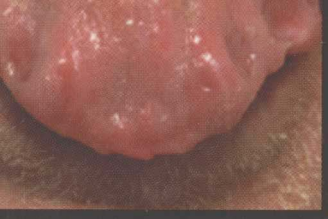

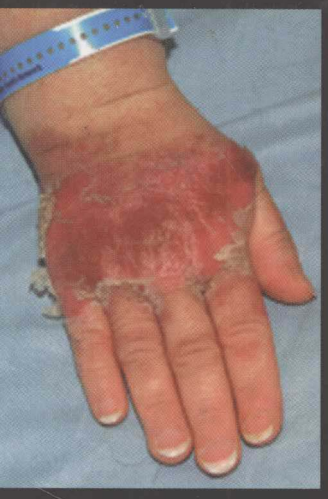

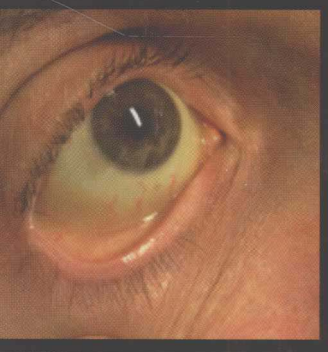

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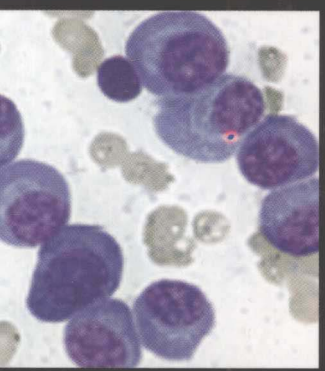

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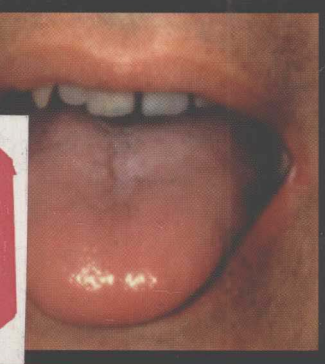

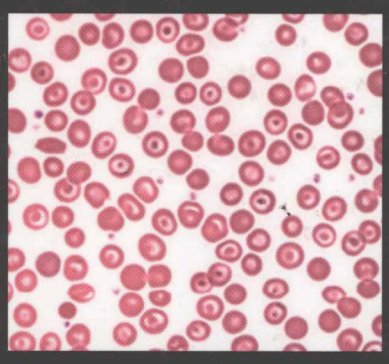

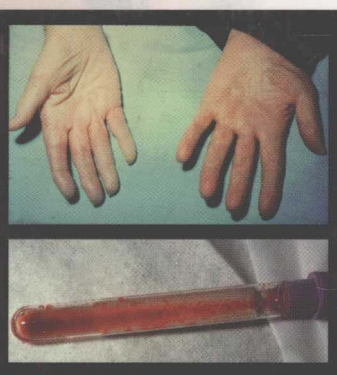

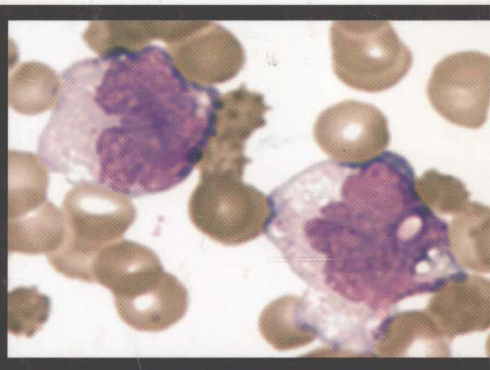

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# Wintrobe's Atlas of Clinical Hematology


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# Preface

Hematology is a very visual subspecialty of medicine. Examining the patient, evaluating bone marrow and other tissue specimens, scrutinizing blood films, and inspecting information derived from recently developed techniques, such as flow cytometry, all require careful observation. The purpose of this text-atlas is to provide clear, accurate, and detailed images to help the reader learn how to interpret these examinations both in normal people and in those with a wide variety of hematologic disorders. The book also provides brief descriptions of the relevant clinical, diagnostic, and pathophysiologic features of the diseases depicted. These accounts and the several accompanying tables contain the most important information, rather than being encyclopedic compilations. Those interested in more extensive coverage of the topics should refer to *Wintrobe's Clinical Hematology*. Because the major purpose of this text-atlas is to help the reader learn how to diagnose hematologic problems, it does not contain information about treatment, which, in any event, is rapidly changing for many of the diseases included. The proposed audience is anyone interested in blood disorders, including laboratory technicians, medical students, physicians in training, oncologists, and hematologists of all levels of experience. We hope that even experts will benefit, for education, as Samuel Johnson stated, is often more a matter of being reminded than informed.

