabilitation can improve speed, tolerance and independence during walking, and may improve balance. There are inadequate long-term data about the effects of exercise and training on death, dependence and disability after a stroke. The future areas of research may concentrate on the optimal exercise prescription and long term health benefits of exercise. The effect of physical training on cognition also may be studied further. The ability to walk independently in their community, indoors or outdoors, is important following stroke. Although no negative effects have been reported, it is unclear if outcomes can improve with these walking programmes when compared to usual treatment.

Self-management

A stroke can affect the ability to live independently and with quality. Self-management programmes are a special training that educates stroke survivors about stroke and its consequences, helps them acquire skills to cope with their challenges, and helps them set and meet their own goals during their recovery process. These programmes are tailored to the target audience, and lead by someone trained and expert in stroke and its consequences (most commonly professionals, but also stroke survivors and peers). A 2016 review reported that these programmes improve the quality of life after stroke, without negative effects. People with stroke felt more empowered, happy and satisfied with life after participating in this training.

PROGNOSIS

Disability affects 75% of stroke survivors enough to decrease their employability. Stroke can affect people physically, mentally, emotionally, or a combination of the three. The results of stroke vary widely depending on size and location of the lesion. Dysfunctions correspond to areas in the brain that have been damaged. Some of

the physical disabilities that can result from stroke include muscle weakness, numbness, pressure sores, pneumonia, incontinence, apraxia (inability to perform learned movements), difficulties carrying out daily activities, appetite loss, speech loss, vision loss and pain. If the stroke is severe enough, or in a certain location such as parts of the brainstem, coma or death can result. Emotional problems following a stroke can be due to direct damage to emotional centers in the brain or from frustration and difficulty adapting to new limitations. Post-stroke emotional difficulties include anxiety, panic attacks, flat affect (failure to express emotions), mania, apathy and psychosis. Other difficulties may include a decreased ability to communicate emotions through facial expression, body language and voice.

Disruption in self-identity, relationships with others, and emotional well-being can lead to social consequences after stroke due to the lack of ability to communicate. Many people who experience communication impairments after a stroke find it more difficult to cope with the social issues rather than physical impairments. Broader aspects of care must address the emotional impact speech impairment has on those who experience difficulties with speech after a stroke. Those who experience a stroke are at risk of paralysis which could result in a self disturbed body image which may also lead to other social issues. 30 to 50% of stroke survivors suffer post-stroke depression, which is characterized by lethargy, irritability, sleep disturbances, lowered self-esteem and withdrawal. Depression can reduce motivation and worsen outcome, but can be treated with social and family support, psychotherapy and, in severe cases, antidepressants.

Emotional lability, another consequence of stroke, causes the person to switch quickly between emotional highs and lows and to express emotions inappropriately, for instance with an excess of laughing or crying with little or no provocation. While these expressions of emotion usually correspond to the person's actual emotions, a more severe form of emotional lability causes the affected person to laugh and cry pathologically, without regard to context or emotion. Some people show the opposite of what they feel, for example crying when they are happy. Emotional lability occurs in about 20% of those who have had a stroke. Those with a right hemisphere stroke are more likely to have an empathy problems which can make communication harder. Cognitive deficits resulting from stroke include perceptual disorders, aphasia, dementia, and problems with attention and memory. A stroke sufferer may be unaware of his or her own disabilities, a condition called anosognosia. In a condition called hemispatial neglect, the affected person is unable to attend to anything on the side of space opposite to the damaged hemisphere. Cognitive and psychological outcome after a stroke can be affected by the age at which the stroke happened, pre-stroke baseline intellectual functioning, psychiatric history and whether there is pre-existing brain pathology. Up to 10% of people following a stroke develop seizures, most commonly in the week subsequent to the event; the severity of the stroke increases the likelihood of a seizure.

