

Lectur 5 in hematology by Dr.Alaa fadhil Alwan

The Inherited Hemolytic Anemias

Hemolytic anemias are characterized by premature destruction of erythrocytes (hemolysis).

Clinically, they are recognized by an increase in the reticulocyte count

There are many different types of hemolytic anemia. At the most basic level, hemolytic anemias can be divided into conditions that are intrinsic to the erythrocyte and those that are extrinsic to the erythrocyte. Virtually all hemolytic anemias that are intrinsic to the erythrocyte are inherited, and virtually all hemolytic anemias that are extrinsic to the erythrocyte are acquired.

Sickle Cell Anemia (Hemoglobin S)

Epidemiology

Hemoglobin S (sickle hemoglobin) is the most common hemoglobinopathy worldwide. Hemoglobin S is found most frequently in equatorial Africa and in people of African descent. There are separate pockets of hemoglobin S in Turkey, along the Mediterranean coast (Sicily, southern Italy, and northern Greece), in Saudi Arabia. These are areas where falciparum malaria is endemic; suggesting that hemoglobin S arose as a protective mechanism against malaria.

Pathophysiology

The abnormality in Hb S is substitution of valine for glutamic acid at the sixth amino acid position. Deoxygenated hemoglobin S tends to polymerize into long rigid structures, which distort the cell into the characteristic sickle shape. Anything that causes deoxygenation of hemoglobin predisposes to sickling, including hypoxia, acidosis, and increased temperature. The polymerization of hemoglobin S is reversible, and cells that have sickled may return to normal shape with re-oxygenation. However, the repeated cycles of sickling and unsickling damage the cell, and, eventually, the erythrocytes becomes irreversibly sickled. The rigid elongated sickle cells obstruct small blood vessels, resulting in tissue infarction. Sickled erythrocytes are also “sticky” and adhere to endothelial cells, predisposing to thrombosis. Common sites of infarction include the spleen, bone and bone marrow, the medulla of the kidney, mesenteric vessels, and pulmonary vessels.

Clinical Manifestations and Complications of Sickle Hemoglobin

Heterozygosity for hemoglobin S (hemoglobin AS) is designated sickle cell trait. Sickle cell anemia is the preferred term for people who are homozygous for hemoglobin S (hemoglobin SS). The term sickle cell disease indicates patients with clinical evidence of sickling and includes sickle cell anemia (hemoglobin SS), sickle/hemoglobin C (hemoglobin SC, and sickle/alfa-thalassemia Since people with sickle trait are asymptomatic, sickle cell trait is not considered a sickle cell disease.

People who are heterozygous for hemoglobin S (hemoglobin AS) are generally asymptomatic, have a normal blood hemoglobin level and complete blood count (CBC), and have a normal life span.

Homozygous Hemoglobin S (Sickle Cell Anemia): The severity of illness in sickle cell anemia (hemoglobin SS) is highly variable and can vary even within families. Many children become symptomatic in infancy after 3 to 4 months of age (before that time they are protected by the high levels of hemoglobin F). Other people have very mild disease and may not be diagnosed until adulthood. The reasons for this variability are not clear; the level of hemoglobin F is a factor (an increase in hemoglobin F decreases the severity of sickle cell disease), but other factors also appear to be important.

People who are heterozygous for both hemoglobin S and some other hemoglobinopathy (hemoglobin C, hemoglobin, or who are heterozygous for hemoglobin S and alfa-thalassemia, are often symptomatic and may have many of the same complications as homozygous sickle cell anemia..

Sickle Cell Crises

Three major categories of sickle cell crises have been identified:

1. Acute vaso-occlusive (painful) crises: Painful crises are the most common type of crisis and are believed to be caused by occlusion of small blood vessels, with consequent infarction of tissues.
2. Sequestration crises: Sequestration crisis can occur during childhood, usually during the first 3 to 4 years, before the spleen has become infarcted. The spleen suddenly becomes enlarged and engorged with blood; this can sequester a major portion of the total blood volume and can be fatal.
3. Acute aplastic crises: This occurs as a complication of infections, usually but not always due to parvovirus B19. Acute parvovirus B19 infection causes a transient stop in production of erythrocytes, which usually lasts about 5 to 7 days.