*Douglas C. Tkachuk, MD, FRCPC*
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# Foreword

It has been my great privilege to study under Dr. M.M. Wintrobe's tutelage for three years and to be asked later, as one of five former fellows, to contribute as writer and editor of the seventh through twelfth editions of *Wintrobe's Clinical Hematology*. In the process, we taught a generation of medical students about the wonders and challenges inherent in the study of the blood and its diseases, as well as the application of the scientific method to clinical practice and research. One of my students was Douglas Tkachuk, now one of the editors and a driving force behind the creation of *Wintrobe's Atlas of Clinical Hematology*. The circle is almost complete.

Dr. Wintrobe was a tough taskmaster as clinician, scientist, and communicator of knowledge; the standards he set for himself and his students were high. He would have been proud of this book that honors his name and that emphasizes the three pillars on which he considered excellence in all of medicine to rest—critical apprehension of physical findings, astute laboratory testing of derived hypotheses, and informed decision-making based on the most up-to-date evidence provided by research in molecular and cellular biology. *Wintrobe's Atlas of Clinical Hematology* makes a superb contribution in these areas. The illustrations of physical findings are excellent and well described in the accompanying text; the reproductions of blood and marrow smears, as well as the histologic sections and other microscopic and submicroscopic illustrations, are among the best I have seen. Most important, wherever possible, clinical and laboratory findings are explained on the basis of the most up-to-date scientific insights available. All these features make the *Atlas* an excellent companion to *Wintrobe's Clinical Hematology* and, indeed, a growing number of textbooks dealing with the fascinating subject of hematology.

In the preface to an early edition of *Clinical Hematology*, Dr. Wintrobe quoted Leonardo da Vinci as saying that, "The love of anything is the fruit of our knowledge, and grows deeper as our knowledge becomes more certain." *Wintrobe's Atlas of Clinical Hematology* contributes to this process in significant measure.

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This book is dedicated to Evy, Claire, Jean, and Jennifer.

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# Contents

CHAPTER

# 1

# Anemia

David Barth, MD, FRCPC, and Jan V. Hirschmann, MD

The World Health Organization (WHO) has defined anemia in adults as a hemoglobin of <13 g/dL in males (a hematocrit [Hct] of about 39) and <12 g/dL in females (Hct about 36). For African-Americans, the hemoglobin is about 0.5 g/dL less. Using these values, anemia is common in the elderly, primarily from the presence of more disease in this population, rather than as a phenomenon of normal aging.

Especially when mild and insidious in onset, anemia often causes no symptoms. When they occur, fatigue and listlessness are common. As the anemia worsens, dyspnea may occur because of the diminished oxygen supplied to the tissues or from high-output cardiac failure, which usually occurs only when the hematocrit drops below 20, unless the patient has underlying heart disease. In patients with coronary artery disease, angina may develop or worsen. When the anemia becomes severe, faintness, dizziness, and diminished concentration can occur from decreased oxygen delivery to the brain. Diminished tissue oxygenation may provoke the compensatory mechanisms of tachycardia and increased force of ventricular contraction, which patients sometimes detect as palpitations.

Physical examination may be unremarkable, but pallor is sometimes apparent in the conjunctiva, palms, and face. Systolic murmurs, usually in the pulmonic area, can develop, probably from a combination of decreased blood viscosity and increased flow across the valves. Retinal examination in severe anemia may reveal hemorrhages that are white-centered (Roth spots), flame-shaped, or round. Some may be pre-retinal. The retinal veins are sometimes tortuous, and cotton wool spots, representing infarction of the nerve fiber layer, may occur. Ischemia of the vessels can lead to leakage of proteinaceous material, causing "hard" exudates.

The classification systems for anemia emphasize either erythrocyte size or the mechanism that reduced the number of red cells. The morphologic scheme divides anemia into three groups, based on mean corpuscular volume (MCV): (1) normocytic (MCV 90–100); (2) macrocytic (MCV >100); and (3) microcytic (MCV <80). In some disorders, the red cells may vary considerably and can cause anemias of more than one category. In hypothyroidism, for example, the red cells may be normocytic or macrocytic. A valuable aspect of this classification is that the measurement of red cell size is immediately available from automated blood counts and that the differential diagnosis of microcytic and macrocytic anemias is small. The diseases causing normocytic, normochromic anemias, however, are more numerous and complex.

