CHAPTER 1

PRINCIPLES OF DIAGNOSIS

INTRODUCTION

Anemia exists when a person's level of circulating hemoglobin is lower than that in healthy persons of the same sex and age group in the same environment (Table 1). The most common kinds of anemia are due to nutritional deficiencies of iron, folic acid, and, less commonly, vitamin B_{12} and protein. Other common causes of anemia are congenital defects of hemoglobin production, namely, sickle cell anemia and other hemoglobinopathies, including thalassemia. Protozoal infections and infestations, particularly malaria and hookworm, are also important direct causes of anemia, and bacterial infections may aggravate an existing anemia and prevent optimal response to hematinics.

Because anemia has multiple etiologies, it presents two problems to laboratory personnel:

1. Ascertaining the presence of anemia.
2. Defining the underlying cause of the anemia.

The laboratory tests that have been developed for the specialized investigation of anemia during the past two decades can identify many types of

Table 1 - Normal lower limits for hemoglobin and PCV* level

	Hemoglobin (g/L)	PCV
Infants (full term)	136	.44
Children, 3 months	95	.32
Children, 1 year	110	.36
Children, 10-12 years	120	.38
Women, nonpregnant	120	.38
Women, pregnant	130	.40
Men	130	.40

* Packed Cell Volume

These are values at sea level. The hemoglobin (Hb) of most persons increases with altitude; for example, levels for men increase by about 10 g/l at 2000 m (6500 ft) and by about 20 g/l at 3000 m (10,000 ft).

anemia; however, most of these types do not represent major public health problems in developing countries, and the extensive laboratory facilities needed to identify them are often not available even in central laboratories in many developing countries.

Fortunately, in the vast majority of cases anemia can be correctly diagnosed and its underlying cause determined with minimal resources.

In many cases, measurement of hemoglobin and examination of a blood film can (with some training) provide all the information necessary for identifying the prevalent kinds of anemia of public health importance in an area. Bone marrow examination can be done relatively easily by a physician and can provide information about the iron status as well as morphologic data needed for diagnosis. In practice, if the prevalent causes of anemia in an area are known diagnostic shortcuts can often be taken, and the causes of anemia can be diagnosed in vulnerable population groups as soon as anemia has been detected. They will have to be established for each region and population group, because the prevalent causes vary.

For example, thalassemia and iron deficiency anemia may have similar blood film appearances. But in a region where thalassemia does not occur, a hypochromic blood film is much more likely to be due to iron deficiency anemia. Similarly, a high prevalence of a hemoglobinopathy, red cell enzyme deficiency, or malaria in a region may suggest a likely cause for anemia. In the occasional region where the diet includes bananas or other food with a high folate content, megaloblastic anemia would more likely be due to B_{12} deficiency, rather than to folate deficiency. Frequently, several causes of anemia may coexist in the same person and therefore diagnosis may be difficult.

How to determine prevalences is discussed in Chapter 2. Apart from assisting in the diagnosis of anemia in individual cases, prevalence data are of great public health importance because they form the basis for any decision on preventive measures, such as supplementary treatment of certain population groups (for example, pregnant women) or fortification of foods. The same methodology is also used to determine the efficacy of any such interventions.

EFFECT OF PATHOPHYSIOLOGY ON MORPHOLOGY

Normoblasts, the precursors of the red cells, are derived (together with granulocytes and megakaryocytes) from a pluripotent stem cell, known as a colony-forming unit (CFU). The next stage of development is progenitor stem cells committed specifically to erythropoiesis. These cells are known as burst-forming units (BFU-E) and give rise to the erythroid colony-forming units (CFU-E). Erythropoiesis is regulated primarily by interleukin 3 and the hormone erythropoietin. The former stimulates BFU-E formation; the latter stimulates proliferation and differentiation of CFU-E and proliferation of late stage BFU-E.

The earliest recognizable erythroid cell in the marrow is the normoblast (Figure 1). The normoblast usually undergoes four cell divisions to produce 16 daughter cells, which mature, extrude their nuclei, and become reticulocytes. With each cell division, the daughter cells become smaller than their parents and progressively change in hue from blue to red as the hemoglobin content increases and the ribosomal ribonucleic acid (RNA) content decreases. This process is led by erythropoietin and some other factors (Figure 2). In a healthy person the basic speed of this maturation process is adjusted to maintain the peripheral blood packed cell volume (PCV) and Hb concentration at normal levels. In patients with anemia due to hemolysis or acute blood loss, there is an increase in circulating erythropoietin and a consequent increase in the number of dividing cells. With this stimulus, provided there is adequate iron, the amount of Hb per cell increases, and the cell size increases as well. "Stimulated" reticulocytes are released earlier from the marrow and are larger (macrocytic) and bluer (polychromatophilic) than other reticulocytes. Such polychromatophilic macrocytes are sometimes referred to as "shift" erythrocytes.

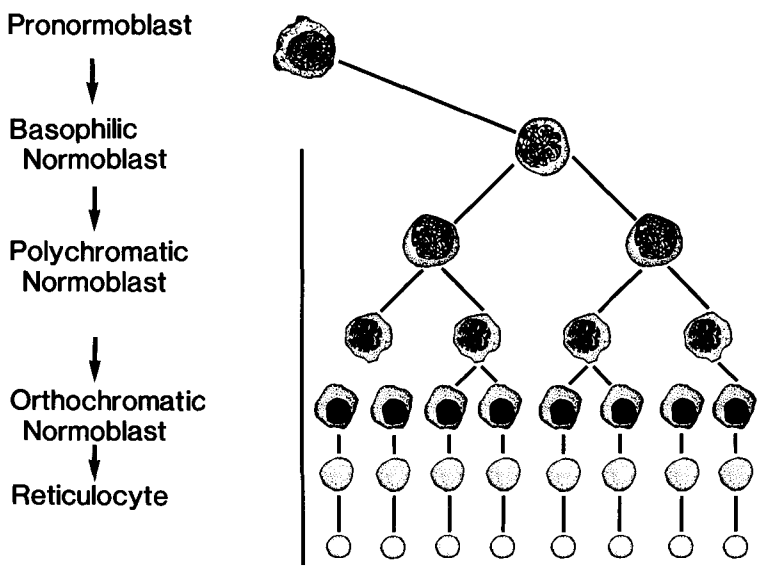


Figure 1 - Erythropoiesis. The pronormoblast has been derived from an erythroid colony forming unit (CFU-E).

If the anemia is severe enough, the stimulus to red cell production may be sufficient for red cells to enter the peripheral blood before they have extruded their nuclei (nucleated red cells, i.e., normoblasts [NRBC]).

Anemia produced by different causes may lead to different morphologic findings. These are described later. Folic acid, vitamin B_{12}, and iron are important nutrients of the red cell and are needed for orderly cell maturation. Deficiencies will produce recognizable morphologic changes in the adult red

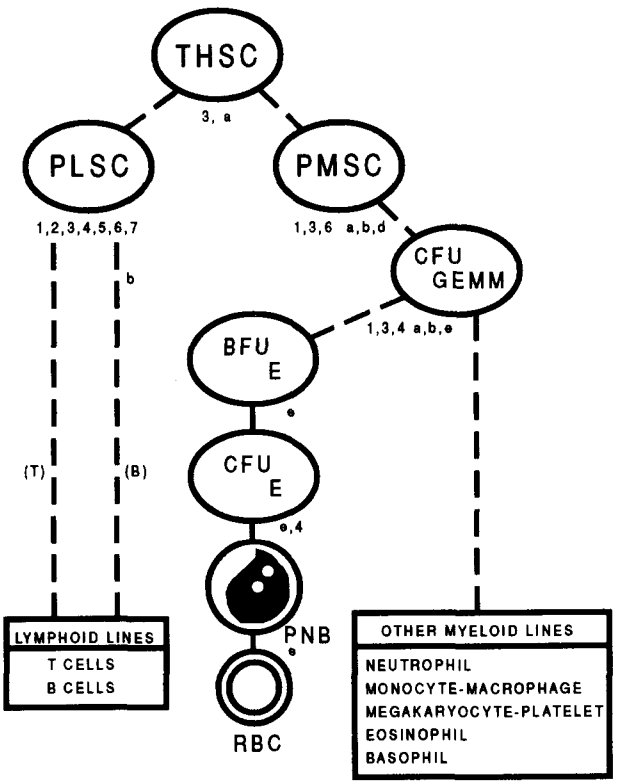


Figure 2 - A scheme showing hematopoiesis and the various sites at which interleukins and growth factors are involved.

THSC= Totipotent Hematopoietic Stem Cell
PLSC= Primitive Lymphoid Stem Cell
PMSC= Primitive Myeloid Stem Cell
CFU= Colony-Forming-Unit
GEMM= Granulocyte, Erythroid, Monocyte, Megakaryocyte
BFU-E= Burst-Forming Unit - Erythroid
CFU-E= Colony-Forming Unit - Erythroid
PNB= Pronormoblast
1-7= Interleukins (IL) 1-7, respectively
a= Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)
b= Granulocyte Colony-Stimulating Factor (G-CSF)
d= Macrophage Colony Stimulating Factor (M-CSF)
e= Erythropoietin

cell. Thus, for instance, deoxyribonucleic acid (DNA), the material which provides the genetic control of cells, must replicate or double if the cell is to divide. If DNA cannot replicate during the DNA synthesis phase of cell division, cell division is delayed. This abnormality occurs when there is a relative or absolute deficiency of folic acid or vitamin B_{12}, for these two substances act as coenzymes in the synthesis of DNA. DNA synthesis will then lag behind the growth of the cell. By the time the cell finally makes adequate DNA to divide, the cytoplasm has grown to an abnormally large extent, and the final product is a large red cell (macrocyte).

