151 Hemoglobin and Hematocrit

HENNY H. BILLETT

Definition

Hemoglobin (Hb) is the protein contained in red blood cells that is responsible for delivery of oxygen to the tissues. To ensure adequate tissue oxygenation, a sufficient hemoglobin level must be maintained. The amount of hemoglobin in whole blood is expressed in grams per deciliter (g/dl). The normal Hb level for males is 14 to 18 g/dl; that for females is 12 to 16 g/dl. When the hemoglobin level is low, the patient has *anemia*. An *erythrocytosis* is the consequence of too many red cells; this results in hemoglobin levels above normal.

The *hematocrit* measures the volume of red blood cells compared to the total blood volume (red blood cells and plasma). The normal hematocrit for men is 40 to 54%; for women it is 36 to 48%. This value can be determined directly by microhematocrit centrifugation or calculated indirectly. Automated cell counters calculate the hematocrit by multiplying the red cell number (in millions/mm^3) by the mean cell volume (MCV, in femtoliters). When so assayed, it is subject to the vagaries inherent in obtaining an accurate measurement of the MCV (see Chapter 152).

Both the hemoglobin and the hematocrit are based on whole blood and are therefore dependent on plasma volume. If a patient is severely dehydrated, the hemoglobin and hematocrit will appear higher than if the patient were normovolemic; if the patient is fluid overloaded, they will be lower than their actual level. To assess true red cell mass, independent radionuclide evaluation of the red cells and plasma (by ^{51}Cr and ^{131}I respectively) must be performed.

Technique

Hematocrit

If the hematocrit must be determined quickly, as is often the case when a patient hemorrhages, it may be necessary to measure the hematocrit directly without the use of an automated counter. The materials needed are:

- Lancets
- Alcohol prep pads
- Gauze pads
- Microhematocrit tubes (heparinized)
- Sealant ("Seal-Ease," "Crit-Seal," etc)
- Microhematocrit centrifuge
- Microhematocrit reader
- If venipuncture is required: tourniquet, syringe, tube containing anticoagulant (EDTA, citrate)

For hematocrits obtained by *fingerstick*, wipe the fingertip pad of the fourth finger of the nondominant hand with the alcohol prep pad. Make certain the area is allowed to dry. Prick the fingertip with the lancet. Place the hematocrit tube near the incision site and allow the blood to flow via capillary action into the hematocrit tube until it is two-thirds to three-fourths full or to a predesignated mark on the tube. Avoid "milking" the finger if possible; this causes the expression of tissue fluids and may result in a falsely low hematocrit. Always fill at least three tubes. For hematocrits obtained by *venipuncture*, draw a sample of blood into the tube containing anticoagulant and mix well. Dip the hematocrit tube into the blood and allow the blood to rise to the desired two-thirds to three-quarters level. Because blood cells naturally sediment, a prior thorough mixing of the blood in the tube is necessary to ensure accurate reading.

After cleaning the outside of the hematocrit tubes of excess blood, invert the tube slowly so that the blood migrates just short of the bottom end of the tube. Seal the bottom of the tube with sealant. Make certain that little or no air is interspersed in the column of blood. If the seal is incomplete, leakage will occur during centrifugation and false readings will be obtained.

Place the tubes in a microhematocrit centrifuge and spin for 3 to 5 minutes at high speed. A shorter spin will not allow for complete sedimentation.

Using either a hematocrit reader or any ruled apparatus, measure the length of the column of the packed red cells and divide it by the length of the whole column of blood (cells and plasma), as in Figure 151.1. To obtain the hematocrit, multiply this number by 100%. Average all readings obtained from the different microhematocrit tubes.

Example: If the column of packed red cells measures 20 mm and the whole blood column measures 50 mm, the hematocrit is 20/50 = 0.4 or (0.4 × 100%) = 40%.

Hemoglobin

Hemoglobin determinations will usually be performed by an automated cell counter from a tube of well-mixed EDTA-anticoagulated blood filled to a predetermined level. In this assay, all forms of hemoglobins are converted to the colored

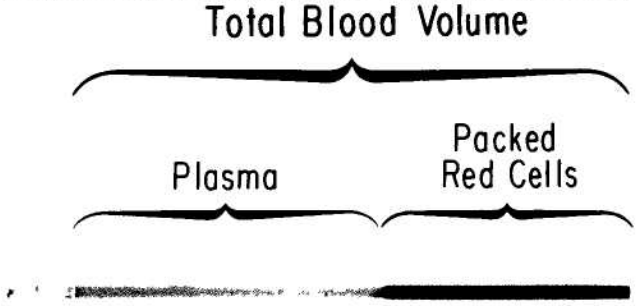

Figure 151.1
Microhematocrit tube after sedimentation. The hematocrit is a ratio of the packed cells to total volume.

protein cyanomethemoglobin and measured by a colorimeter. An inadequate sample, whether due to insufficient volume or inadequate anticoagulation, may give false readings. If it is necessary to determine the level of anemia quickly, the hematocrit is an easier, more convenient test.