Microcytic anemias represent disordered hemoglobin synthesis from inadequate iron, abnormal globin formation, or deficiencies in heme and porphyrin synthesis that occur in some types of sideroblastic anemia, such as those due to lead poisoning and pyridoxine deficiency. The commonest cause of microcytic anemia is iron deficiency. The second most frequent type is the anemia of chronic disease, in which microcytosis occurs in about 30% of cases. One of the components of the pathophysiology of this disorder is reduced transfer of iron from the macrophages in bone marrow to the plasma. Abnormal globin formation causing microcytic anemia occurs in the thalassemias and some hemoglobinopathies, such as hemoglobin C and E.

Macrocytic anemias may occur from several mechanisms. One is abnormal DNA synthesis, most commonly produced by deficiencies of folic acid and vitamin $B_{12}$, causing abnormally large erythrocyte precursors (megaloblasts) in the bone marrow. Other etiologies are inherited disorders of DNA synthesis or medications that interfere with it. Macrocytic anemia also occurs frequently in the myelodysplastic syndromes because of altered erythrocyte maturation caused by a clonal expansion of abnormal hematopoietic stem cells. Macrocytosis, usually with an MCV of 100 to 110, but typically without anemia, is present in about 60% of alcoholics. The cause is not deficiency of folic acid or vitamin $B_{12}$, but a direct effect of ethanol itself on the bone marrow. Another source of macrocytosis

is the presence of young erythrocytes released early from the marrow because of anemia caused by hemorrhage or hemolysis. Some of these large erythrocytes are identifiable on peripheral blood smears because they still contain nuclei (nucleated red cells). Others, although matured beyond the nucleated stage, possess residual blue-staining nuclear RNA, as well as red-staining hemoglobin, leading to a purplish color with the Romanowsky stains ordinarily used for peripheral blood films. These large erythrocytes are called polychromatophilic ("lover of many colors") or polychromatic ("many colors") cells. The presence of a few is common on normal smears, but numerous polychromatophilic cells can lead to macrocytosis.

Normocytic anemias have many disparate causes. With acute hemorrhage or hemolysis, the bone marrow responds maximally by increasing red cell production and releasing young erythrocytes prematurely. In the other forms of normocytic anemia, however, the bone marrow response is reduced because of intrinsic bone marrow disease, insufficient iron, or inadequate erythropoietin effect. Categories of intrinsic bone marrow disorders include: (1) diminished erythrocyte precursors, such as in aplastic anemia or following cancer chemotherapy; (2) infiltration of the marrow with abnormal tissue, such as with fibrosis or leukemia; and (3) myelodysplastic disorders, in which abnormal red cell maturation leads to erythrocyte death in the marrow. In iron deficiency a normocytic anemia typically occurs before further progression leads to a microcytic one. Inadequate erythropoietin effect can develop from: (1) impaired production in the kidney because of renal disease; (2) reduced stimulation, possibly the cause of anemia in some endocrine disorders, such as hypothyroidism and hypogonadism; or (3) interference with both production and its bone marrow effects, caused by the presence of inflammatory cytokines, which is part of the pathogenesis of the anemia of chronic disease. Other components include a diminished red cell life span and impaired iron utilization.

In assessing anemias, it is useful to know whether the bone marrow has responded with a robust increase in red cell production. One assessment is to get a marrow sample to detect hyperplasia of the red cell precursors. A simpler, indirect measure is to enumerate the immature red cells in the peripheral blood by finding those with residual ribosomal RNA. When mixed with certain dyes, such as new methylene blue, that stain RNA, such immature erythrocytes show at least two blue granules or a network of material (reticulum). These young red cells are, therefore, called *reticulocytes*. Automated counters use a chemical, such as acridine orange, which binds to RNA and fluoresces. The reticulocyte count is expressed as a total number per volume of blood or as a percentage of the red cells. When a percentage is used, it should be corrected for the severity of anemia by multiplying it by the patient's hemoglobin (or hematocrit) divided by the normal hemoglobin (hematocrit). With a hematocrit of 20 and a reticulocyte count of 6%, for example, the corrected value would be $6\% \times (20/45) = 2.6\%$. When the anemia is severe (Hct $\leq$25) and polychromatophilia is prominent on the smear, a second correction is necessary. Usually, polychromatophilia lasts for 1 day in circulating red cells. When immature cells are released especially early, the blue color may persist for 2 to 3 days. For the reticulocyte percentage to reflect erythrocyte production in these circumstances, it should be divided by 2. The reticulocyte percentage that emerges from these corrections is called the *reticulocyte index*.