Other Complications of Sickle Cell Disease

1. Infections: Infections are the most common cause of death in sickle cell disease. Overwhelming pneumococcal sepsis is the major risk, due to impaired splenic function and splenic auto-infarction. Starting in infancy, children with sickle cell anemia are usually maintained on penicillin prophylaxis against pneumococcal sepsis. Gram-negative rods are the most common type of infectious agent in adults. Osteomyelitis is common in patients with sickle cell anemia
2. Cerebrovascular accidents: Strokes are a major cause of morbidity in sickle cell disease, occurring in 5 to 8% of patients by the age of 14 years. They usually occur in young patients, with a median age below 10.
3. Acute chest syndrome (acute lung syndrome): The acute chest syndrome is the second most common cause of hospitalization (after vasoocclusive crises) and causes approximately 25% of the deaths from sickle cell disease. Manifestations include pulmonary infiltrates on chest radiograph, fever, chest pain, hypoxemia, tachypnea, cough, and dyspnea. Infection, fat embolism from infarcted bone marrow, other pulmonary embolism or vascular occlusions, hypoventilation and atelectasis due to rib infarcts or surgery, and pulmonary edema are all possible causes of the acute chest syndrome
4. Altered splenic function and splenic infarcts: Infants and young children with sickle cell anemia often have mild splenomegaly. Later, sickling in the spleen leads to progressive splenic infarction, and by adulthood, the spleen is typically reduced to a small fibrous nodule (autosplenectomy)..
5. Renal disease: Infarction of the medulla of the kidney is common, resulting in hematuria (gross or microscopic) and loss of concentrating ability.
6. Priapism: Priapism is a common problem in males with sickle cell anemia, due to infarction of the corpora cavernosa of the penis. Repeated episodes result in impotence.
7. Gallstones: (pigment) gallstones are common in sickle cell disease, due to increased hemoglobin turnover and bilirubin production;
8. Leg ulcers
9. Aseptic necrosis of the femoral heads
10. Retinopathy
11. Complications of pregnancy: The maternal mortality rate is increased. They also have an increased rate of spontaneous fetal loss and low birth weight infants.

Diagnosis of Sickle Hemoglobin

Patients with sickle trait have a normal CBC and blood smear. The blood smear shows target cells and the characteristic sickled erythrocytes with sharply pointed ends. Howell-Jolly bodies may be present after splenic infarction, and nucleated red blood cells (RBCs) may be present. Common tests for sickle hemoglobin include sickle solubility tests and hemoglobin electrophoresis. The solubility test is usually positive if hemoglobin S comprises more than 10 to 20% of the hemoglobin. It is positive in patients with both sickle trait and sickle cell anemia and therefore cannot be used to distinguish homozygous from heterozygous

hemoglobin S. The other test commonly used to detect hemoglobin S is hemoglobin electrophoresis

Treatment of Sickle Cell Disease

General supportive measures, including folic acid supplementation and prophylactic penicillin, are important. Prophylactic penicillin has been shown to reduce mortality from *S. pneumoniae*, and should be started as soon as possible after birth and continued at least through age 5 years. Febrile episodes must be presumed to represent sepsis until proven otherwise and managed accordingly.

Treatment of acute painful vaso-occlusive crises is predominantly supportive; the key measures are adequate hydration and pain control. Morphine is generally the preferred parenteral agent for severe pain....

Red cell transfusions should be considered for acute cerebrovascular accidents, intractable or recurrent episodes of the acute chest syndrome, or priapism that does not respond to conservative measures. Exchange transfusion allows the hemoglobin S concentration to be decreased rapidly and is helpful in such instances

The acute chest syndrome is treated with supplemental oxygen, empiric antibiotics pending culture results, adequate analgesia, and transfusions as needed.

An important advance in preventive therapy is the use of hydroxyurea. Hydroxyurea has been shown to decrease the number of painful crises, hospitalizations, red cell transfusions, and episodes of the acute chest syndrome in patients with sickle cell anemia. Hydroxyurea increases the percent of fetal hemoglobin in the erythrocytes of many patients, which interferes with polymerization of hemoglobin S and therefore decreases sickling, but, the use of a chemotherapy drug for a non-neoplastic condition has raised a concern regarding possible carcinogenic (particularly leukemogenic) potential, especially in children

Transfusion therapy in sickle cell disease: The anemia in sickle cell disease is usually well compensated and does not require transfusions. Red cell transfusion to maintain hemoglobin S levels <30% is recommended for patients who have had cerebrovascular accidents and is commonly used in acute cerebrovascular accidents, acute chest syndrome, and priapism that does not respond to conventional therapies.