The cytoplasm of the red cell is composed largely of hemoglobin. The hemoglobin consists of paired α and ß chains made up of amino acids. Abnormalities of the synthesis of hemoglobin will affect the appearance of the cytoplasm and therefore the red cell. Hemoglobin is composed of two basic parts, the protein globin chain and the heme ring, which contains iron. If production of either of these two parts becomes reduced, the production of hemoglobin is also reduced and can result in iron deficiency anemia, sideroblastic anemia (a condition in which the proper incorporation and use of iron is blocked), or thalassemia (genetic abnormalities in which the production of globin is reduced). The red cells produced in all three of these conditions are small, distorted, and pale (microcytic and hypochromic).

BASIC BLOOD STUDIES FOR DETECTION OF ANEMIA: PACKED CELL VOLUME (PCV) DETERMINATION, HEMOGLOBIN (Hb) DETERMINATION

Before the condition of a patient suspected of having an anemia is evaluated, the hematology laboratory must first establish the presence and severity of anemia. The PCV and the Hb level are used to make this determination. Because each of these measurements provides slightly different information, both should be measured, but measuring one or the other usually suffices at least to establish the presence and the severity of anemia.

Packed cell volume (PCV) determination

The PCV is expressed as the fractional volume of red blood cells per volume of blood; it is obtained by centrifuging anticoagulated venous or capillary blood and measuring the relative amounts of packed red cells and plasma. The procedure is simple and reproducible, and it has proved to be of value in estimating the degree of anemia, regardless of the alteration in the size, shape, and thickness of red blood cells in the various kinds of anemia. Normal lower limits of PCV in men, women, and children of different age groups are shown in Table 1.

Examination of the plasma above the packed cells in the PCV tube may sometimes provide valuable information concerning the underlying cause of

the anemia. Elevated serum bilirubin, as seen in patients with hemolytic and megaloblastic anemia, will be noted as deep yellow plasma. In iron deficiency or inflammation, the plasma may be paler than normal. High white blood cell or platelet counts may be suggested by a layer of grayish cells lying above the packed red cells.

Hemoglobin determination

This test is also used to assess the presence and severity of anemia (Table 1). Numerous methods have been proposed for estimating the hemoglobin concentration of blood. The degree of reliability varies with the methods, and the choice of the method is frequently based on the availability of equipment and the degree of accuracy needed for a particular application. The most reliable method, and the one recommended when it is available, is the hemiglobincyanide method (p. 63). This method has many advantages, such as the availability of a satisfactory standard, and the ability to measure all forms of clinically significant hemoglobin. It is the essential method for scientific studies of anemia and especially for establishing the prevalence of anemia in public health surveys. Other methods, such as the oxyhemoglobin assay, can be used in routine diagnosis (p. 66), but their shortcomings must be recognized, and they should always be calibrated and controlled by reference to the hemiglobincyanide method.

BASIC STUDIES TO DEFINE CAUSE OF ANEMIA: PERIPHERAL BLOOD SMEAR, RETICULOCYTE COUNT, BONE MARROW EXAMINATION

When the presence of anemia has been established, the laboratory worker should try to determine the nature and cause. This requires other tests: the peripheral blood smear, the reticulocyte count, serum iron and iron-binding capacity where available and, in selected cases, a bone marrow examination.

Peripheral blood smear

The examination of the peripheral blood smear is one of the most important laboratory tests in the evaluation of anemia. This simple test provides valuable information, and yet it is frequently neglected. It is essential that the blood smears be of the highest quality. Otherwise, little information will be obtained or, more importantly, artifacts may give misinformation and thus lead to an erroneous diagnosis. The procedure for making and staining the blood film is given on p. 79.

The examination itself should be performed in a systematic way so that nothing is overlooked. The following procedure is suggested. Check the label. Examine the whole film by direct inspection. Then place a drop of mounting fluid (or immersion oil) on the blood film and cover with a cover glass. Then examine the blood film using the low power objective (X10) to assess the

quality of the film (that is, even distribution of white cells, small tails without trailing, and platelet number and distribution) and to estimate roughly the number of white blood cells and platelets. If present, large microorganisms, such as filarial larvae or trypanosomes, may be seen at this power. Scanning for nucleated red cells and abnormal white cells can also be done. Next, examine the film using the high power objective (X40) to assess the number and types of white blood cells and to determine if nucleated red cells or abnormal white cells or blast cells are present. In a normal well-spread film, one should usually see 1-3 leukocytes and about 10-15 platelets with about 200 red cells in each high-power field. Under this power the slide can be scanned relatively rapidly. It is also examined under this power for red cell morphology and the presence and number of platelets. Malarial parasites may also be seen and should be searched for, specifically if malaria infection is a possibility.

Finally, examine the film under oil immersion. The detailed features of red cell, white cell, and platelet morphology can be seen and the presence of malarial parasites confirmed. In areas where *Borrelia* infection occurs, the causative *Spirochaeta* may be detected.

Red cell morphology should be evaluated in the thin area of the slide, where most of the red cells touch but do not overlap (see Photomicrographs p. 13-16). The observer must ascertain if the red cells are macrocytic, microcytic, or normocytic. The mature, small lymphocyte provides an excellent size marker for this purpose because its nucleus is 7-8 μm in diameter, the same size as a normal red cell. Observations should also be made to determine red cell uniformity, variations in size (anisocytosis), or variations in shape (poikilocytosis). Abnormal forms, such as target cells, burr cells, acanthocytes, fragmented cells (schistocytes), sickle cells, and spherocytes, should be noted. The red cells should then be examined for the presence of inclusion bodies, such as nuclear fragments (Howell-Jolly bodies), aggregated ribosomes (stippling), or malaria parasites. Pale cells (hypochromia), variation in color of cells (polychromasia), and lack of uniform color should also be noted. These various identifiable abnormalities are associated with certain diseases; they are listed in Table 2 and illustrated in Photomicrographs p. 13.

Reticulocyte Count

Reticulocytes are juvenile red cells that still contain remnants of ribonucleic acid and ribosomes. This material stains with certain dyes. The procedure for performing the reticulocyte count is described on p. 78. Normally, 0.5%-2.5% of the circulating red cells are reticulocytes. When the bone marrow responds with an appropriate increase in erythropoiesis as a result of the stimulus of anemia (for example, in hemolysis), the reticulocyte count increases. If the bone marrow is not functioning adequately as, for example, in aplastic anemia, erythropoiesis will be impaired and the reticulocyte count will be lower than expected for the degree of anemia. The

Table 2 - Red cell abnormalities

Abnormal Morphology	Associated Clinical Conditions
Macrocytes	Megaloblastic anemia (folate or Vitamin B12 deficiency), liver disease, reticulocytes, myelodysplasia
Microspherocytes	Hereditary spherocytosis, immune hemolytic anemia, Hb C disease, splenectomy, stored blood, burns
Microcytes	Iron deficiency, thalassemia, sideroblastic anemia
Ovalocytes (Elliptocytes)	Hereditary ovalocytosis, thalassemia, pernicious anemia, iron deficiency anemia, myelofibrosis
Sickle cells	Hb SS disease and other sickle cell diseases
Target cells	Hemoglobinopathy (e.g., Hb CC disease, Hb SC disease), liver disease, iron deficiency, splenectomy
Schistocytes (fragmented cells)	Microangiopathic hemolytic anemia, thalassemia, drug hemolytic anemia, mechanical hemolytic anemia
Hypochromia	Iron deficiency, thalassemia, sideroblastic anemia; sometimes in anemia of chronic inflammation
Polychromasia	Hemolytic anemia, hypoxia, myelophthisic anemia, megaloblastic anemia, acute blood loss
Basophilic stippling	Lead poisoning, thalassemia
Howell-Jolly bodies (nuclear remnants)	Hemolytic anemia, megaloblastic anemia, splenectomy
Cabot rings	Megaloblastic anemia, lead poisoning
Heinz bodies	G6PD deficiency, drug- or toxin-induced injury, unstable hemoglobin, Hb H, splenectomy
Siderocytes or sideroblasts	Sideroblastic anemia, myelodysplasia, splenectomy
Acanthocytes	Abetalipoproteinemia, liver disease

adequacy of a response to treatment can be assessed by the extent of the rise in reticulocyte count. However, the relative reticulocyte count expressed as a percentage may be misleading. It is better to calculate the absolute reticulocyte count, but this requires knowledge of the total red cell count. When the latter is not known, an estimate of the true reticulocyte count, corrected for anemia, can be obtained by multiplying the count by either of the following fractions (p. 78):

$$\frac{\text{PCV}}{0.45} \quad \text{or} \quad \frac{\text{Hb}}{150}$$

In evaluating effective red cell production, another factor that should be taken into account is the maturation time of the reticulocytes in the circulation. This "maturation time" is the time taken for the normal reticulocyte to lose its stainable stroma. Normally, this is one day. However, in severe anemia, reticulocytes are often released prematurely and remain in circulation and become mature red cells. In such circumstances, the observed reticulocyte count should be divided by the maturation time (in days) to obtain a reticulocyte index. The normal reticulocyte response to anemia when the marrow is functioning well is shown in Table 3.

Table 3 - Reticulocyte response expected for different degrees of anemia*

Hematocrit	.45	.40	.35	.30	.25	.20	.15
Hemoglobin levels (g/l)	150	133	117	110	83	66	50
Reticulocyte count (%)	1	2	5	10	15	20	30
Corrected reticulocyte count (%)	1	1.8	4	7	8.3	9	10
Estimated maturation time	± 1 day			± 2 days			3 days
Reticulocyte index	1	2	5	5	7.5	10	10

* These figures are intended only as rough guides; they indicate the level of response when the bone marrow is functioning normally.

Bone marrow examination

The aspiration and biopsy of bone marrow can be very useful in diagnosing anemia. It must be stressed, however, that bone marrow examination is secondary to a good peripheral blood smear examination. The peripheral blood, along with the clinical findings, should usually provide a diagnostic clue as to what to expect from the bone marrow examination. The bone marrow examination is used predominantly to confirm the diagnosis of the disease process. It should be performed only for a definite diagnostic reason.