Reticulocyte enumeration is especially important in the classification of anemia by physiologic mechanisms or red cell kinetics. It has three categories: (1) hypoproliferative anemia, in which the bone marrow cannot increase its erythrocyte production; (2) maturation defects, in which bone marrow hyperplasia occurs, but many cells die in the marrow, a situation called *ineffective erythropoiesis*; and (3) acute hemorrhage or hemolysis, in which the red cell production increases and erythrocytes leave the marrow intact but die prematurely in the peripheral circulation.

Assigning an anemia to one of these categories utilizes the reticulocyte index and the results from a bone marrow sample. In the absence of anemia, the reticulocyte index is 1. With a moderately severe anemia (Hct <30) and a normal bone marrow, the reticulocyte index should exceed 3. This response typically occurs with hemolysis or acute hemorrhage. The reticulocyte index is less than 2 in hypoproliferative and maturation defect disorders. In a normal bone marrow sample, the ratio of erythroid to myeloid cells (E:M ratio) is about 1:3. With a moderately severe anemia, exuberant red cell production occurs and the E:M ratio should exceed 1:1. This kind of hyperplasia occurs with both maturation disorders and hemolysis. With the ineffective erythropoiesis of maturation defects, many red cells die within the bone marrow, whereas with hemolysis, the erythrocyte destruction is in the peripheral blood. In both cases, the serum lactate dehydrogenase (LDH) and indirect bilirubin levels may increase.

The reticulocyte index, the bone marrow findings, and these serum studies allow an accurate designation of the category of anemia. In hypoproliferative anemias, the erythrocytes are usually normocytic, the reticulocyte index is <2, the E:M ratio is <1:2, and the indirect bilirubin and LDH are normal. Early iron deficiency and the anemia of chronic disease are hypoproliferative disorders. Hypoproliferation also occurs when erythropoietin production (renal failure) or response (endocrine disorders) is diminished or when the bone marrow is damaged by injury to stem cells (e.g., cancer chemotherapy), by altered marrow structure (e.g., fibrosis), or by autoimmune or unknown mechanisms (e.g., pure red cell aplasia). Helping to distinguish among these possibilities is examining the blood smear for polychromatophilia, which is present in marrow damage and iron deficiency, but diminished in renal failure and the anemia of chronic disease.

In maturation defects, the reticulocyte index is <2, the E:M ratio is >1:1 with severe anemias, the serum LDH and indirect bilirubin are elevated (except in iron deficiency), and polychromasia is present. Examples of nuclear maturation defects, which cause macrocytosis, are vitamin $B_{12}$ and folate deficiencies. Cytoplasmic maturation defects produce microcytic erythrocytes and include thalassemias, certain hemoglobinopathies, and some sideroblastic anemias.

In hemolysis, the reticulocyte index is >3, the E:M ratio is >1:1, serum LDH and indirect bilirubin are characteristically elevated, and polychromatophilia is prominent. In acute hemorrhage, the bone marrow takes 7 to 10 days to achieve a robust erythrocyte production. In the first few days, the anemia appears hypoproliferative. Later, the picture resembles ineffective erythropoiesis as bone marrow production increases, but the red cell precursors are not mature enough to leave the marrow. The reticulocyte index is <2; the E:M ratio is increased, yet still <1:1; and polychromatophilia is increased but not markedly. When the marrow finally achieves its maximal response, the findings are similar to those of hemolysis, with the reticulocyte index >3, the E:M ratio >1:1, and polychromasia prominent. Hemorrhage is distinguishable from hemolysis by the serum LDH and indirect bilirubin, which are normal because the red cells are not being destroyed, either in the bone marrow or in the peripheral blood.

For most clinicians, dividing anemia according to red cell size is the easiest approach, especially since the differential diagnosis of microcytic and macrocytic anemias is small. One use of the physiologic classification is in analyzing normocytic anemias, where the number of possibilities is large and that system of categorization provides a framework for distinguishing among them.

## MICROCYTIC ANEMIA

The major diagnostic considerations in microcytic anemia are iron deficiency, anemia of chronic disease, and thalassemias. Less common causes include hemoglobinopathies and certain types of sideroblastic anemias. As discussed in the section on normocytic anemias, the anemia of chronic disease is microcytic in about 30% of cases, but usually with an MCV of 70 to 80 fl and unaccompanied by significant morphologic changes on peripheral smear. In iron deficiency, thalassemias, and hemoglobinopathies, the cell size is often smaller, and the blood film can disclose dramatic changes in red cell morphology.

Iron deficiency usually arises from chronic blood loss. The major cause in younger women is menstruation. In nonmenstruating women and in men, the most common source is gastrointestinal hemorrhage. Other less common reasons include hematuria, nosebleeds, hemoptysis, or intrapulmonary hemorrhage from such disorders as idiopathic pulmonary hemosiderosis, microscopic polyangiitis, or Goodpasture syndrome. A rare cause is intravascular hemolysis from such diseases as paroxysmal nocturnal hemoglobinuria or mechanical fragmentation of erythrocytes from prosthetic heart valves, in which destruction of red cells leads to excretion of iron in the urine in the form of ferritin, hemosiderin, or hemoglobin.