EPIDEMIOLOGY

Stroke was the second most frequent cause of death worldwide in 2011, accounting for 6.2 million deaths (~11% of the total). Approximately 17 million people had a stroke in 2010 and 33 million people have previously had a stroke and were still alive. Between 1990 and 2010 the number of strokes decreased by approximately 10% in the developed world and increased by 10% in the developing world. Overall, two-thirds of strokes occurred in those over 65 years old. South Asians are at particularly high risk of stroke, accounting for 40% of global stroke deaths. It is ranked after heart disease and before cancer. In the United States stroke is a leading cause of disability, and recently declined from the third leading to the fourth leading cause of death. Geographic disparities in stroke incidence have been observed, including the existence of a "stroke belt" in the southeastern United States, but causes of these disparities have not been explained.

The risk of stroke increases exponentially from 30 years of age, and the cause varies by age. Advanced age is one of the most significant stroke risk factors. 95% of strokes occur in people age 45 and older, and two-thirds of strokes occur in those over the age of 65. A person's risk of dying if he or she does have a stroke also

increases with age. However, stroke can occur at any age, including in childhood. Family members may have a genetic tendency for stroke or share a lifestyle that contributes to stroke. Higher levels of Von Willebrand factor are more common amongst people who have had ischemic stroke for the first time. The results of this study found that the only significant genetic factor was the person's blood type. Having had a stroke in the past greatly increases one's risk of future strokes. Men are 25% more likely to suffer strokes than women, yet 60% of deaths from stroke occur in women. Since women live longer, they are older on average when they have their strokes and thus more often killed. Some risk factors for stroke apply only to women. Primary among these are pregnancy, childbirth, menopause, and the treatment thereof (HRT).

HISTORY

Episodes of stroke and familial stroke have been reported from the 2nd millennium BC onward in ancient Mesopotamia and Persia. Hippocrates (460 to 370 BC) was first to describe the phenomenon of sudden paralysis that is often associated with ischemia. Apoplexy, from the Greek word meaning "struck down with violence", first appeared in Hippocratic writings to describe this phenomenon. The word *stroke* was used as a synonym for apoplectic seizure as early as 1599, and is a fairly literal translation of the Greek term. In 1658, in his *Apoplexia*, Johann Jacob Wepfer (1620–1695) identified the cause of hemorrhagic stroke when he suggested that people who had died of apoplexy had bleeding in their brains. Wepfer also identified the main arteries supplying the brain, the vertebral and carotid arteries, and identified the cause of a type of ischemic stroke known as a cerebral infarction when he suggested that apoplexy might be caused by a blockage to those vessels. Rudolf Virchow first described the mechanism of thromboembolism as a major factor. The term *cerebrovascular accident* was introduced in 1927, reflecting a "growing awareness and acceptance of vascular theories and (...) recognition of the consequences of a sudden disruption in the vascular supply of the brain". Its use is now discouraged by a number of neurology textbooks, reasoning that the connotation of fortuitousness carried by the word *accident* insufficiently highlights the modifiability of the underlying risk factors. *Cerebrovascular insult* may be used interchangeably. The term *brain attack* was introduced for use to underline the acute nature of stroke according to the American Stroke Association, who since 1990 have used the term, and is used colloquially to refer to both ischemic as well as hemorrhagic stroke.

RESEARCH

Angioplasty and Stenting

Angioplasty and stenting have begun to be looked at as possible viable options in treatment of acute ischemic stroke. Intra-cranial stenting in symptomatic intracranial arterial stenosis, the rate of technical success (reduction to stenosis of <50%) ranged from 90-98%, and the rate of major peri-procedural complications ranged from 4-10%. The rates of restenosis and stroke following the treatment were also favourable. This data suggests that a randomized controlled trial is needed to more completely evaluate the possible therapeutic advantage of this preventative measure.

Neuroprotection

Neuroprotective agents including antioxidants which combat reactive oxygen species, or inhibit programmed cell death, or inhibit excitatory neurotransmitters have been shown experimentally to reduce tissue injury caused by ischemia. Until recently, human clinical trials with neuroprotective agents have failed, with the probable exception of deep barbiturate-induced coma. Disufenton sodium, the disulfonyl derivative of the radical-

scavenging phenylbutylnitrone, was reported to be neuroprotective. This agent is thought to work at the level of the blood vessel lining. However the favourable results evidenced from one large-scale trial were not reproduced in a second trial. So that the benefit of disufenton sodium is questionable. Hyperbaric oxygen therapy has been studied as a possible protective measure, but as of 2014, while the benefits of this have not been ruled out, further research is said to be needed. Modulating microglial activation and polarization might mitigate hemorrhagic stroke injury and improve brain repair.