Stem cell therapy (bone marrow transplant): Bone marrow transplant (BMT) or other hematopoietic stem cell therapy has the potential of completely reversing the abnormality in sickle cell disease. Bone marrow transplants have been performed in a number of patients, with generally favorable results

THE THALASSEMIAS

The thalassemias are characterized by a quantitative abnormality of globin chain synthesis. Classic thalassemia is characterized by a complete lack of globin chain synthesis or a decreased amount of a structurally normal globin chain. The majority of thalassemias involve either the alpha or beta globin chains. There is a high prevalence of thalassemia in areas with endemic malaria, including parts of Africa, the Mediterranean basin, the Middle East, India, Southeast Asia, and southern China (the "thalassemia belt"). Epidemiologic evidence suggests that the thalassemias arose as a defense against malarial infection,

The decrease in globin chain synthesis has two consequences:

- 1• Decreased hemoglobin synthesis, resulting in anemia and microcytosis
- 2• Aggregation of the excess free globin chains produced by the non-thalassemic gene (alpha chains in the case of beta thalassemia; beta chains in the case of alpha thalassemia). The aggregates of unpaired globin chains attach to and damage the erythrocyte cell membrane, resulting in hemolysis. In severe cases, the majority of erythroid precursors are destroyed in the marrow (ineffective erythropoiesis). Those that escape into the circulation are prematurely destroyed by macrophages in the spleen and liver.

Complications of Thalassemias

The complications of thalassemia are related to four factors:

1• Chronic anemia: Chronic anemia causes growth retardation, delayed sexual maturation, cardiac dilatation and congestive heart failure, decreased work capacity, and all of the other complications associated with chronic anemia.

2• Marked expansion of the bone marrow: The bone marrow becomes greatly expanded due to marked erythroid hyperplasia. Widening of the diploic spaces in the skull gives a characteristic “hair on end” appearance on radiographs. Hypertrophy of the frontal bones results in frontal bossing. Hypertrophy of the maxillae results in prominent Cheeks and dental malocclusions, giving a characteristic “chipmunk” facies. Extramedullary hematopoiesis causes enlargement of the spleen and liver.

3• Iron overload: There is chronic hyperabsorption of iron by the gastrointestinal tract. Iron deposition in the heart causes cardiomyopathy and cardiac arrhythmias. Deposition in the liver causes portal fibrosis and may result in hepatic cirrhosis. Patients with hepatic cirrhosis are at risk of developing hepatocellular carcinoma (hepatoma).

4• Chronic hemolysis: Chronic hemolysis causes splenomegaly, hepatomegaly, and bilirubin gallstones. Hypersplenism may develop, increasing transfusion requirements.

The clinical manifestations of the different types of thalassemia (alfa versus Beta) are generally similar.

Alfa -Thalassemia

Pathophysiology

Most cases of alfa-thalassemia are due to deletions in the alfa globin genes. There are two genes for the alfa globin chain on chromosome 16; the mutation may involve only one or both alfa genes on each chromosome. Since each person has four alfa genes, there can be a deletion in one, two, three, or all four alfa genes. The severity of illness depends on the number of alfa genes with mutations.

- Single-gene mutation ($\alpha\alpha/\alpha-$): Clinically silent, without microcytosis or anemia
- Two-gene mutation ($-\alpha/-\alpha$ or $—/\alpha\alpha$; α -thalassemia minor or α -thalassemia trait): Mild microcytic anemia; serious complications are uncommon
- Three-gene mutation ($\alpha\alpha/-\alpha$; hemoglobin H disease): Moderately severe, microcytic anemia. The excess β chains precipitate as β_4 tetramers (hemoglobin H). The clinical picture is variable; patients may or may not have splenomegaly, iron overload, and the skeletal complications seen in severe thalassemia.
- Four-gene mutation ($—/—$; hydrops fetalis): Incompatible with life.

Beta -Thalassemia

Pathophysiology

There is a single gene for the beta globin chain on chromosome 11. Most mutations in beta-thalassemia are single-nucleotide substitutions. The mutation may result in a complete lack of beta chain synthesis (β^0 -thalassemia) or a decrease in beta chain synthesis (β^+ -thalassemia). The severity of illness depends on how much β chain is synthesized and whether the person is heterozygous or homozygous for the mutation. The β^- -thalassemias have been divided into three main clinical syndromes:

1• β^- -Thalassemia minor: Heterozygosity for β^- -thalassemia results in a mild clinical syndrome called β^- -thalassemia minor. The hemoglobin and the MCV are mildly or moderately decreased (hemoglobin ~ 9 – 12 g/dL and MCV ~ 65 – 75 fL), and the patient has few symptoms or complications.

2• β^- -Thalassemia major (Cooley's anemia) (Homozygosity for β^- -thalassemia results in a severe clinical syndrome characterized by severe anemia and microcytosis (hemoglobin ~ 3 – 5 g/dL and MCV < 65 fL), total or near total absence of Hb A, marked ineffective erythropoiesis, marked expansion of the bone marrow with skeletal complications, splenomegaly, and iron overload due to hyperabsorption of iron.