Iron deficiency occasionally develops from inadequate dietary intake or iron malabsorption. Little absorbable iron is present in the majority of foods, including most fruits and vegetables. Good sources are meat, poultry, fish, beans, and peas. Because daily iron loss is slight in adult males, primarily small amounts in the alimentary canal, they need little dietary iron, and deficiency from inadequate dietary intake is uncommon. When iron utilization is increased in infancy and during growth, or when concurrent blood loss occurs, as in menstruation, dietary intake may be insufficient, especially because women and children tend to consume less than the recommended minimal daily requirement. The problem increases during pregnancy, when some iron is diverted to the fetus for hematopoiesis, and with breast feeding, when iron is lost in the milk. Iron is absorbed throughout the gastrointestinal tract, but especially in the duodenum. With small intestinal disease, such as celiac sprue, or with gastric resection, which may accelerate the movement of intestinal materials through the duodenum and thereby diminish absorption time, iron deficiency may develop.

The clinical features of iron deficiency are generally similar to other anemias, but three uncommon but distinctive findings are pica, koilonychia, and blue sclera. Pica is the craving for, and ingestion of, certain unusual substances, such as starch, dirt, cardboard, and ice (pagophagia). Pagophagia is especially suggestive of iron deficiency. Virtually pathognomonic of iron deficiency is koilonychia, in which the fingernails become thin, brittle, and concave (spoon-shaped) in the distal half. Thinning of the sclera from impaired epithelial growth causes a blue tint because of the more visible choroid beneath.

With mild and recent iron deficiency, the red cell indices (MCV, mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC]) and the blood film are normal, but with time and increasingly severe anemia, the MCV and MCHC diminish. An early change in the erythrocytes is anisocytosis, indicated on automated counters by an increase in red cell distribution width (RDW). Later morphologic changes include poikilocytosis, microcytosis, and hypochromia. Tiny microcytes, elongated pale elliptical red cells (pencil cells), and target cells may be visible, but many of the erythrocytes may appear normal. Often, thrombocytosis is apparent. In iron deficiency anemia, the serum iron is decreased, the total iron binding capacity is elevated, and the saturation is <20%. The serum ferritin is decreased. Usually, the diagnosis is established by these tests, but occasionally obtaining a bone marrow sample for iron staining or a trial of iron therapy may be necessary to

confirm the presence of iron deficiency (see Microcytic Anemia Tables and Diagrams).

## HEMOLYTIC ANEMIAS

In hemolytic anemias, red cell destruction significantly shortens the normal life span of the erythrocyte in the peripheral circulation, which is about 120 days. Classifications of hemolytic anemia emphasize either the site of destruction—intravascular versus extravascular—or the site of the abnormality provoking it—as intrinsic or extrinsic to the red cell. In intravascular hemolysis, the red cells are destroyed within the bloodstream, whereas extravascular hemolysis indicates destruction within macrophages present in organs, such as the spleen, liver, or bone marrow.

Intravascular hemolysis is typically severe and arises from several mechanisms. One is mechanical damage to the red cell caused by: (1) fibrin present in the vessel lumen from such diseases as disseminated intravascular coagulation or vasculitis; (2) physical trauma from red cells passing through prosthetic valves or small vessels of the feet during hard marching; (3) thermal injury from burns. Intravascular hemolysis may also occur from infections, such as malaria, or from toxins, such as venom from some poisonous snakes. A third type is complement-mediated damage to erythrocytes caused by cold agglutinins, incompatible red cell transfusions, and paroxysmal nocturnal hemoglobinuria. Initially, the hemoglobin released into the circulation during intravascular hemolysis binds to haptoglobin, reducing its serum level. When the hemoglobin exceeds the binding capacity of haptoglobin, it makes the plasma appear pink. Free hemoglobin is filtered in the kidneys, and the urine may appear red. The dipstick testing for blood is positive, but the urine microscopy is negative for increased red cells. The renal tubular epithelium cells take up some of the hemoglobin, transforming it into hemosiderin, which is visible within these cells on iron stains of the urinary sediment. Evidence of recent or ongoing intravascular hemolysis, thus, includes a reduced serum haptoglobin level (which also occurs in extravascular hemolysis), the presence of plasma or urine hemoglobin, and detection of hemosiderin in renal tubular cells in the urinary sediment.