Bibliography

Abdelgader EA. Diab TA, Kordofani AA and Abdalla SE.: *Haemoglobin Level, RBC Indices and Iron Status in Pregnant Females in Sudan*. Basic Research Journal of Medicine and Clinical Sciences 2014.

Afifi RA, Ali DK and Talkhan HM.: *Pregnancy Outcome and the Effect of Maternal Nutritional Status.* Journal of the Egyptian Society of Parasitology. 2013.

Agarwal DK, Agarwal KN, Roychoudhary S.: *Target in National Anaemia Prophylaxis Programme for Pregnant Women.* Indian Paediatr 2000.

Agarwal KN, Agarwal DK, Sharma A, Sharma K, Prasad K, Kalita MC.: *Prevalence of Anaemia in Pregnant Women*. Indian J Med Res 2006.

Agarwal T, Kochar GK and Goel S.: *Impact of Iron Supplementation on Anemia During Pregnancy.* Ethno Med. 2008.

Allen L, de Benoist B, Dary O, Hurell R, editors. *Guidelines on Food Fortification with Micronutrients.* Geneva, Switzerland: WHO; 2006.

Allen L.H.: *Biological Mechanisms that might Underlie Iron's Effects on Fetal Growth and Preterm Birth.* J Nutr 2001.

Allen LH.: *Anemia and Iron Deficiency: Effect on pregnancy outcome*. Am J Clin Nutr 2000.

Allen LH.: *Pregnancy and Iron Deficiency Unreasoned Issues.* National Nutr Rev, 2001.

Alquaiz JM, Addulaghani HM, Khawaja RA and Ahamed SS.: *Accuracy of Various Iron Parameters in the Prediction of Iron Deficiency Anaemia among Healthy Women of Child Bearing Age,* Saudi Arabia. Iranian Red Crescent Medical Journal, 2012.

Anderson AS, Campbell D, Shepherd R.: *Nutrition Knowledge, Attitude to Healthier Eating and Dietary Intake in Pregnant Compared to Non pregnant Women.* Journal of Human Nutrition and Dietetics. 2003.

Andrews NC.: *Disorders of Iron Metabolism chap 46. In Blood: Principles and Practice of Hematology.* Editors Handin RI Lux SE Stossel JP 2003.

Artiss JD, Vinogradev S, Zak B.: *Spectrophotometric Study of Sensitive Reagents for Serum Iron.* Clin Biochem 2001.

Arturo AL, Marti GP, Comunian G and Munoz S.: *Prevalence of Anaemia During Pregnancy.* Archivos Latinoamericans de Nutricion 2002.

Ashwood ER.: *Clinical Chemistry of Pregnancy In Tietz Fundamentals of Clinical Chemistry.* Am J Clin Nutr 2006.

Asif N, Hassan K, Mahmuds, Zaheer H, Naseem L, Zafar T and Shams R.: *Comparison of Serum Ferritin Levels in Three Trimesters of Pregnancy and their Correlation with Increasing Gravidity.* International Journal of Pathology 2007.

Bakeit ZAN, Megeid FYA, Badr NAA and Alsohaibani EA.: *Micronutrients Status and Correlation between Some Micronutrients Deficiency and Pregnancy Characteristics of Pregnant Women in Hafr Al-Baten.* World Journal of Medical Sciences. 2011.

Barrett FR, Whittaker PG, Williams JG.: *Absorption of Non Haem Iron from Food During Pregnancy*. BMJ 2004.

Batool Z, Zafar I M, Anwar S, Bano S, Gul M and Nazir F.: *An Investigation into Socio-economic Factors Explaining Dietary Intake in Pregnant Women*. A study in Urban areas of District Faisalabad, Punjab, Pakistan. Pakistan Journal of Nutrition 2012.