The bone marrow examination is usually diagnostic for the conditions listed in Table 4. It also provides useful information in other conditions. The technique for examining the bone marrow is described on p. 84.

Table 4 - Conditions for which bone marrow examination may be diagnostic

1. Iron deficiency anemia*
2. Anemia of infection*
3. Megaloblastic anemia
4. Hematological malignancies (e.g., leukemias and multiple myeloma)
5. Myelofibrosis
6. Aplastic anemia
7. Metastatic tumor
8. Dyserythropoietic anemia
9. Sideroblastic anemia*
10. Refractory anemia (e.g., myelodysplasia)*
11. Certain metabolic disorders (e.g., Gaucher's disease)

*Requires examination of the iron stain.

The rules that apply to the quality of the preparation of peripheral blood smears also apply to the preparation of film for bone marrow smears. The bone marrow smears must be of good quality. The smear should be examined systematically, first by direct inspection, then by microscopy with the low power (X10). By using low power, the worker can assess the cellularity of the specimen and note if it is heterogenous (that is, composed of normal bone marrow elements) or homogenous (that is, packed with cells of a single type, suggesting leukemia). The cellularity varies with the age of the patient. In early childhood, the marrow normally contains little fat. In adults, the marrow usually has about 50% fat cells, but may have up to 70%. In the elderly, it may have up to 75% fat cells. The number of megakaryocytes can be easily seen on low power.

Cancer cells, if present, can also be identified; they tend to appear in clumps. Abnormal patterns suggesting diseases can frequently be noted when the marrow is examined under low power. After the entire slide has been examined under low power, the marrow should be examined under high dry and oil immersion magnification. The relative numbers of erythroid and myeloid cells should be noted, and the morphology of the red cell precursors then examined for megaloblastic or other changes. Maturation of the white cell elements and megakaryocytes should be assessed. When the marrow is examined in such a thorough, completely systematic manner, much useful information will be obtained.

Red cell count and absolute values

The red blood cell count (RBC) can be obtained by means of a hemocytometer counting chamber in the same way as a leukocyte count (WBC) (p. 113). However, it is too inaccurate to be of much value in diagnosis. The usefulness and practicability of such counts have increased enormously with the advent of electronic cell counters. In laboratories that have this facility, classification of anemia is largely based on "absolute values," which are calculated from the results of the RBC, Hb and PCV, as follows:

Mean cell volume (MCV) = 1000 (PCV ÷ RBC (in femtoliters)
Mean cell hemoglobin (MCH) = Hb ÷ RBC (in picograms)
Mean cell hemoglobin concentration = Hb ÷ PCV (in % concentration)

The normal ranges are shown in Table 5.

Table 5 - Normal reference range of absolute values

	Normal range	Significance		
MCV	78 - 98 fl	Normal	-	Normocytic
		Increased	-	Macrocytic
		Decreased	-	Microcytic
MCH	26 - 32 pg	Normal	-	Normochromic
		Decreased	-	Hypochromic
MCHC	31 - 34 %	Normal	-	Normochromic
		Decreased	-	Hypochromic

In macrocytic anemias the MCV is increased above 100 fl, the MCH is increased and the MCHC is within the normal range. In microcytic hypochromic anemias the MCV is less than 70 fl, and the MCH and MCHC are also low. These measurements may be useful for distinguishing between mild iron deficiency anemia and thalassemia trait. In both conditions the MCV and MCH are likely to be low, but the MCHC is often normal in thalassemia trait and is usually low in iron deficiency anemia.

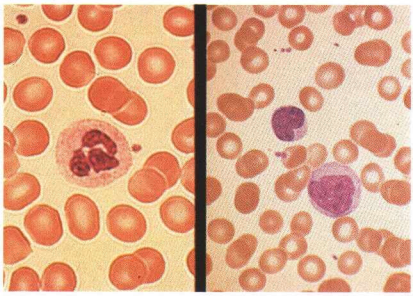
In normocytic normochromic anemias all the values are usually within the normal range, but the MCV may be increased in aplastic anemia and in hemolytic anemias, and in chronic blood loss if the reticulocyte count is high.

Note:
All blood smears and bone marrow aspirates are stained with Wright's Stain unless otherwise specified. All bone marrow biopsies are stained with H & E, unless otherwise specified.

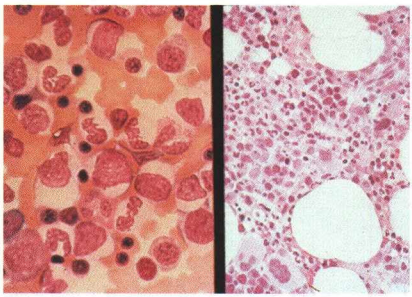
Code for magnification:

(L.P.) = Low power magnification (dry)
(H.P.) = High power magnification (dry)
(L.O.) = Low oil magnification
(H.O.)= High oil magnification

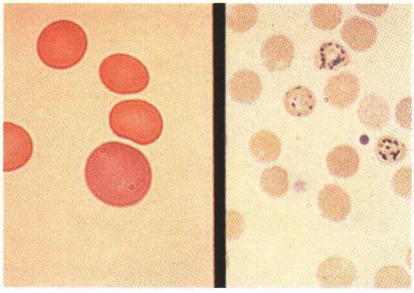
Anemia - General Morphology



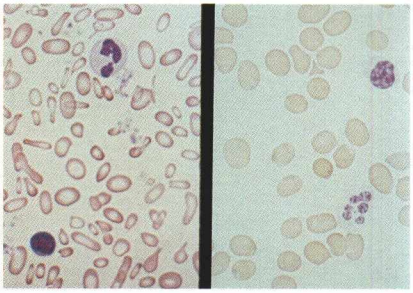
Normal blood cells: Left, Normal neutrophil (center) with segmented nucleus and normal cytoplasmic granules. A platelet is immediately adjacent. The remaining cells are normocytic, normochromic red cells (L.O.). Right, A normal lymphocyte (above) and monocyte (below) are in the center. The red cells are smaller than the lymphocyte nucleus (H.P.).



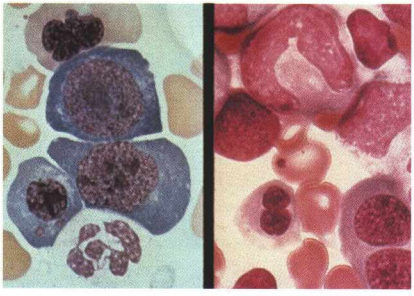
Normal bone marrow: Left, This normal bone marrow aspirate shows the expected 2:1 ratio between myeloid and erythroid precursors—the latter being identified by their shrunken pyknotic ("coal black") nuclei (L.P.). Right, This bone marrow biopsy section has normal cellularity and cell distribution. The larger cells are megakaryocytes (L.P.).



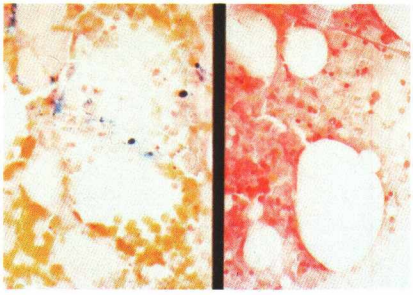
Left, The larger, gray-pink erythrocyte in the center of this field is called a polychromatophilic or "shift" cell (H.O.). Right, Reticulocytes: The dark purple retuculin in red blood cells newly entering the blood is demonstrated by this New Methylene Blue stain (L.O.).



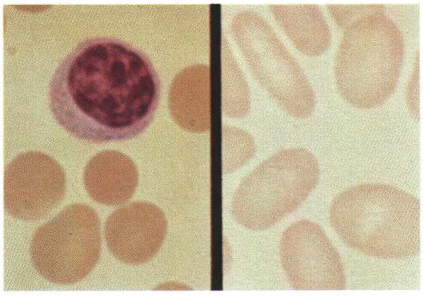
Left, Iron deficiency anemia: Peripheral blood smear in severe iron deficiency anemia. Microcytosis, hypochromia, marked anisocytosis and poikilocytosis are present along with a normal lymphocyte and neutrophil (L.P.). Right, Pernicious anemia: Peripheral blood showing macrocytosis and neutrophil hypersegmentation as well as a normal lymphocyte (L.P.)



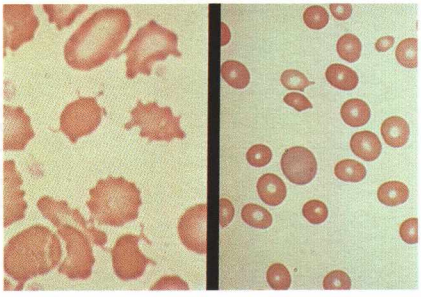
Pernicious anemia: Left, Bone marrow aspirate showing megaloblastic changes (nuclear dyspoiesis; nuclear/cytoplasmic disproportion; open, beaded nuclear chromatin) in erythroid precursors of varying degrees of maturation (H.O.). Right, The major abnormalities here are two "giant metamyelocytes." Binucleate megaloblasts are also present (H.O.).



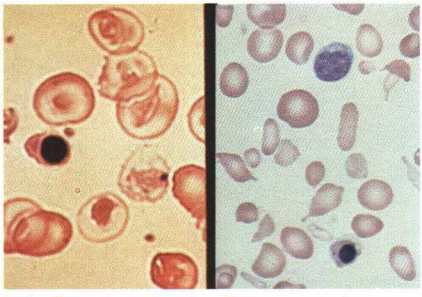
Iron-stained bone marrow: Left, Normal iron stores are seen as dark blue-staining material. Right, The absence of iron is a characteristic finding in iron deficiency anemia (L.P.).