In most cases of hemolytic anemia, the red cell destruction is extravascular. The differential diagnosis includes: (1) an abnormal environment in the circulation because of infections, medications, or immunologic processes; (2) erythrocyte membrane abnormalities; (3) red cell metabolic defects; and (4) abnormalities in hemoglobin structure.

The other major classification system for hemolytic anemias differentiates disorders intrinsic to the red cell, which are typically hereditary, and those extrinsic to the red cell, usually acquired diseases. The intrinsic disorders include: (1) abnormal hemoglobins; (2) enzyme defects; (3) membrane abnormalities. The extrinsic disorders are: (1) immunologic; (2) mechanical factors; (3) infections and toxins; (4) liver disease (spur cell anemia); and (5) hypersplenism.

Abnormalities in red cell morphology may be apparent on peripheral smear, such as sickle cells, bite cells, schistocytes, and spherocytes. Other findings may include red cell agglutination from the presence of increased Igm, organisms such as malarial parasites, and ingestion of erythrocytes by macrophages (erythrophagocytosis), which especially suggests immune hemolytic anemias, but also can occur with infections or toxins. The peripheral smear in hemolytic anemia should reveal substantial polychromatophilia caused by the increased release of immature red cells from the bone marrow. The reticulocyte index is $>3$ and the absolute reticulocyte count is $>100{,}000/mm^3$. The indirect bilirubin is elevated and represents >80% of the total bilirubin. The serum LDH may be increased and the serum haptoglobin diminished. With suspected intravascular hemolysis, helpful tests include urine and plasma hemoglobin measurements, as well as iron stains of urinary sediment. With extravascular hemolysis, Coombs tests detect immunoglobulin and complement on the red cell surface, indicating an immune hemolysis. A hemoglobin electrophoresis is indicated for suspected hemoglobinopathies.

## HEMOGLOBINOPATHIES AND THALASSEMIAS

Hemoglobin A (HbA), which constitutes more than 90% of the adult hemoglobin, consists of four polypeptide chains, two α and two β ($\alpha_2\ \beta_2$). Hemoglobin $A_2$, composed of two α and two δ ($\alpha_2\delta_2$), is present in small quantities. Hemoglobin F ($\alpha_2\gamma_2$) constitutes $<1\%$ of the normal adult's hemoglobin, but is the main hemoglobin during fetal life. As β-chain production begins just before birth, the level of HbF decreases and represents about 75% of the hemoglobin at delivery. By 6 months of age, it has diminished to 5%. The thalassemias are inherited disorders with reduced or absent synthesis of one or more globin chains. Two major consequences occur: reduced production of functioning hemoglobin, leading to hypochromic, microcytic erythrocytes; and continued production of the unaffected chains, which have decreased solubility or diminished oxygen-carrying capacity that causes damage to the red cell or its precursors, leading to ineffective erythropoiesis and hemolytic anemia. The thalassemias are labeled according to the chain with impaired production. With β-thalassemia, β-chains are absent or diminished; with α-thalassemia, α-chains are affected. These are the two most important thalassemias, although others exist.

The β-thalassemias are common in the Mediterranean area (thalassemia in Greek means "sea in the blood"), India, Southeast Asia, and the Middle East. The clinical spectrum includes severe (thalassemia major), moderate (thalassemia intermedia), and mild (thalassemia minor) cases.

β-Thalassemia major, or homozygous disease, is caused by the inheritance of two β-thalassemia alleles, resulting in little or no β-chain production although α-chain synthesis remains normal. Because of diminished HbA synthesis, anemia is severe, and the red cells produced contain diminished hemoglobin, making them very hypochromic. The accumulation of free α-chains leads to their deposition in red cell precursors, causing erythrocyte destruction in the bone marrow (ineffective erythropoiesis). Red cells containing these precipitates that do reach the peripheral blood are prematurely destroyed by macrophages in the liver, spleen, and bone marrow. Because HbF is present in substantial quantities at birth, anemia emerges only when γ-chain synthesis diminishes. Adequately transfused children grow and thrive until iron overload problems begin to develop. In untreated or insufficiently transfused patients, growth is subnormal. Increased erythropoiesis in response to the anemia leads to expanded marrow cavities that can eventuate in long-bone fractures and expansion of skull and maxillary areas, causing abnormal contours of the face and head. Increased erythrocyte destruction in the spleen causes splenomegaly, which can lead to hypersplenism with thrombocytopenia and leukopenia. On blood smear, the erythrocytes demonstrate anisocytosis and poikilocytosis, with elliptocytes, teardrop cells, and other bizarrely shaped red cells. Hypochromia is pronounced, and microcytosis is apparent, although the cells are flat and they spread out on drying, giving them a diameter larger than expected, based on the MCV. Target cells and nucleated red cells are typically numerous, and basophilic stippling is common. Red cell inclusions, representing excess α-chains, may be apparent. Findings on bone marrow examination include erythroid hyperplasia, basophilic stippling, and diminished hemoglobin in the red cell precursors, which also show inclusions. Iron content is increased.