Batra J, Sood A.: *Iron Deficiency Anaemia: Effect on Cognitive Development in Children*: A review. Indian Journal of Clinical Biochemistry 2005.

Baynes RD, Skikine B. Iron absorption: In: Brock, JH, Halliday JW, Pipard MJ and Powell LW (eds).: *Iron metabolism in Health and Disease*, London, UK : WB Saunders, 2004.

Beard JL Hendricks MK, Perez EM.: *Maternal Iron Deficiency Anaemia Affects Post Partum Emotions and Cognition* J. Nutr 2005.

Berry GK.: *Measure of Agreement Statistical Methods in Medical Research*, Oxford Black Well Scientific, 2000.

Bhale DV. Bhale PV, Mande SA and Guddetwar S.: *Study of Serum Ferritin Levels in Anaemia of Pregnancy*. International Journal of Recent Trends in Science and Technology 2013.

Bhalerao VR, Galwankar M, Kowli SS, Kumar R, Chaturvedi RM.: *Contribution of the Education of the Prospective Fathers to the Success of Maternal Health Care Programme*. J Postgrad Med. 2000.

Bleyere MN, Amonkan AK, Kone M, Sawadoga D and Yapo PA.: *High Variability of Iron Status in Adolescent During Pregnancy in Cote D Ivoire*. J Blood Disorders Tranf. 2013.

Bothwell TH, Baynes RO, Macfarlane BJ, Macphail AP.: *Nutritional Iron Requirements and Food Iron Absorption.* J Intern Med 2003.

Brahin L, Nicholas S, Gogate A, Gogate S, Karande A.: *High Prevalence of Anaemia among Women in Mumbai, India*. Food Nutrition Bull 2003.

Breymann C.: *Iron Deficiency and Anemia in Pregnancy*: *Modern Aspects of Diagnosis and Therapy*. Blood Cells Molec Dise. 2002.

Brown SA, Seifert SA, Rayburn WF. *Management of Envenomations During Pregnancy*. Clin. Toxicol. (Phila) 2013.

Brune M, Rossander L and Hallberg L: *Iron Absorption and Phenolic Compounds: Importance of Different Phenolic Structure*, Eur J clin Nutr. 1989.

Chancy SG. Principles of Nutrition: *Micronutrients in Textbook of Bio Chemistry with clinical correlations* (ed by TM Devlin) 1986.

Chang SC, Obrien KO, Nathanson MS, Mancini J, Witter FR *Hemoglobin Concentrations Influence Birth Outcomes in Pregnant African-American Adolescents.* J Nutr. 2003.

Chard T, Lilford RJ. How useful is a test? J. stud Ed. *Progress in Obstetrics and Gynaecology.* 2006.

Cook JD, Sikikne BS, Lynch SR, Reusser ME. *Estimation of iron sufficiency in US population.* Blood. 1986.

Daftary SN and Chakravarti S.: *Manual of Obstetrics Update Edition of the Classic Halland and Brews Manual of Obstetrics*, 3rd edition. Published by Elsevier, A division of Reed Elsevier India Private Limited. 2011.

Derman D. *Iron Absorption from Cereal Based Meal Containing Cane Sugar Fortified with Ascorbic Acid.* Br J Nutr, 2001.

Dutta DC. Anemia in pregnancy. *Text book of obstetrics including Perinatology & Contraception 6th edition*, Culculta, India 2004.

Earl R, Woteki EM. Eds. *Iron Deficiency Anaemia Recommended Guidelines for the Prevention, Detection and Managements*

among US Children and Women of Child Bearing Age. Washington DC. National Academy Press, 2001.

Eshaghian S, Horwich TB, Fonarow GC. *An Unexpected Inverse Relationship between Hb AIC Levels and Mortality in Patients with Diabetes and Advanced Systolic Heart Failure*. Am Heart J 2006.

F.M.O.H. *Guideline for Malaria Control for Physicians in Nigeria*. Fed. Ministry of Health, Lagos, 2000.