3• β -Thalassemia intermedia: beta-Thalassemia intermedia is homozygous beta-thalassemia that is not transfusion dependent. It is genetically and clinically heterogeneous. The hemoglobin is intermediate between β -thalassemia major and minor (~6–9 g/dL), as is the incidence of clinical complications.

Diagnosis of Thalassemia

A microcytic anemia that is not due to iron deficiency is most likely thalassemia. The first step after identification of a microcytic anemia is to check the serum ferritin or serum iron/transferrin/transferrin saturation. If the results do not indicate iron deficiency, start a workup for thalassemia which includes history of ethnic background and family history, examination of a well-stained blood smear, and hemoglobin electrophoresis.

The blood smears in mild thalassemia shows microcytosis, hypochromia, and target cells. There may be basophilic stippling. In more severe cases, there is marked microcytosis and anisocytosis, bizarre poikilocytes, polychromasia, and nucleated erythrocytes.

β -Thalassemia is generally diagnosed by the presence of increased hemoglobin A2 on hemoglobin electrophoresis. Hemoglobin F is often also slightly increased. The mean hemoglobin A2 level in β -thalassemia is about 5% (normal is less than about 3%).

Alfa-Thalassemia trait (two gene mutations) is generally diagnosed by exclusion: a microcytic anemia that is not due to iron deficiency and has normal hemoglobin A2 is most likely alfa-thalassemia. The hemoglobin electrophoresis is normal, except for the first few weeks of life, when four gamma tetramers (hemoglobin Bart's) are present. Study of family members can also be helpful in confirming the suspicion of alfa-thalassemia. Hemoglobin H disease can be diagnosed by the presence of hemoglobin H on electrophoresis.

Prenatal Diagnosis of Thalassemia

Molecular analysis of the globin genes for thalassemia can be done on amniotic cells obtained by amniocentesis or on chorionic villus cells obtained by chorionic villus biopsy.

Treatment of Thalassemia

The cornerstones of thalassemia therapy are red cell transfusion and iron chelation. Patients with thalassemia minor usually do not require transfusion just folic acid tab. Patients with thalassemia intermedia or hemoglobin H disease may or may not require transfusions to prevent complications of anemia and erythroid hyperplasia. Patients with severe thalassemia are frequently maintained on hypertransfusion regimens in which periodic red cell transfusions given to maintain the blood hemoglobin >9 to 10 g/dL. This prevents the skeletal complications of thalassemia by shutting off the erythropoietin-driven erythroid hyperplasia, allows normal growth and sexual development, and delays the onset of splenomegaly. A typical transfusion requirement is one to three donor units every 3 to 5 weeks.

Iron overload is a serious complication of transfusion therapy (each red cell unit contains 200–250 mg of iron), and chelation therapy is required to prevent the development of secondary hemochromatosis. Splenomegaly eventually develops in patients with severe thalassemia even with transfusion therapy, and splenectomy will be required. The usual indication for splenectomy is an increase in transfusion requirement. Bone marrow transplant is potentially curative for thalassemia. Patients with related HLA-matched donors and severe thalassemia should be considered for BMT early, before serious complications occur.

RED CELL ENZYME DEFECTS

Some defects in erythrocyte metabolic enzymes are associated with hemolytic anemia. By far the most common of these is glucose-6-phosphate dehydrogenase (G6PD) deficiency.

Glucose-6-Phosphate Dehydrogenase Deficiency

Epidemiology

Glucose-6-phosphate dehydrogenase deficiency is one of the most common genetic diseases in the world, affecting hundreds of millions of people. The gene for G6PD is located on the X chromosome, so G6PD deficiency is inherited as an X-linked trait. As for all X-linked traits, men develop the disease, whereas women are usually asymptomatic carriers. It is most common in areas of endemic malaria, and it (like sickle hemoglobin and the thalassemias) probably arose as a defense mechanism against malaria.

Pathophysiology

G6PD is the first enzyme in the hexose monophosphate shunt, in the absence of sufficient glutathione, hemoglobin is oxidized and precipitates in the cell. This damages the cell, resulting in hemolysis. The aggregates of oxidized hemoglobin are taken out of the cell by the spleen, resulting in characteristic “bite” or “blister” cells. The level of G6PD is highest in reticulocytes and declines with increasing age of the red cell.