Thalassemia intermedia is usually the result of the inheritance of two β-thalassemia mutations: both mild or one mild, one severe. The anemia is typically moderately severe, but transfusions may not always be necessary. The blood smear is similar to that of thalassemia major.

Thalassemia minor occurs from the inheritance of a single β-thalassemia mutation and a normal β-globin gene on the other chromosome. No clinical problems emerge, and anemia is mild or absent. The smear, however, is abnormal, with microcytic, hypochromic red cells. The MCV is 50 to 70 fl. Poikilocytosis, target cells, and basophilic stippling are typically present. Hemoglobin electrophoresis demonstrates $HbA_2$ that is about twice normal and an $HbA_2$:HbA ratio of 1:20, rather than the normal 1:40. HbF is increased in many patients.

Production of α-globin chains is regulated by four gene loci. Four α-thalassemia types occur, depending on how many gene foci are affected. No hematologic abnormalities develop with α-thalassemia-2, in which one focus fails to function. In α-thalassemia-1, two foci are affected, and the condition is mild, with slight or absent anemia. The blood smear shows microcytosis, hypochromia, and slight anisocytosis and poikilocytosis.

When three gene foci are defective, α-chain synthesis is substantially decreased, and excess β-chains form tetramers called HbH, which are soluble and do not precipitate in the marrow to cause damage of erythrocyte precursors. They are present in the circulating red cells, but precipitate as they age, forming inclusion bodies. The spleen, which enlarges in this disorder, prematurely destroys these cells, causing hemolytic anemia. This disease most commonly occurs in Asians, and the anemia is usually moderate, with hematocrits of 20 to 30. The blood smear shows substantial hypochromia, microcytosis, basophilic stippling, and polychromasia. Abnormal erythrocytes apparent on blood smear include target cells, teardrop cells, and nucleated and fragmented cells. Heinz-body preparations disclose precipitated HbH, visible as multiple small erythrocyte inclusions. On hemoglobin electrophoresis, about 3% to 30% of the total is HbH.

When four genes are defective, α-chains are absent, and tetramers of γ-chains called Hb Bart, form in the fetus. This hemoglobin oxygenates poorly, producing tissue hypoxia, and they are unstable, resulting in hemolysis and anemia. The fetus develops heart and liver failure, resulting in massive edema (hydrops fetalis) and intrauterine death. The disease almost always occurs in Southeast Asians.

## NORMOCYTIC ANEMIAS

Many of the causes of normocytic anemia, such as hemolysis, iron deficiency, leukemia, and myelodysplastic syndromes, are discussed and illustrated in other sections. The main causes considered here are pure red cell aplasia, aplastic anemia, the anemias caused by renal disease and endocrine disorders, and the anemia of chronic disease.

### Pure Red Cell Aplasia

In this disorder, a normocytic anemia occurs with diminished reticulocytes (<1%), absence of polychromasia on the peripheral blood smear, and almost no erythroblasts in the bone marrow (<0.5% of the marrow differential count), despite normal megakaryocytes and white cell precursors. It may develop without apparent cause or be associated with a wide variety of systemic diseases. It occurs in about 5% of patients with thymoma, and this tumor accounts for approximately 10% of cases of pure red cell aplasia. Hematologic malignancies, especially chronic lymphocytic and large granular lymphocytic leukemias, have been associated, as have some solid tumors, rheumatic diseases (such as Sjögren syndrome and systemic lupus erythematosus [SLE]), and infections, primarily parvovirus B19. Numerous medications have been implicated, including phenytoin, azathioprine, and isoniazid. Sometimes, pure red cell aplasia occurs during pregnancy without any apparent explanation and typically disappears following delivery. In many patients, no cause

is found. Often, in these cases, an IgG that inhibits erythropoiesis is present in the serum.