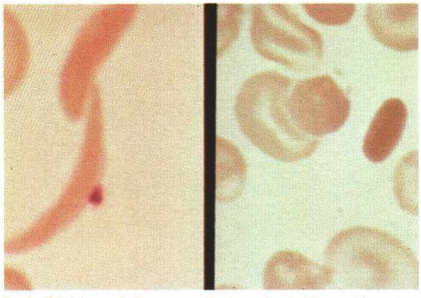
Left, Hereditary spherocytosis (H.S.): This view of the peripheral blood shows microspherocytes and a normal lymphocyte. Right, Hereditary elliptocytosis (H.E.): A significant number of elliptocytes (oval erythrocytes) are seen in this field (H.O.).



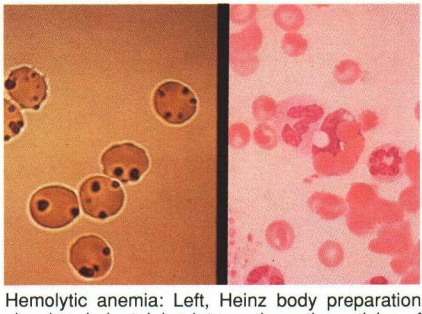
Hemolytic anemia: Left, Anisocytosis, Peripheral blood smear in a patient with marked hepatorenal failure. It shows marked red cell membrane abnormalities - particularly "burr cells" and "spur cells" (H.O.). Right, Microangiopathy - A polychromatic ("shift") cell and red cell fragments are seen centrally (L.O.).



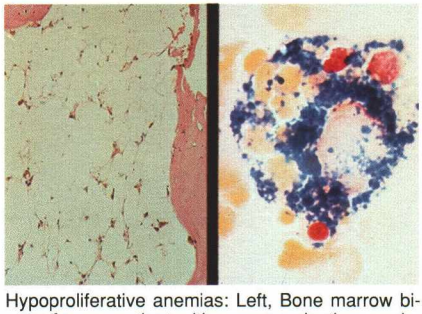
Left, Beta-thalassemia: The smear shows an orthochromatic normoblast to the left. Also seen are targeting, hypochromia, and a Howell-Jolly body (H.O.). Right, Alpha-thalassemia, E hemoglobinopathy: A normoblast, a lymphocyte, and anisocytosis are present (L.O.).



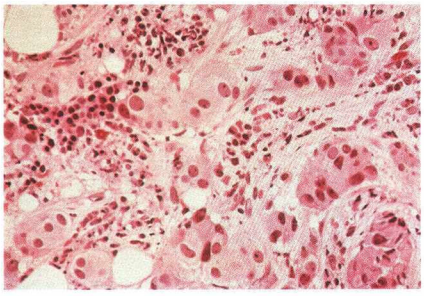
Left, Sickle cell disease: A classic sickle cell is seen in this field. Right, The cell to the right of center demonstrates a classic finding in hemoglobin C disease. It represents crystallized hemoglobin C. Also present are targeting and aniscytosis (H.O.).



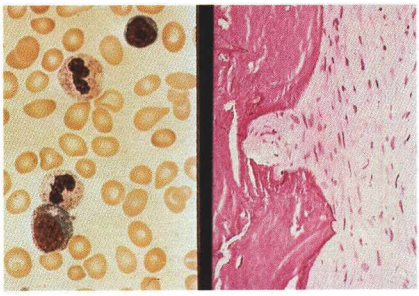
Hemolytic anemia: Left, Heinz body preparation showing dark staining intraerythrocytic particles of denatured globin (H.O.). Right, Erythrophagocytosis. Four red blood cells (center) have been engulfed by a cell of the monocyte macrophage line (L.O.).



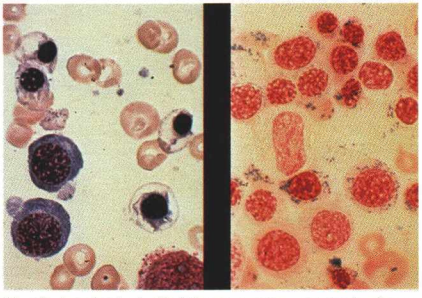
Hypoproliferative anemias: Left, Bone marrow biopsy from a patient with severe aplastic anemia. Virtually no hematopoietic cells are seen in this section (L.P.). Right, Anemia of chronic disease - This iron-stained bone marrow aspirate shows heavy dark blue iron globules which are seen in the storage cells (H.O.).



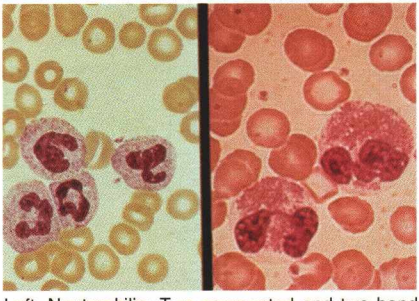
Metastatic breast carcinoma is seen in this view of an H & E-stained bone marrow biopsy. The malignancy has virtually replaced normal marrow elements (L.P.).



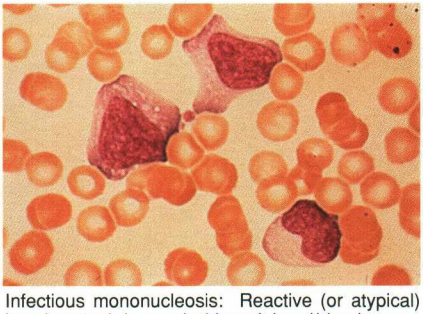
Agnogenic myeloid metaplasia: Left, This blood smear shows some teardrop-shaped red cells and a characteristic leukoerythroblastic reaction (L.O.). Right, This H & E-stained preparation shows virtual replacement of the marrow cavity with light pink-staining fibrous tissue (H.P.).



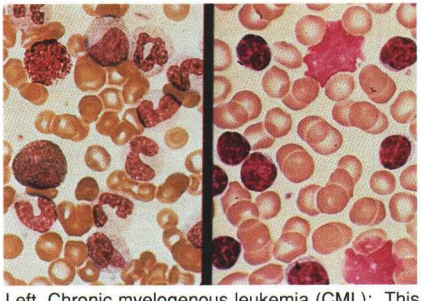
Myelodysplasia: Left, A bone marrow aspirate showing marked erythroid dyspoiesis. The diagnosis was refractory anemia with ring sideroblasts (RARS). Right, An iron stain in the same patient showing perinuclear rings of iron-laden mitochondria (L.O.).



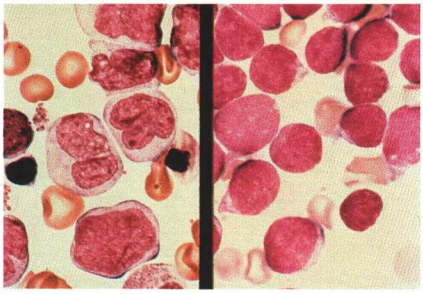
Left, Neutrophilia: Two segmented and two band neutrophils are seen. Some red cells are slightly hypochromic (L.O.). Right, Eosinophilia: Two eosinophils are shown from the peripheral blood of a patient with the hypereosinophilic syndrome (H.O.).



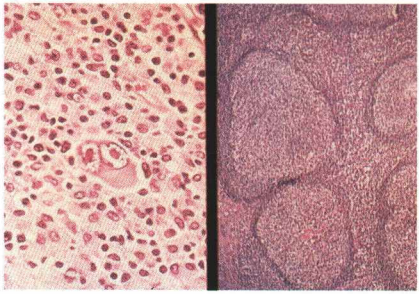
Infectious mononucleosis: Reactive (or atypical) lymphocytosis is seen in this peripheral blood smear. Pleomorphic, reticular nuclei; peripheral basophilia of cytoplasm; and scalloped cell borders are characteristic. Slight rouleaux is also present (H.O.).



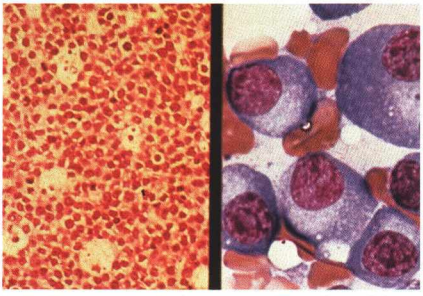
Left, Chronic myelogenous leukemia (CML): This blood smear shows marked neutrophilia with left shift and two abnormal eosinophils (H.P.). Right, Chronic B-cell lymphocytic leukemia: The blood smear shows a neoplastic lymphocytosis. Two of the lymphocytes have been irreversibly damaged (L.O.).



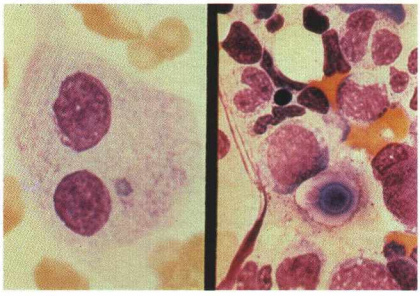
Left, Acute non-lymphoblastic leukemia, M-4 type: These blasts have both myeloid and monocytic characteristics (H.O.). Right, Acute lymphoblastic leukemia, L-1 type: The lymphoblasts in this field are moderately uniform morphologically (H.O.).



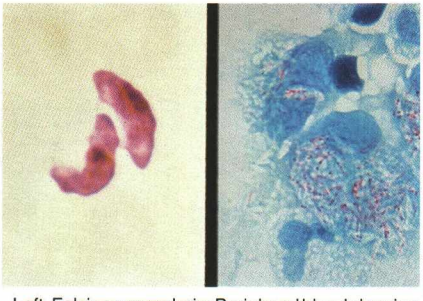
Left, Hodgkin's disease: This H & E-stained lymph node biopsy shows lymphocyte depletion and a classic Reed Sternberg Cell (center) (H.P.). Right, Non-Hodgkin's lymphoma, follicular, small cleaved cell type: This H & E-stained lymph node biopsy shows a clear follicular pattern (L.P.).