Clinical Manifestations of G6PD Deficiency

The clinical severity of G6PD deficiency is variable. The majorities of patients are not anemic and have no hemolysis in the baseline state; minorities of patients have chronic, ongoing hemolysis. It is likely that the majority of people with G6PD deficiency are never diagnosed as such. Under normal conditions, patients have no hemolysis or anemia. Episodes of hemolysis may be precipitated by infection, oxidative drugs or chemicals, surgery, and acidosis. Infections are probably the most common cause of hemolytic episodes. Most infection-associated hemolytic episodes are self-limited and mild, although fulminant hemolysis with disseminated intravascular coagulation and acute renal failure can occur. Drugs and chemicals that can cause hemolysis include primaquine and other antimalarial agents, sulfa antibiotics, naphthalene and phenazopyridine. Episodes of hemolysis are indicated by sudden onset of jaundice, pallor, dark urine, and abdominal or back pain. The hemoglobin level typically drops about 3 to 4 g/dL. The blood smear may show “bite” and “blister” cells, polychromasia, schistocytes, and microspherocytes. Serum bilirubin and lactic dehydrogenase levels are increased; haptoglobin is decreased. A reticulocyte response becomes evident at about 5 days and is maximal at 10 days. Since reticulocytes have protective G6PD levels, hemolysis stops, and the hemoglobin returns to normal after about 2 to 4 weeks even if the oxidative drug is continued. Uncooked fava beans are a notorious cause of hemolysis in patients with G6PD Mediterranean (favism).

Diagnosis of Glucose-6-Phosphate Dehydrogenase Deficiency

Glucose-6-Phosphate Dehydrogenase deficiency should be an important consideration in any case of acute nonimmune hemolytic anemia. The peripheral smear should be examined for “bite” or “blister” cells and cells with eccentric “puddles” of hemoglobin; however, the smear is often surprisingly normal.

⇨ The G6PD level can be normal in a patient with G6PD deficiency shortly after a hemolytic episode, giving a false-negative result. This occurs if the reticulocyte count is high since reticulocytes have higher G6PD levels than older cells. If you get a normal G6PD level in a patient who you really think has G6PD deficiency, repeat the laboratory test in a few weeks.

Treatment of Glucose-6-Phosphate Dehydrogenase Deficiency

The main treatment for G6PD deficiency is to avoid conditions that predispose to hemolysis. Patients with G6PD deficiency should not be given medications that cause hemolysis. Infections should be treated promptly. Give folic acid tab

The Red Cell Membrane defect

Hereditary Spherocytosis

Epidemiology

Hereditary spherocytosis (HS) is the most common inherited hemolytic anemia in people of Northern European descent inherited in an autosomal dominant fashion

Pathophysiology

The fundamental cause in most cases of HS is defective vertical attachment between the phospholipid bilayer and the cytoskeleton scaffold. Hereditary spherocytosis is genetically and clinically heterogeneous; the majority of cases result from mutations in the gene for ankyrin. Spherocytic RBCs are less flexible than normal (biconcave disk) RBCs, and, consequently, they are selectively trapped and destroyed in the spleen.

Clinical Manifestations

The clinical manifestations of HS are highly variable, from asymptomatic without anemia to severe chronic hemolysis. The majority of older patients have relatively mild or moderate anemia, and the primary manifestations are hyperbilirubinemia (which may be intermittent) and mild splenomegaly. Bilirubin gallstones are common. Like other patients with chronic hemolytic anemias, patients with HS may have exacerbation of anemia associated with infections, aplastic crises due to parvovirus B19 infection, or megaloblastic anemia associated with folate deficiency.

Diagnosis of Hereditary Spherocytosis

The CBC shows a MCV that is normal to slightly low, with an increased mean corpuscular hemoglobin concentration (MCHC). The blood smear shows microspherocytes. The reticulocyte count is increased. A direct antiglobulin (Coombs') test should be done to exclude an immune hemolytic anemia.

- A hemolytic anemia with spherocytes with a negative direct antiglobulin test is most likely HS. The classic laboratory test for HS is the osmotic fragility test.
- It is important to remember that increased osmotic fragility is not specific for HS; any cause of spherocytosis results in increased osmotic fragility.

Treatment of Hereditary Spherocytosis

The standard treatment for HS is splenectomy. Since the spleen is involved in both, causing the membrane loss and removing the spherocytes from the circulation, splenectomy is highly effective. However, because of the risk of overwhelming infection following splenectomy, splenectomy should be performed only on patients having significant complications from anemia, such as growth retardation. It should be delayed until at least 3 to 5 years of age if possible. Folic acid supplementation is also recommended

Acquired Hemolytic Anemias

Acquired hemolytic anemias are divided into two main types: immune and non-immune. Unlike the inherited hemolytic anemias, which predominantly involve abnormalities intrinsic to the erythrocyte, the acquired hemolytic anemias (with one exception) involve abnormalities that are extrinsic to the erythrocyte. The exception to the acquired extrinsic rule is paroxysmal nocturnal hemoglobinuria (PNH), which is an acquired genetic lesion resulting in increased susceptibility of red blood cells (RBCs) to hemolysis by the complement cascade.