### Aplastic Anemia

In aplastic anemia, a reduction in red cells occurs in the setting of pancytopenia in the peripheral blood and hypocellularity of the bone marrow. Certain forms, such as Fanconi anemia, are hereditary, whereas some acquired types have identifiable causes, such as medications, benzene exposure, or infections with certain viruses. Transfusion-associated graft-versus-host disease consists of fever, pancytopenia, and a generalized morbilliform eruption a few days to weeks following receipt of blood products containing competent lymphocytes. Aplastic anemia may develop in patients with paroxysmal nocturnal hemoglobinuria or as a complication of certain rheumatic diseases, such as eosinophilic fasciitis, SLE, or Sjögren syndrome. In the hemophagocytic syndrome, most commonly associated with viral infections or certain malignancies, pancytopenia, fever, hepatosplenomegaly, and lymph node enlargement occur, and the bone marrow, often hypocellular, shows macrophages ingesting erythrocytes.

Despite the many causes identified, most cases of aplastic anemia are unexplained. Many probably originate from immunologic damage to the bone marrow. Whether a cause is identified or not, the usual presentation is anemia and/or bleeding because of thrombocytopenia; infections are uncommon initially.

### Anemia of Chronic Renal Disease

Anemia typically occurs with chronic renal disease only after the creatinine clearance decreases below 40 mL/min, which corresponds to a serum creatinine of about 2.5 mg/mL. The anemia tends to worsen as the renal function decreases, but it usually stabilizes at a hematocrit of 15 to 30. The cause of the kidney disease is not usually important in determining the severity of anemia, but it is typically less severe with polycystic kidney disease. Several factors cause the decrease in red cells, the most important, however, being inadequate renal production of erythropoietin, a glycoprotein hormone synthesized in the kidney and responsible for the proliferation, maturation, and differentiation of erythrocytes in the bone marrow. In addition, the red cell survival is shortened in uremia, and various toxins ordinarily excreted by the kidney accumulate in the serum and appear to depress erythropoiesis. The processes involved in the anemia of chronic disease, discussed later in this section, may also contribute.

The anemia is normochromic, normocytic, and most red cells are unremarkable on the peripheral smear. Burr cells (echinocytes), however, may form via unknown mechanisms, and sometimes schistocytes appear.

### Anemia of Endocrine Disorders

Anemia, usually normocytic, occurs in several endocrine disorders. About 30% of patients with hypothyroidism have anemia, and about one-third of these are macrocytic. The anemia, usually mild, seems to be from the hormone deficiency itself, and its severity is related to the duration and degree of the hypothyroidism. Approximately 10% to 25% of patients with hyperthyroidism, usually with severe, prolonged disease, are anemic. The mechanism is uncertain.

Most patients with adrenal insufficiency have anemia, usually normocytic, normochromic. In those with autoimmune causes, pernicious anemia, producing a macrocytic anemia, is present in about 10%. Androgen deficiency also is a cause of normochromic, normocytic anemia. Hypopituitarism causes anemia through deficiencies of the previously mentioned thyroid, adrenal, and androgenic hormones.

A small number of patients with hyperparathyroidism have a normocytic, normochromic anemia, with bone marrow examinations typically demonstrating fibrosis. The increased parathyroid hormone may also decrease erythropoiesis.

### Anemia of Chronic Disease

Anemia of chronic disease is quite common among certain illnesses lasting longer than 1 to 2 months, especially those with infection, noninfectious inflammation, or malignancy. In about 25% of cases, however, the only chronic diseases present in the patient, such as congestive heart failure or diabetes mellitus, have none of these features. Usually, the anemia is mild to moderate, with a hematocrit of >25 but, in about 20% of patients, it is more severe. Although the red cells are typically normocytic, normochromic, in about 30% they are microcytic (usually 70–79 fl), and in about 50% they are hypochromic (MCHC 26–32). The red cells on peripheral smear may display mild poikilocytosis and anisocytosis, but markedly small and thin cells, often seen in iron deficiency, are absent.

As in iron deficiency, the serum iron is reduced, but so is the iron-binding capacity, which is typically elevated in iron deficiency. As in iron deficiency, the saturation may be <10%. The serum ferritin, which is characteristically <15 μg/L in iron deficiency, is at least 30 μg/L and usually much higher in anemia of chronic disease. When the two disorders coexist in an individual patient, the serum ferritin is usually <30 μg/L. In ambiguous circumstances, definitive evidence to determine whether the patient has iron deficiency, anemia of chronic disease, or both simultaneously requires bone marrow samples stained for iron. A trial of oral iron therapy is an alternative; supplemental iron has no effect on a pure case of the anemia of chronic disease.

The main pathogenesis of the anemia of chronic disease appears to be the effects of cytokines on erythropoiesis. They impair the proliferation and differentiation of erythroid precursors, diminish erythropoietin production, and decrease the bone marrow response to erythropoietin. They also affect iron metabolism by increasing the retention of it in the bone marrow stores and by decreasing its availability for erythroid precursors. An additional contribution to the anemia is a mild to moderate decrease in erythrocyte lifespan.