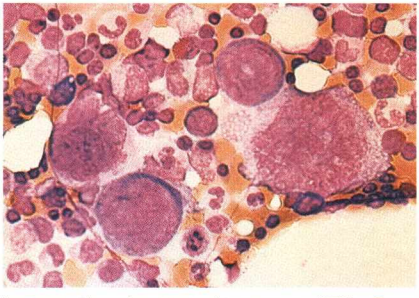
Left, Non-Hodgkin's lymphoma (B cell type) in a patient with AIDS. Lymph node biopsy H & E stain. Right, Multiple myeloma: All plasma cells in this bone marrow aspirate field are neoplastic myeloma cells. The nuclei are pleomorphic and eccentric, and the cytoplasm is gray-blue. One cell is binucleate (H.O.).



Left, Gaucher's disease: This bone marrow aspirate shows a giant binucleate storage cell filled with glucocerebrosides. The fibrillar pattern is characteristic (H.O.). Right, Cryptococcosis: This bone marrow aspirate is from an immunocompromised host. The *Cryptococcus neoformans* organism is near the center (H.O.).



Left, Falciparum malaria: Peripheral blood showing two crescent-shaped gametocytes. Right, AIDS: Mycobacterium avium intracellulare infection. Massive numbers of red, acid-fast organisms are seen in the macrophages of this marrow aspirate (H.O.).



Immune thrombocytopenic purpura (ITP): Bone marrow aspirate. Megakaryocytosis is reflected in this field, where four such cells are seen. These range from a megakaryoblast (top) to a mature megakaryocyte (middle right) (H.P.).

The MCHC is higher than normal when the red cells include a high proportion of spherocytes; a high MCHC also occurs in dehydration.

For severe anemia, absolute values do no more than confirm the features that should be apparent in a blood film. However, they provide greater sensitivity than is possible by microscopic examination in a routine laboratory at the borderline between normal and abnormal.

DIAGNOSIS OF ANEMIA: PRACTICAL APPROACH

The diagnosis of the cause of anemia should be approached on a step-by-step basis, incorporating information obtained from the initial clinical and laboratory evaluation. The basic steps are these:

1. Evaluation of clinical information obtained from a review of the history and physical examination.
2. Evaluation of the basic blood studies, which include Hb, PCV, reticulocyte count, and examination of the peripheral blood smear; also MCV, MCH, and MCHC if a blood cell counter is available.
3. Determination of serum iron (and total iron-binding capacity) and/or ferritin levels if these tests are available and reliable.
4. Examination of aspirated bone marrow, when necessary.
5. Specialized laboratory procedures when necessary for making a definitive diagnosis. Some of these procedures may require referral to a central laboratory.

It is often difficult to find the cause of anemia because the patient may have a combination of conditions, each of which may contribute to the anemia. Thus, for example, a patient with thalassemia may also have nutritional anemia and/or infection. Megaloblastic and iron deficiency anemia often occur together. However, one factor will usually predominate to point to the major cause of the anemia. It is impractical to provide a detailed description of all possible combinations and permutations produced when multiple etiologies are present; therefore, only the systematic approach is given here. However, remember that an unexpectedly poor response to specific anti-anemic therapy may indicate an anemia with more than one cause.

History

This information is invaluable for determining the direction of subsequent studies. For example, anemia found in poorly nourished infants or in patients on insufficient diets suggests a nutritional cause. A history of jaundice suggests a possible hemolytic process or parasitic infection - for example, malaria. Ingestion of certain drugs, exposure to chemicals such as cleaning fluids used in the household, or the presence of preexisting renal disease suggest these factors as causes of anemia. A history of bleeding (gynecologic, gastrointestinal) points to the cause of an iron deficiency anemia. Family and social histories, including ethnic and geographical considerations, frequently

contain valuable information that can be important in suggesting the most likely diagnosis. Certain traditions, genetic backgrounds, exposures, and diets can be associated with particular health problems. Information on the frequency of a particular form of anemia in a region can sometimes be derived from previous public health surveys, which might reveal nutritional, genetic, or infectious disease problems. The surveys, however, must have used appropriate methodology and have been statistically validated.

Physical examination

The physical examination may also provide very important information; for example, pallor, bruising, shock, palpable spleen, enlarged lymph nodes, and jaundice are all associated with identifiable types of clinical disease. Similarly, ulcers on the lower leg may occur in sickle cell anemia and thalassemia. Residual neurological abnormalities, which may follow sickle cell crisis involving the central nervous system or long-standing vitamin B_{12} deficiency, may suggest the diagnosis. We must emphasize, however, that this book is not a clinical treatise. The clinician wanting detailed information on clinical appearances of patients should refer to standard textbooks on clinical hematology.

Initial laboratory studies

Once the clinical data have been evaluated and the initial impressions obtained, the laboratory worker determines whether anemia is present and decides upon the direction of the subsequent tests. Examining the peripheral blood film can be a vital help in determining that direction, as can reliable red cell indices (p. 77). The size of the red cells often determines if an anemia is macrocytic, microcytic, or normocytic (We have classified the anemias into these three types because we believe this is the simplest approach for the laboratory worker).

LABORATORY DIAGNOSIS OF MACROCYTIC ANEMIA

(see Figure 3)

Blood smear

If a patient's red cells are macrocytic, that is, predominantly larger than the nucleus of the small mature lymphocyte in the peripheral blood, the laboratory worker should consider three main causes: 1) megaloblastic anemia (due to vitamin B_{12} deficiency or folate deficiency, or both), 2) alcoholism and liver disease; and 3) conditions with a large number of reticulocytes in circulation.

Frequently, other features in the peripheral blood are useful in distinguishing these causes of macrocytosis. For example, if a deficiency of folate or vitamin B_{12} is the cause, the blood smear may show thrombocytopenia and leukopenia. Characteristically, there will be hypersegmented polymorphonuclear leukocytes containing six or more nuclear lobes. In addition, there is an overall shift to multisegmentation and more than 5% of the polymorpho-

nuclear leukocytes will have five or more lobes. The red cells show great variation in size and shape; many have a distorted and abnormal appearance. Howell-Jolly bodies may be seen, as may other abnormal red cell inclusions or stippling. Occasionally a circulating megaloblast will be seen. If these morphologic characteristics are present, the laboratory worker should pursue the diagnostic workup for megaloblastic anemia as outlined on pages 20-3. In less severe megaloblastic anemia, macrocytosis alone may be present. If iron deficiency is also present, a dimorphic picture may be seen.

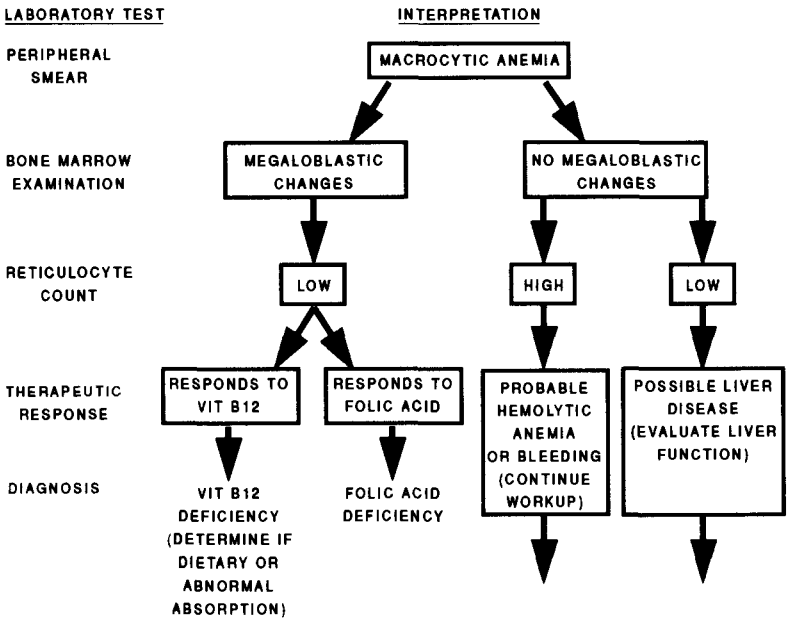


Figure 3 - Scheme for investigating patients with macrocytic anemia. This figure represents a convenient approach to the diagnosis of this form of anemia. It is intended only as a guide and might require modification to suit local conditions or resources.

The morphology of the red cells of a patient with liver disease is characteristically different. As a rule, the red cells are less macrocytic and tend to be more uniform in size and shape. Large target cells frequently occur. With these findings, the laboratory worker should evaluate the liver function and ascertain if there is a history of alcohol excess. However, when macrocytes are present, megaloblastic anemia must be suspected unless or until proved otherwise by marrow aspiration (Figure 3). Alcoholism may also be associated with folate deficiency.

The third cause of macrocytic red cells is marked reticulocytosis. The reticulocytes are responsible for the macrocytosis. On a Romanowsky-stained film they appear polychromatophilic. This condition is easily confirmed by performing a reticulocyte count, because in the other two conditions causing macrocytic cells, the reticulocyte count is normal or low. If the

reticulocyte count is elevated, the laboratory worker should consider the hemolytic processes mentioned under the section *Laboratory diagnosis of normocytic anemia* (p. 27). Other causes of macrocytosis are aplastic anemia and myelodysplasia.

Investigation for suspected megaloblastic anemia

The common causes of megaloblastic anemia are shown in Table 6. Ninety-five percent of the cases will be due to either folate deficiency or vitamin B_{12} deficiency, and in developing countries these conditions are caused predominantly by nutritional deficiency. Less common, but also important to consider, is deficiency as a result of malabsorption due to sprue or abnormal bacterial flora or parasites in the intestine. Another cause of megaloblastic anemia is lack of intrinsic factors necessary to ensure absorption of vitamin B_{12}. This is the cause of classic "pernicious anemia."