Hemoglobin Electrophoresis

Hemoglobin electrophoresis measures the mobility of hemoglobin in an electric field; it can therefore detect only those abnormalities in hemoglobin that alter the charge. Electrophoretic mobilities are affected by pH and by the medium in which the test is conducted. Screening tests typically use a hemolysate of anticoagulated blood electrophoresed on cellulose acetate at pH 8.6 to 8.8. If necessary, a further electrophoresis in starch gel at pH 6.2 to 6.8 is performed. At that stage, the work will usually be performed by a specialized laboratory.

Hemoglobin electrophoresis will not readily assess situations where there are neutral amino acid substitutions or where the hemoglobin is normal but the constituent chains are not produced in equal numbers (thalassemias). The diagnosis of alpha thalassemia of a mild to moderate degree cannot be made by hemoglobin electrophoresis; the diagnosis of beta thalassemia may be made by inference from an increase in the Hb A_2.

A standard electrophoresis would look like Figure 151.2.

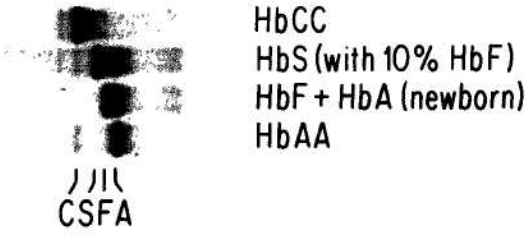

Figure 151.2
A standard hemoglobin electrophoresis (cellulose acetate, pH 8.6).

Basic Science

The molecular weight of hemoglobin is approximately 64,500 daltons. Hb is composed of two pairs of dissimilar chains, α and β, each defined by a specific amino acid sequence and incorporating an iron-containing heme group. Two α–β dimers combine to form a hemoglobin tetramer. This allows for the "heme–heme" interaction necessary for effective oxygen uptake (deoxyhemoglobin → oxyhemoglobin) and delivery (oxyhemoglobin → deoxyhemoglobin). The oxygen affinity of hemoglobin is a function of this heme–heme interaction and of pH (Bohr effect), and is a measure of how many hemoglobin molecules have oxygen bound to them for a given level of oxygen tension. In a normal individual the major hemoglobin is Hb A, constituting approximately 97% of the total hemoglobin. Variations and/or amino acid substitutions in these chains exist. Some are deleterious to the normal function of hemoglobin, whereas others may have relatively normal oxygen affinity and stability. Hemoglobins containing different types of chains make up the remainder of the hemoglobin content in red cells ($\alpha_2\delta_2$ = Hb A_2 approximately 2%; $\alpha_2\gamma_2$ = Hb F approximately 1%).

Substitutions in the normal hemoglobin amino acid sequence may result in hemoglobins that have different subunit interactions and varying affinities for oxygen. For example, a substitution of the sixth amino acid on the beta chain causes Hb S, or sickle hemoglobin. Hb S has a lower oxygen affinity and surrenders its oxygen more readily. Hb F, a normal minor hemoglobin constituent, has a higher oxygen affinity.

If the oxygen dissociation curve is abnormal, the body will adjust the hemoglobin level to ensure adequate oxygen distribution to the tissues. Thus in a rare disease like hemoglobin Hotel Dieu, the difficulty in extracting oxygen from a variant hemoglobin with increased oxygen affinity could result in a lack of oxygen for the tissues (tissue hypoxia) and a compensatory erythrocytosis. The smaller fraction of oxygen released from the hemoglobin is thereby offset by the increased number of hemoglobin molecules. Similarly, in sickle cell anemia, the decreased oxygen affinity allows these patients more tissue oxygen at any given hemoglobin level.

Clinical Significance

Many anemias are detected by routine laboratory screening performed before the patient is symptomatic. When the patient does have symptoms from an abnormality in the hemoglobin level, the symptoms are often a nonspecific weakness or fatigue. The only finding on physical examination may be pallor; additional changes in the nail beds (such as spooning), glossitis (red tongue), or hepatosplenomegaly (enlarged liver or spleen) may give a clue to the etiology of the anemia. Symptoms are usually related to the level of hemoglobin, its abruptness of onset and its duration. A patient with pernicious anemia may feel well at the same level of hemoglobin that would cause severe weakness in a patient with acute gastrointestinal hemorrhage. This is due to volume compensation by plasma and shifts in the oxygen dissociation curve which occur over time.

When first confronted with an abnormal hemoglobin or hematocrit level, the next step is to assess the red cell indices (see Chapter 152), peripheral smear (Chapter 155), and the reticulocyte count (Chapter 156) in light of the patient's history and physical examination.

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Edited by

H. KENNETH WALKER, M.D.
Professor of Medicine and Associate Professor of Neurology
Emory University School of Medicine
Atlanta, Georgia

W. DALLAS HALL, M.D.
Professor of Medicine
Emory University School of Medicine
Atlanta, Georgia

J. WILLIS HURST, M.D.
Candler Professor of Medicine (Cardiology)
Emory University School of Medicine
Atlanta, Georgia

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