Fairbanks UF, Beutler E. *Iron Metabolism in Williams Hematology* (ed. by E Beutler, MS Lichtmen, BS Coller J.J Kipps, U Seligsohn), McGraw Hill, USA. 2001.

Fairbanks VF, Klee GG,: *Biochemical Aspects of Hematology*. In : Tietz Fundamentals of clinical chemistry (ed by CA Burtis, ER Ashwood), WB Sanders, USA, 2000.

Fairweather-tait SJ : *Iron Nutrition in the UK : Getting the Balance Diet*. Proc Nutr Soc. 2004.

Flemming DJ, Jacques PF, Dallal GE, Tucker KL, Wilson PW, Wood RJ.: *Dietary Determinants of Iron Stores in a Free Living Elderly Population,* 2000.

Galy B, Ferring AD, Saver SW. *Iron Rregulatory Proteins Secure Mitochondrial Iron Sufficiency and Functions. Cell Metabolism,* 2010.

Garg A and Kashyap S. *Effect of Counselling on Nutritional Status During Pregnancy*. Indian Journal of Pediatrics. 2006.

Gautam CS, Saha L, Sekhari K, Saha PK. *Iron Deficiency in Pregnancy and the Rationality of Iron Supplements Prescribed During Pregnancy*. Medscape J Med. 2008.

Gillespie S.:*Malnutrition in South East Asia, A regional profile, Rosa Publication no. 5*, UNICEF, Regional office for South Asia Nov. 2001.

Gillespie SR. *Major Issue in the Control of Iron Deficiency.* Ottawa : Micronutrient Initiative, 2000.

Hallberg L, Hulten L: *Iron Requirements, Iron Balance and Iron Deficiency in Menstruating and Pregnant Women.* Iron Nutrition in Health and Disease. George Libbey, London 2006.

Hiremath DA and Hiremath LD.: *Essentials of Community Medicine a Practical Approach,* 1st edition, Jaypee Brothers Publishers Pvt Ltd, New Delhi, 2004.

Hoffbrand AV, Pettit JE, Moss PAH.: *Essential Haematology,* Black Well Science, Italy, 2001.

Hopkin RJ.: *Availability of Iron from Vegetarian and Non Vegetarian Sources*. Am J Clin Nutr 2003.

International Nutritional Anaemia Consultative Group (INAC): *Iron deficiency in women*. Washington D.C. Nutrition Foundation 2005.

Jain S.: *Childhood and Adolescent in India II IMA Report on Anaemia,* 2006.

Janne J, Vihko R.: *Serum Ferritin in the Diagnosis of Anaemia during Pregnancy.* Acta Obstet Gynecol Scand 2000.

Johansson and Anderson: *Nutritional Intake by Rural Females.* J Health Popul Nutr. 2001.

Karaoglu L, Pehlivan E, Egri M, Deprem C, Gunes G, Genc MF: *The Prevalence of Nutritional Anaemia in Pregnancy in an East Anatolian Province,* Turkey. BMC Public Health. 2010.

Khan ME, Khan I, Mukerjee N.: *Involving Men in Safe Motherhood.* J Fam Welfare. 2000.

Lacasse A, Rey E Ferreira E, Morin C and Berard A.: *Epidemiology of Nausea and Vomiting of Pregnancy: Prevalence, Severity, Determinants and the Importance of Race/Ethnicity.* Bio Med Central Pregnancy and Child birth. 2009.

Ladipo OA.: *Nutrition in Pregnancy: Mineral and Vitamin Supplements*. Amer J Nutr. 2000.

Lokare PO, Karanjekar VD, Gattani PL, Kulkarni AP.: *A Study of Prevalence of Anemia and Socio Demographic Factors Associated with Anemia among Pregnant Women in Aurangabad City*, India. Ann Nigerian Med. 2012.

Lynch SR.: *Ascorbic Acid and Iron Nutrition,* ASDCJ Dent Child 1981.

Lynch SR.: *The Potential Impact of Iron Supplementation during Adolescence on Iron Status in Pregnancy*. J Nutr

2000.