Sources of vitamin B_{12} and folic acid (folate)

Vitamin B_{12} is not synthesized by plants in general, although some bacteria are exceptions. Therefore, strict vegetarians or patients who eat only a vegetable diet are certain to become vitamin B_{12} deficient. Although some bacteria in the large bowel in humans can make vitamin B_{12}, it would not be absorbed from there because its absorption site is located in the terminal ileum of the small bowel. Foodstuffs with the highest content of vitamin B_{12} are liver and kidney, but other meat, dairy products, poultry, fish, and shellfish also contain large amounts. Folic acid is present in most foodstuffs; green vegetables, liver, and yeast are particularly rich in folic acid, but the activity of the vitamin can be destroyed by boiling, especially if this is prolonged and at an acid pH.

Bone marrow examination for confirmation of diagnosis

A diagnosis of megaloblastic anemia based on examination of the peripheral blood should generally be confirmed by bone marrow aspiration. If the diagnosis is correct, the marrow specimen in this condition will be very hypercellular. There is an increase in erythroid elements and an accumulation of the early erythroid forms due to a selective death of the mature forms that are unable to complete their maturation process. The diagnostic morphologic finding is disassociation between nuclear and cytoplasmic development in the erythroblast. The nucleus maintains a primitive appearance (frequently containing nucleoli) in spite of an adequate amount of hemoglobin in the cytoplasm. The persistence of a nucleus containing nucleoli in a cell in which hemoglobin production has begun is diagnostic of retarded nuclear maturation. The nucleus itself has an open, fine lace-line appearance, for these cells have grown large because of delayed cell division. The granulocytic series shows giant and abnormally shaped metamyelocytes (see photomicrographs p. 13).

Table 6 - Causes of megaloblastic anemia

1. Folate deficiency
   a. inadequate diet
   b. pregnancy and lactation
   c. alcoholism
   d. steatorrhea or sprue
   e. other causes of malabsorption, including partial gastrectomy

2. Vitamin B12 deficiency
   a. pernicious anemia
   b. gastrectomy
   c. sprue
   d. long-term dietary deficiency, especially vegans
   e. parasites (Diphyllobothrium latum)

3. Drug-induced megaloblastic anemia
   a. 6-mercaptopurine
   b. 5-fluorouracil
   c. cytosine arabinoside
   d. vinca alkaloids
   e. diphenylhydantoin
   f. antifolate compounds

4. Congenital disorders (very rare)
   a. orotic aciduria
   b. congenital dyserythropoietic anemia
   c. juvenile pernicious anemia

5. Leukemia and myelodysplasia

6. Erythroleukemia

Differentiating vitamin B_{12} deficiency from folic acid deficiency

In most developing countries, patients with megaloblastic anemia may have either vitamin B_{12} or folate deficiency. The type of deficiency (that is, whether folate or vitamin B_{12}) can usually be determined with a high degree of probability from the patient's dietary history, the clinical data, and a knowledge of the prevalence of these deficiencies in the local population.

Establishing a definite diagnosis, however, requires measuring the serum and red cell folate and the serum vitamin B_{12} level, and/or possibly doing a radioactive B_{12} absorption test. These tests require more resources than are usually present in the basic laboratory and are usually done only in large central laboratories, if at all. If a central laboratory capable of performing B_{12} and folate assays is accessible, the patient's blood should be drawn before treatment and sent to that laboratory. For this purpose, about 10 ml of blood is collected, avoiding contamination as much as possible. If red blood cell folate is to be determined, some of the blood is mixed with EDTA

(anticoagulant) and kept cold (4°C). The remainder of the blood is allowed to clot, and the serum is removed without contamination. The serum is stored cold (frozen, if possible) until examined. Some methods for assay of B_{12} and folate may be invalidated if the patient is receiving antibiotics. A radioactive B_{12} absorption test requires special arrangements for obtaining doses of the isotope to be administered to the patient, and subsequent measurement of the radioactivity in the patient's urine or plasma by a central or regional laboratory.

Treatment and therapeutic trials

The patient's clinical picture may indicate whether folic acid or B_{12} should be given. When the diagnosis is not clear, a therapeutic trial may be useful for differentiating between the two causes of anemia. Therapy must take precedence over diagnosis when the patient is seriously ill, has a hemoglobin of less than 60 g/L, is in the later stages of pregnancy, or has a neurologic disease. Blood samples should be collected as described above for later assays, and the patient should be given both folate and B_{12} in large therapeutic doses. In order to check response to treatment, blood counts, including reticulocytes, should be performed on the 3rd, 6th, 10th, 14th, and 21st day after treatment. Failure to respond adequately indicates either that the patient does not have megaloblastic anemia or that it is complicated by iron deficiency or infection.

When treatment is less urgent, a therapeutic trial can be carried out. However, it is time-consuming and is probably worthwhile only in a major hospital under ideal conditions when the patient can have frequent measurements of hemoglobin and reticulocyte counts. The therapeutic trial must not be undertaken on critically ill patients; that is, in the presence of angina pectoris, congestive heart disease, or thrombocytopenia with bleeding. Under optimum conditions, the test is done in the following sequence:

The patient is continued on his or her usual diet and given 0.2 mg (200 μg) of folic acid daily by mouth. The reticulocyte response is noted daily for one week. If there is no response or if the response is inadequate, the patient is then treated with 1 to 2 μg of vitamin B_{12} daily by intramuscular injection, and any response of reticulocytes and other blood count parameters is noted.

Full response to a therapeutic trial is shown by reticulocytosis beginning on the third day and reaching a peak on the sixth or seventh day, with a rise in Hb of 10 g/L, and normal leukocyte and platelet counts after seven days. A partial response may occur if the anemia is partly due to concomitant iron deficiency or if it is due to vitamin B_{12} deficiency. If there is only a partial remission with folic acid, the B_{12} trial must not be delayed for more than one week.

Therapeutic preparations

Folic acid is usually dispensed as a 5-mg tablet. This tablet is insoluble in water and cannot be easily divided into the correct dose for the trial. Tablets

containing 100-500 μg are available but in some products this is in combination with iron. These are suitable, provided that the preliminary hematologic investigation has excluded iron deficiency as the cause of the anemia.

Vitamin B_{12} is usually dispensed as cyanocobalamin in ampoules containing 1000 μg/ml, and can be given in doses of 200-1000 μg.

LABORATORY DIAGNOSIS OF HYPOCHROMIC MICROCYTIC ANEMIA

(see Figure 4)

If, during the initial evaluation of the peripheral blood film, the laboratory worker finds that the red cells are paler or smaller than normal, he or she must consider the common causes of hypochromic microcytic anemia: iron deficiency, thalassemia, and abnormalities of iron metabolism. Although the blood film can give some clues to the probable causes, the morphologic abnormalities are not as helpful as with macrocytic anemia. For instance, in iron deficiency anemia the red cells are characteristically hypochromic and microcytic, but the extent of these abnormalities depends on the level of Hb or PCV - a patient may have a normal blood smear in iron deficiency anemia until his or her PCV falls below about 0.34 or 0.35 and the Hb below 100 to 110 g/L. At that point the careful observer may detect mild microcytosis. When the PCV is less than 0.30 and the Hb is less than 100 g/L, microcytosis

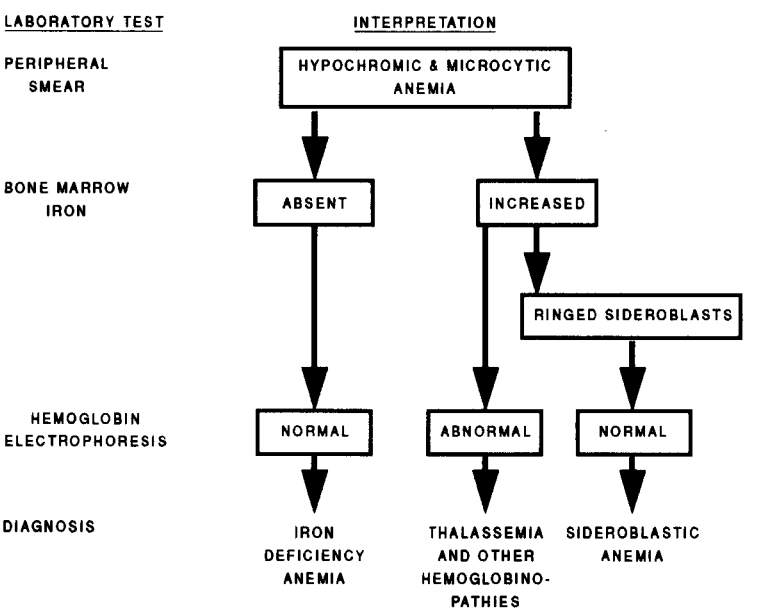


Figure 4 - Scheme for investigating patients with microcytic hypochromic anemia. This figure represents a convenient approach to the diagnosis of this form of anemia. It is intended only as a guide and might require modification to suit local conditions or resources.

becomes clearly evident and hypochromia may be noticed. A useful indication is that hypochromic cells have a central pale area larger than half the diameter of the cell. If the patient's PCV is below 0.27 and the Hb below 90 g/L, hypochromia and microcytosis will be prominent with increasing anisocytosis, poikilocytosis, a few target cells, and elongated and elliptical forms. The platelet count is often increased. If acute bleeding occurs with the iron deficiency, polychromasia (p. 13) may also be seen, but the ability to produce reticulocytes is reduced compared with that in patients who have normal iron stores.