IMMUNE HEMOLYTIC ANEMIAS

The easiest way to approach the immune hemolytic anemias is to divide them according to the mechanism of hemolysis and the type of mediating antibody:

- Warm-reactive antibodies, usually immunoglobulin G (IgG), versus cold-reactive antibodies, usually IgM

Immune hemolytic anemias can be further divided: (1) primary or idiopathic versus secondary and (2) acute versus chronic. An important additional category of immune-mediated hemolysis is immune hemolytic anemia due to drugs or medications.

Cold-Reactive Immune Hemolytic Anemia

Cold-reactive immune hemolytic anemia is generally mediated by IgM antibodies that react maximally at approximately 4 to 18°C. They bind to cells at the cooler temperature of the extremities, fix complement, and then dissociate from the cell surface after the RBC returns to the warmer temperatures of the central circulation. The complement components remain on the cell surface after the antibody dissociates. IgM antibodies may cause intravascular hemolysis if the antibody is present in high titer; more often hemolysis is extravascular, predominantly in the liver. IgM antibodies are large and can bridge the distance between two RBCs; thus, IgM antibodies by themselves are able to agglutinate RBCs. (In contrast, IgG antibodies are smaller and alone are not able to agglutinate RBCs.) There are many causes of cold-reactive immune hemolytic anemia. Most of these fall into the category of cold agglutinin disease. Cold agglutinin disease, in turn, is divided into primary (idiopathic) and secondary forms. Cold-reactive immune hemolytic anemia is less common than the warm-reacting type, accounting for approximately 10 to 20% of immune hemolytic anemia.

Causes of Cold-Reactive Immune Hemolytic Anemia

Cold agglutinin disease:

- Primary (idiopathic)
- Secondary:
 - Infections
 - Autoimmune disorders
 - Lymphoproliferative disorders
 - Paroxysmal cold hemoglobinuria (PCH)

Primary (Idiopathic) Cold Agglutinin Disease

Primary cold agglutinin disease occurs with no obvious precipitating cause. It usually occurs in older individuals (peak age about 70 years) and is more common in women than men. The course is usually chronic, lasting for months or years. The anemia associated with idiopathic cold agglutinin disease is usually modest; the main symptoms are related to agglutination of erythrocytes on exposure to cold rather than the anemia. Agglutination occurs in the fingers, toes, nose, and ears and causes cyanosis of those areas (acrocyanosis). Some patients have a lymphoproliferative disorder such as chronic lymphocytic leukemia (CLL), Waldenström's macroglobulinemia, or non-Hodgkin's lymphoma.

Secondary Cold Agglutinin Disease

Secondary cold agglutinin disease is most often related to infections, primarily *Mycoplasma pneumoniae* or Epstein-Barr virus (EBV) (infectious mononucleosis). It can occasionally occur with other viral infections (adenovirus, cytomegalovirus, rubella, mumps, HIV), bacterial infections (*Legionella*, *Escherichia coli*, *Listeria*), malaria, syphilis, and others. The patients are usually young and otherwise healthy. The onset is abrupt, usually as the infection is resolving. The patients present with pallor, jaundice, and the other signs of acute hemolytic anemia. Massive acute intravascular hemolysis and acute renal failure may occur but are fortunately rare. Infection-related cases tend to be transient and self-limited, lasting only a few weeks.

Diagnosis of Cold Agglutinin Disease

Cold agglutinins cause several abnormalities on the routine CBC, including a decreased RBC count, increased mean corpuscular volume (MCV), and increased mean corpuscular hemoglobin concentration (MCHC). Large round clusters of RBCs are seen on the blood smear. Repeating the CBC and smear after rewarming the sample to 37°C resolves the difficulties, demonstrating the temperature dependence of the reaction. Spherocytes may be

present on smear, particularly in cases of acute cold agglutinin disease related to infection. A direct antiglobulin test (DAT or Coombs' test) detects the presence of complement components (but not IgG) on the surface of the patient's RBCs.

Treatment of Cold Agglutinin Disease

Primary Cold Agglutinin Disease

Patients should avoid cold temperatures and take precautions to keep the extremities warm. In patients who require further therapy, cyclophosphamide or chlorambucil given orally may be helpful. Corticosteroids and splenectomy (the mainstays of therapy for warm immune hemolytic anemia) are generally not helpful. Plasmapheresis to remove the agglutinin may provide temporary benefit.