Margaret R.: *The Obstetric Hematology Manual, Sue Pavord and Beverly Hunt*. Published by Cambridge University Press, 2010.

Martinez B, Rincon F, Ibanez MV.: *Effects of Ascorbic Acid and Ferrous Sulphate on Trace Elements Extractability by Dialyzation of Weaning*. Food Chem. 2004.

Mazza J.: *Manual of Clinical Hematology,* Philadelphia: Lippincott Williams and Wilkins, 2002.

Mishra V, Thapa S. Retherfard RD, Dai X.: *Effect of Iron Supplementation During Pregnancy on Birth Weight: Evidences from Zimbabwe*. Food Nutr Bull 2005.

Mittal and Srivastava.: *Nutritional Status and Food Related Traditions of Oraon Tribes of New Mal*, West Bengal 2006.

Morck TA, Lynch SE and Cook JD.: *Inhibition of Food Iron by Coffee*. Am J Clin Nutr. 2003.

Mridula D, Mishra C P, Chakravorty: *A Dietary Intake of Expectant mother.* Indian Journal of Nutrition and Dietetics. 2003.

Mudaliar AL and Meanon MK.: *Clinical Obstetrics.* Orient Longman; Hyderabad. 2005.

Noronha JA, Bhaduri A and Bhat V.: *Prevalence of Anaemia Among Pregnant women: A community based study in Udupi District.* Health and Population. 2008.

Panigrahi S and Sahoo PS.: *Nutritional anaemia and its epidemiological correlates among women of reproductive age in an Urban slum of Bhubaneswar,* Orissa. Indian Journal of Public Health. 2011.

Pant BR.: *Women and Nutrition in Himalayan Region*: A case study. Himalayan Ecology 2008.

Rasheed P, Koura MR, Al-Dabal BK, Makki SM. Anaemia in pregnancy: *A Study among Attendees of Primary Health Care Centers.* Ann Saudi Med 2008.

Raza N, Sarwar I, Munazza B, Ayub M and Suleman M.: *Assessment of Iron Deficiency in Pregnant Women by Determining Iron Status*. J Ayub Med coll Abbottabad. 2011.

Reddy V, Pralhad Rao N, Sastry GJ, and Kashinath K.: *Nutrition Trends in India*, NIN, ICMR, Hyderabad; 2003.

Rhode BM, Shustick C, Christon NV, Maclean LD.: *Iron Absorption and Therapy after Gastric Bypass.* Obes Surg 2000.

Rizwan F. Qamarunisa, Habibullah and Memon A.: *Prevalence of Anaemia in Pregnant Women and its Effects of Maternal and Fetal Morbidity and Mortality.* Pak J Med Sci 2010.

Roberts JM, Balk JL, Bodnar LM, Belizan JM, Bergel E.L: *Nutrient Involvement in Preeclampsia*. J Nutr. 2003.

Rund D, Filon D, Stuss N.: *Mean Corpuscular Volume of Heterzygotes for Beta Thalassemia Correlates with the Severity of Mutations*. Blood 2000.

Rush D.: *Nutrition and Maternal Mortality in the Developing World*. Am J clin Nutr 2000.

Sachdeva R, Kaur P, Kochhar A and Chawla P.: *Impact of Iron Status of Pregnant Women on the Anthropometry of New Born in Industrial and Non Industrial Areas*. Anthropologist 2009.

Steer PJ.: *Maternal Haemoglobin Concentration and birth weight*. Am J Clin Nutr 2002.

Sukchan P, Liabsuetrakul T, Chongsuvivat W, Songwathana P, Sornsrivichai V and Kuning M.: *In Adequacy of Nutrients Intake among Pregnant Women in Deep South of Thailand.* BMC Public Health. 2010.

Toteja GS, Saxena BN.: *Prevalence of Anemia among Pregnant Women and Adolescent Girls in 16 Districts of India*, Food Nutr Bull. 2006.

Yip R, Dallmann PR. Iron In : *Present Knowledge in Nutrition* (ed Ziegler, LJ Filer), Washington, 2006.

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