Patients with thalassemia and other hemoglobinopathies may have very similar blood films, but the extent of the red cell abnormalities also depends on the severity of the anemia and, therefore, on the type of thalassemia. For instance, on the blood films of some patients with thalassemia, particularly the heterozygous form, microcytic red cells may be the only morphologic abnormality, and the film may be difficult to differentiate from a film indicating iron deficiency. However, with severe (homozygous) thalassemia major, the blood films show striking abnormalities not usually seen with iron deficiency. The red cells are markedly microcytic and hypochromic, with bizarre variations in size and shape. Target cells comprise from 5% to 30% or even more of the red cells; nucleated red cells and shift erythrocytes may be seen. In thalassemia, cells with coarse basophilic stippling are frequently seen. The reticulocyte count is frequently elevated (up to 8%), the leukocyte count is often elevated, and the platelet count may be elevated.

The third cause of hypochromic microcytic anemia is an inability to use iron properly for effective heme production. Anemia caused by this inability is called sideroblastic anemia. It may be congenital or secondary to various pathological processes that produce different levels of blockage of heme synthesis. The morphologic changes, however, are very similar in all. Although the findings on the blood smear in severe cases may be helpful in distinguishing this group, milder forms may be very hard to differentiate from iron deficiency or thalassemia. Variation in size and shape of the red cells is prominent. Characteristically, the red cells appear to be dimorphic, that is, composed of two populations, one relatively normochromic and normocytic and the other hypochromic and microcytic. In severe cases, there are numerous very pale target cells. These cells and other cells, characteristically, may contain small groups of three or four coarse granules (a type of coarse stippling). When the blood film is stained with an iron stain, the stippled granules are shown to contain iron.

Subsequent investigation of patients with hypochromic anemia

A definitive diagnosis of the cause of hypochromic anemia may require bone marrow examination, hemoglobin electrophoresis, and other specialized tests, especially serum iron estimation. However, the likely diagnosis can be predicted by a knowledge of the patient's history and physical features, as

well as the prevalent public health situation in the particular area. If the laboratory resources do not include serum iron assay, it is practical to assume that iron deficiency is the tentative diagnosis of hypochromic anemia, provided that the following basic criteria are met:

1. The blood film is compatible with or suggestive of iron deficiency anemia.
2. The patient has a recognizable reason to have iron deficiency anemia: for example, dietary lack; a known source of bleeding, such as hookworm; or a suspected gastrointestinal tumor.

If these conditions are fulfilled, the patient may be given a therapeutic trial with iron. The trial should consist of the equivalent of 300 mg of ferrous sulfate three times a day for four weeks. Iron may be given immediately to a patient bleeding from a suspected tumor of the gastrointestinal tract; this should in no way delay diagnostic investigation. If there is a reticulocyte response with an increase in Hb[1], the diagnosis is confirmed. If not, further studies are warranted. In an area where thalassemia is prevalent, it is important not to persist with this treatment, because a prolonged trial with iron may be harmful to patients with thalassemia. Furthermore, trial tests often fail because the patient does not take the medicine prescribed.

Bone marrow

The bone marrow examination is a diagnostic tool for distinguishing iron deficiency from the other categories of hypochromic anemia, and it should be performed if it can be reliably interpreted and if the potential information to be gained will help to confirm the diagnosis. By definition, iron deficiency means that the bone marrow has no iron stores. This absence is usually determined by an iron stain of the marrow preparation (p. 88). In thalassemia and in conditions of abnormal iron use, iron stores are above normal. Furthermore, if the iron stain preparation is examined carefully, other useful diagnostic information may be obtained.

NOTE: The laboratory worker must recognize that a diagnosis of iron deficiency anemia does not conclude the diagnostic process. Iron deficiency anemia may be due to inadequate iron intake, increased use as in pregnancy, or blood loss. If the iron deficient patient has had an adequate dietary intake of iron, the source of blood loss must be found and identified. In all adult males with iron deficiency, whatever their diet, a source of bleeding should be looked for. The worker must also remember that there may be more than one cause for a patient's hypochromic anemia, for example, the coexistence of hookworm-induced blood loss, malnutrition, and hemoglobinopathy.

[1] In a patient with continuing blood loss, there may be a reticulocyte response without parallel improvement in Hb and PCV.

Other tests to diagnose microcytic hypochromic anemia

Serum iron

The serum iron test (p. 110) is useful for establishing the cause of hypochromia. Normally 13-32 μ mol/L (0.7-1.8 mg/L), the serum iron concentration is low (i.e., <13) in persons with iron deficiency anemia and in chronic infections, and is within normal limits or increased in patients with thalassemia, hemoglobinopathy, or sideroblastic anemia, and in patients who have had many blood transfusions.

Iron-binding capacity

When it can be reliably performed, the total iron-binding capacity measurement (which indirectly measures transferrin) may provide useful information. Iron-binding capacity is increased in persons with iron deficiency anemia and reduced in those with thalassemia, hemolytic anemia, and the anemia of chronic infections. Methods for measuring it are based on saturating the plasma with iron, removing the excess unbound iron by adsorbing it (for example, onto magnesium carbonate), and then estimating the iron in the iron-saturated serum. The method recommended by the ICSH (British Journal of Hematology 1978, 18, 281-90) is fairly reliable; although this and other methods lack a completely satisfactory way to ensure that all unbound iron has been removed.

Ferritin

Measurement of serum ferritin is widely used as a test for iron deficiency and iron overload. A method based on immunoassay has been recommended by ICSH (see Flowers C A et al, American Journal of Hematology, 1986, 23, 141-51). A reference standard consisting of human ferritin is available from WHO.

In normal adults the serum ferritin concentration is in the 15-300 μg/L range. Different ranges should be established for males and females. Concentration is lower in children. In an adult a level less than 15 μg/L indicates an absence of storage iron, but in patients with acute or chronic disease that results in secondary anemia, the concentration increases to about 50 μg/L despite an absence of iron stores. In such cases, the erythrocyte sedimentation rate (ESR) provides guidance on the significance of the serum ferritin level (Figure 5).

Hemoglobin electrophoresis

Hb A_2 and Hb F determinations are very useful for differentiating thalassemia from abnormal use of iron, and these should be the final tests in the general evaluation flow chart; that is, they should be used if the patient has a hypochromic microcytic anemia associated with increased iron store in the marrow. Hb A_2 and HbF are increased in patients with ß-thalassemia.

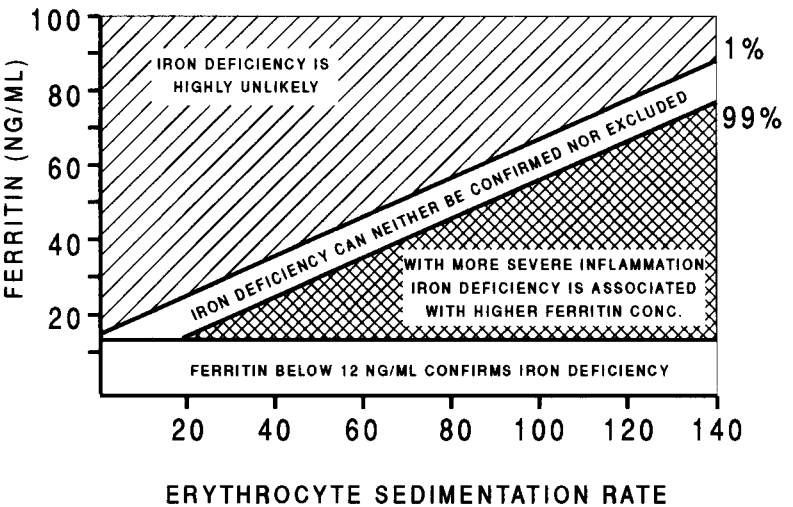


Figure 5 - Relation of ferritin level to iron deficiency and to secondary anemias due to inflammation. (From DL Witte et al, Am Journ Clin Path , (1986)85;202-6. Reproduced with permission.)

However, α-thalassemia, a genetic abnormality resulting from decreased synthesis of another part of the hemoglobin molecule, may be missed because no characteristic changes are seen on the electrophoretic patterns of Hb A, Hb A_2, or Hb F. These cases can sometimes be identified by examining blood films obtained from family members for morphologic findings or, in some cases, by demonstrating precipitated Hb H on films stained by brilliant cresyl blue (p. 95).

An excessive amount of iron that occurs in nucleated red cells as ferritin aggregates ("sideroblasts") is seen in patients with hemolytic anemias, megaloblastic anemias, iron overload, and thalassemia. When non-ferritin iron is deposited in mitochondria, ring sideroblasts (sideroblastic anemia) develop. This abnormal use of iron has many etiologies. It may be congenital, but most cases are acquired (see Table 7).

LABORATORY DIAGNOSIS OF NORMOCYTIC ANEMIA

(see Figure 6)

Initial laboratory studies

Normocytic anemias should be separated into two general categories: those with increased red cell production and those with decreased red cell

Table 7 - Causes of sideroblastic anemias

1. Hereditary - sex-linked

2. Acquired
   a. primary
      i. refractory anemia
      ii. myelodysplastic syndrome

   b. secondary

      i. myeloid leukemia, myelosclerosis, polycythemia, myeloma, erythroleukemia
      ii. vitamin B6 abnormalities - antituberculosis chemotherapy, celiac disease, alcoholism, other mitochondrial enzyme defects
      iii. block at mitochondrial level of hemosynthesis - lead poisoning, chloramphenicol
      iv. idiopathic - rheumatoid arthritis, carcinoma, megaloblastic anemia, phenacetin

production. The blood smear provides useful information, since increased red cell production is often associated with large polychromatophilic red cells. The reticulocyte count is the primary test for separating the patients into the two groups.

NOTE: The level of expected reticulocytes depends upon a normally functioning bone marrow and rises as the PCV level falls.

Acute blood loss

Blood loss that occurs suddenly or over a relatively short period of time (2-6 weeks) can be associated with normocytic normochromic anemia and reticulocytosis provided that the marrow has a normal ability to increase production. Generally, there is clear evidence of blood loss by history or physical examination (menorrhagia, hematemesis, melena, trauma). However, occasionally the evidence is less clear, and one must actively pursue the diagnosis of blood loss by a more extensive clinical evaluation.