Secondary Cold Agglutinin Disease

Infection-related cold agglutinin disease is usually transient, lasting only a few weeks. Supportive therapy is usually all that is needed. It is important for the blood bank to test for red cell antibodies other than the cold agglutinin (alloantibodies). If an alloantibody is detected, units lacking the corresponding antigen should be chosen. It is important to maintain the temperature of the transfused blood at 37°C, using a blood warmer, to prevent the transfused cells from agglutinating as soon as they enter the patient.

Warm-Reactive Immune Hemolytic Anemia

Warm-reactive immune hemolytic anemia is more common than the cold reactive variant. It can be primary (idiopathic), secondary to a wide variety of different conditions, or drug-related. Most cases (about 80%) are due to some underlying condition, although this may not be apparent at the onset of hemolysis. Hemolytic anemia may be the presenting feature of SLE or other autoimmune disorder.

The idiopathic variety is more common in women and the older population. The antibody is almost always an IgG

Causes of Warm-Reactive Immune Hemolytic Anemia

Primary (idiopathic)

Secondary

Infections

Autoimmune disorders: SLE, rheumatoid arthritis

Lymphoproliferative disorders: CLL, non-Hodgkin's lymphomas, myeloma

Hodgkin's lymphoma

Thymoma

Ovarian dermoid cyst or teratoma

Carcinomas

Hypogammaglobulinemia

Acquired immunodeficiency syndrome (AIDS)

Drug related

Clinical Manifestations

The severity of illness varies from an asymptomatic increase in red cell turnover completely compensated by increased marrow production to a fulminant illness with shock, and acute renal failure. The majority of patients experience an insidious onset of fatigue, weakness, and shortness of breath on exertion. Angina may be a presenting feature in older patients with coronary artery disease. Jaundice may be present. Mild splenomegaly and hepatomegaly may be present on examination, as well as mild lymphadenopathy; however, marked hepatosplenomegaly or lymphadenopathy suggests an underlying lymphoproliferative disorder. The course in adults tends to be chronic, lasting months to years, with periodic exacerbations.

Diagnosis

The hemoglobin level is variable, from severely decreased to nearly normal. The MCV is typically slightly increased due to reticulocytosis. The peripheral blood smear shows polychromasia and microspherocytes; in severe cases, nucleated RBCs may be present. The reticulocyte count is increased. The bilirubin and lactic dehydrogenase are elevated, and the haptoglobin is decreased.

The most important diagnostic test is the direct antiglobulin test (DAT), which is positive in >95% of cases. The antibody screen (indirect antiglobulin test) is positive in about 80% of cases.

Evaluation

Initial evaluation should focus on determining the severity of illness and discovering any possible causes of a secondary hemolytic anemia. Key points to consider:

- Identify any drugs or medications that the patient has been taking. All medications that may cause drug-related immune hemolytic anemia should be stopped as much as possible.
- Determine if the patient has had any transfusions, particularly within the past 3 months.
- History of pregnancies (including miscarriages or abortions) is critical in women.

Treatment

Any possible primary cause (such as an underlying autoimmune disease or lymphoproliferative disorder) should be treated appropriately. It is preferable to avoid red cell transfusions, but they should be given if needed. The blood bank will usually not be able to find totally compatible units for transfusion but may be able to select least incompatible units. The mainstay of initial therapy is corticosteroids, such as prednisone (1–2 mg/kg/day in divided doses). Most patients show a response within 3 weeks. Corticosteroids block the macrophage Fc receptors and thus prevent RBC phagocytosis. They can also decrease antibody production by the spleen, but this takes several weeks. Prednisone is usually continued at the original dose until the hemoglobin is 10 g/dL; the dose is then slowly tapered over 3 to 4 months. About 80% of patients initially respond; however, about two-thirds of responders relapse as the drug is tapered. Some patients can be maintained on no corticosteroids or on a low dose. Patients requiring more than 10 to 20 mg of prednisone per day, or patients having intolerable side effects on prednisone, should be considered for splenectomy.

Splenectomy is the mainstay of therapy for patients who fail to respond to corticosteroids or who require excessive doses to maintain remission.

Splenectomy eliminates the primary site of red cell destruction and also removes an important site of antibody production. Drugs like cyclophosphamide or azathioprine, intravenous immunoglobulin, plasmapheresis, vincristine or vinblastine, and danazol. Intravenous immunoglobulin and plasmapheresis may be helpful, but the effects are transient

NON-IMMUNOLOGIC ACQUIRED HEMOLYTIC ANEMIAS

Causes of Non-immunologic Acquired Hemolytic Anemia

1. Mechanical trauma: Malfunctioning mechanical heart valve, Microangiopathic hemolytic anemias: thrombotic thrombocytopenic purpura (TTP), hemolytic-uremic syndrome (HUS), preeclampsia/eclampsia, malignant hypertension, disseminated intravascular coagulation (DIC)
2. Acanthocytosis: Hereditary abetalipoproteinemia, End-stage liver disease, Severe starvation, anorexia nervosa
3. Severe hypophosphatemia: Intravenous hyperalimentation lacking phosphorous supplementation, Severe starvation, Alcoholism, Prolonged therapy with phosphate-binding antacids
4. Wilson's disease; copper poisoning

5. Oxidative drugs or chemicals
6. Severe burns
7. Venoms
8. Infections: Direct infection of erythrocytes: malaria, babesiasis, bartonellosis, trypanosomiasis, Clostridium perfringens septicemia Other: gram-positive and gram-negative septicemia, leptospirosis, Borrelia, others
9. Paroxysmal nocturnal hemoglobinuria (PNH)

Paroxysmal Nocturnal Hemoglobinuria

Pathophysiology

Paroxysmal nocturnal hemoglobinuria is an acquired genetic **disorder** that results in increased susceptibility of RBCs to lysis by the complement system. The condition results from an inability to synthesize the glycosyl phosphatidylinositol (GPI) anchor, which anchors a variety of molecules on the surface of erythrocytes and other cells.

Clinical Manifestations

Paroxysmal nocturnal hemoglobinuria occurs at all ages from childhood to old age but is most commonly diagnosed in the fourth and fifth decades. The course is highly variable, from clinically benign to a chronic disabling disorder. The name paroxysmal nocturnal hemoglobinuria derived from patients who experienced episodes of dark urine on awakening from sleep; however, this classic presentation occurs in only about one-fourth of patients. The majority of patients have an insidious onset of fatigue, weakness, and jaundice. The attacks are not precipitated by exposure to cold, distinguishing PNH from paroxysmal cold hemoglobinuria. Patients may experience abdominal or back pain, nausea, headaches, malaise, and fever associated with exacerbations of hemolysis. Unexplained pancytopenia may also be a presenting manifestation of PNH.

Complications

Paroxysmal nocturnal hemoglobinuria is associated with a number of significant complications, including:

- **Thromboemboli:** Thrombotic disease is a common complication in PNH and Thrombi predominantly occur in the venous system, including unusual sites such as the cerebral veins, mesenteric veins, portal vein, and hepatic vein (Budd-Chiari syndrome), as well as in the extremities. The reason for the increased risk of thrombosis in PNH is unknown.
- **Infections:** Patients with PNH may be at increased risk of infections due to leukopenia, leukocyte dysfunction, or corticosteroid therapy.
- **Iron deficiency:** Chronic intravascular hemolysis with hemoglobinuria results in iron deficiency, which further exacerbates the tendency for anemia.
- **Leukopenia, thrombocytopenia, or pancytopenia:** Nearly all patients with PNH develop leukopenia, thrombocytopenia, or pancytopenia.
- **Hemorrhage:** Patients with PNH and severe thrombocytopenia are at increased risk for hemorrhage.
- **Transformation to acute myelogenous leukemia:** Transformation to acute myelogenous leukemia is a rare but well-known complication of PNH.
- **Development of aplastic anemia**

Diagnosis

The CBC shows anemia, which can vary from mild to severe. The anemia is usually mildly macrocytic but can be microcytic and hypochromic if iron deficiency has developed. Leukopenia and/or thrombocytopenia are common. Lactic dehydrogenase and bilirubin may be elevated during exacerbations of hemolysis. A test for urine hemosiderin will be positive. The traditional diagnostic tests are the acidified serum (Ham's) test and the sucrose hemolysis test. The sucrose hemolysis test is more sensitive, but the Ham's test is more specific. Demonstration of a decreased expression of GPI-anchored proteins on RBCs or leukocytes by

flow cytometry with CD55 and CD 59 has been shown to be more sensitive than either of these tests

Treatment

Standard treatments for PNH include corticosteroids and androgens. Both are helpful in some patients and should be tried. Patients who respond should be maintained on minimally effective doses to avoid side effects.

Transfusions may be needed for severe anemia. Thrombotic episodes are treated with anticoagulants or thrombolytic agents.

Bone marrow transplantation has been tried in a few younger patients with histocompatible donors, and has been relatively successful.

Prognosis

The median survival in PNH is approximately 10 to 15 years.