Hemolytic anemia

Hemolytic anemia is produced by an increased rate of red cell destruction (hemolysis) that cannot be compensated for by the increased red cell production. The destruction may be due to defects within the cell (intrinsic cell defects) or to external sources (extrinsic causes) (Table 8). Often, other

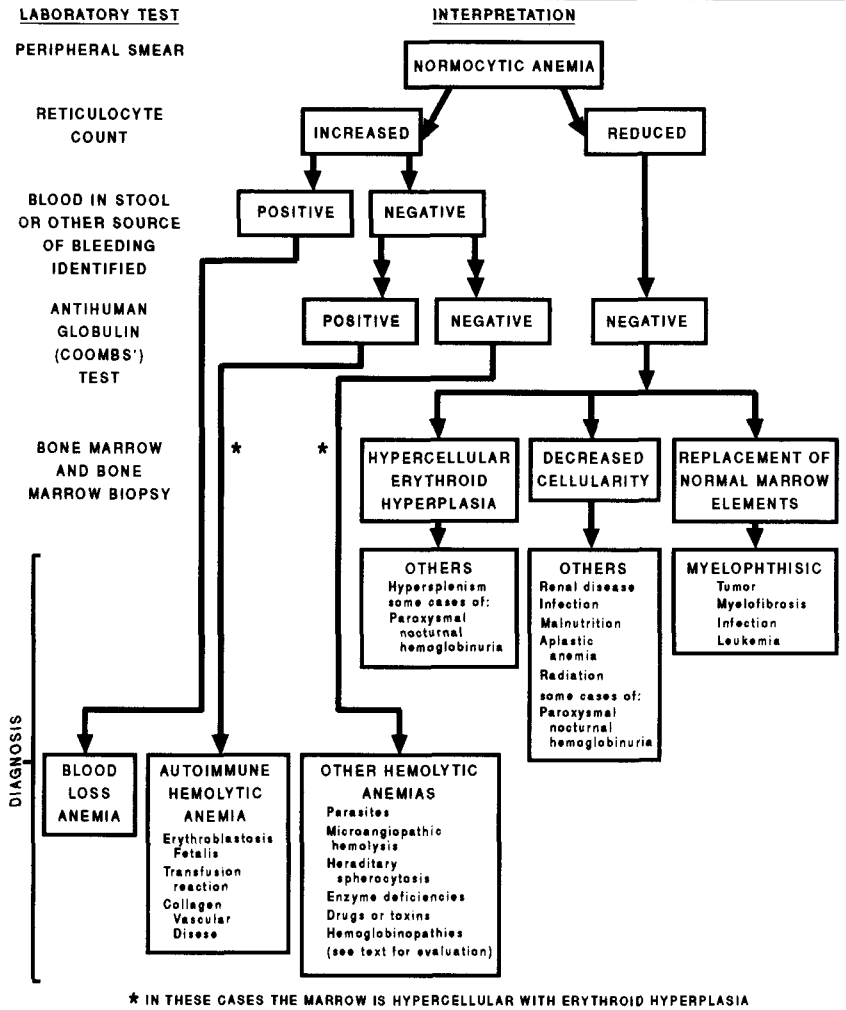


Figure 6 - Scheme for investigation of patients with normocytic anemia. This figure represents a convenient approach to the diagnosis of this form of anemia. It is intended only as a guide and might require modification to suit local conditions or resources.

clinical data on the patient will give clues to the proper diagnosis; such data include, a family history of hemoglobinopathy or hereditary spherocytosis, a recent blood transfusion, malaria, splenectomy, or a known metastatic tumor.

Blood film

The peripheral blood film should be examined for the presence of "microspherocytes", that is red cells which appear small, dense, and spherical (photomicrographs p. 14). When these cells are present as the primary red cell abnormality, the laboratory worker should consider two main causes: 1) anemia due to immune hemolytic anemia, and 2) hereditary spherocytosis.

Table 8 - Common causes of hemolytic anemia

Intrinsic causes (congenital)	Extrinsic causes (acquired)
1. Hemoglobinopathies (homozygous or doubly heterozygous S, C, and D disease)	1. Autoimmune a. warm antibodies b. cold antibodies
2. Thalassemia	2. Drug (methyldopa, penicillin)
3. Enzyme deficiencies (e.g., G6PD deficiency)	3. Snake venoms
4. Hereditary spherocytosis	4. Parasitism (malaria)
5. Hereditary hemolytic ovalocytosis anemia (elliptocytosis)	5. Microangiopathic hemolytic anemia
6. Unstable hemoglobins	6. Hypersplenism
	7. Alloimmune bodies
	8. Chemical substances (industrial and domestic)
	9. Severe infections (sepsis)
	10. Burns

Microspherocytes also occur in hemoglobinopathies, especially Hb C disease, and in hypersplenism, but in these conditions they occur only in small numbers and are associated with other morphologic changes. In contrast to microcytic hypochromic red cells, these red cells are densely packed with hemoglobin, and the MCHC (p. 77) is increased. The MCV is usually normal, and the MCH is normal, whereas in hypochromic anemia the MCV, MCH, and MCHC are all reduced.

When hereditary spherocytosis is considered as a possible diagnosis, blood smears obtained from members of the patient's family should be examined for microspherocytes. The finding of spherocytes in family members would further support the diagnosis of hereditary spherocytosis.

If the spherocytes are found in association with other red cell abnormalities, other conditions must be considered. For instance, microspherocytes in the presence of numerous target cells are highly suggestive of hemoglobin C disease, and hemoglobin electrophoresis should be performed. If, on the other hand, there are fragmented red cells (burr cells, schistocytes, and helmet cells), the cause is most likely microangiopathic hemolytic anemia (Table 9), Clostridium sepsis, or possibly splenectomy.

There are other causes of hemolytic anemia in which spherocytes are not a feature. Examples include glucose-6-phosphate dehydrogenase (G6PD)

Table 9 - Conditions associated with fragmented red cell hemolytic anemia

1. Microangiopathic hemolytic anemia
   a. malignant hypertension
   b. eclampsia and pre-eclampsia
   c. severe burns
   d. diffuse metastatic tumor
   e. severe renal disease
   f. thrombotic thrombocytopenia purpura
   g. hemolytic uremic syndrome

2. Mechanical hemolytic anemia
   a. severe aortic valvular disease
   b. march hemoglobinemia

deficiency, hemoglobinopathies other than HbC and thalassemia, unstable hemoglobins, chemical damage, drugs, snake bites, and malaria. To identify these, one should consider the G6PD test, hemoglobin electrophoresis, the sucrose lysis test, stains for inclusion bodies, and a blood smear search for parasites. Bone marrow examination is not very helpful in differentiating between these abnormalities, because it shows predominantly the same findings in all, i.e., a marked increase in erythroid cellularity and a reduction in fat. In this group of conditions, the antiglobulin test (see below) is negative.

Antihuman globulin test (Coombs' test)

This test identifies immune hemolytic anemia and should be performed when hemolysis is suspected (p. 92). Drug-induced hemolysis may also be associated with a positive antiglobulin test. If the test is negative in the presence of spherocytes, the laboratory worker should consider hereditary spherocytosis as a possible cause of the hemolysis.

The abnormal hemoglobins

Hb S and Hb C are the most prevalent of the abnormal hemoglobins, and produce the most severe disease in the homozygote. They are therefore the most important in some developing countries. The frequency of the Hb S gene is generally very high in Africa south of the Sahara (5% to 20%), and it is found in other areas, for example, Cyprus, Greece, parts of India, Central America, the Caribbean, and the Middle East. Hb C is very common in West Africa, especially west of the Niger River.

The increased hemolysis in patients with Hb C disease or sickle cell disease is due to the increased rigidity of the red cell caused by the tendency of Hb C

and of deoxygenated Hb S to crystallize. In sickle cell disease vascular occlusion also occurs due to the aggregation of the rigid (irreversibly sickled) cell.

The abnormal hemoglobins are generally produced by a single gene defect that causes an amino acid substitution in the globin part of the hemoglobin molecule. When this substitution occurs at a critical site, that is, at those sites that interact with the heme ring or other hemoglobin molecules in the cell, clinical disease develops. These substitutions provide the basis for a diagnostic test, because each amino acid carries a slightly different total electrical charge. Therefore when one amino acid is substituted for another in the hemoglobin molecule, it migrates differently in an electrical field (Hb electrophoresis) (p. 105).

The best screening method is hemoglobin electrophoresis on cellulose acetate because this provides the best separation of many hemoglobins, including Hb A_2, which can be eluted and quantified. When patients are suspected of having a hemoglobinopathy, the sickling test may also be a useful screening test. Further studies, such as family studies, may be needed to elucidate genetic combinations of different hemoglobinopathies.

Normocytic anemia associated with a low reticulocyte count

In this category, anemia is due to reduced proliferation of erythroid cells. This may be caused by a number of different disorders (Table 10), which are usually secondary to systemic disease. In practice, most patients with this type of anemia have infection, chronic renal disease, other chronic diseases, drug or chemical toxicity, or malnutrition. Sometimes hypoproliferative anemia may coexist with another cause, making recognition difficult. The laboratory worker will often find the patient's clinical symptoms more useful than most of the hematologic tests in making a diagnosis of the anemia.

Blood film

Anemia caused by acute or chronic infection is sometimes suggested by the leukocyte morphology. For instance, the white blood cells may be elevated with an increase in the proportion of polymorphonuclear leukocytes (PMNs). There may be an orderly deviation to the younger forms, with many band cells and, occasionally, myelocytes; the mature PMNs may show the classic changes of infection, that is, toxic granulation, Döhle bodies, or, in severe cases of septicemia, vacuoles in the cytoplasm (photomicrographs p. 15). The red cells may be hypochromic, reflecting impaired hemoglobin synthesis. In order to differentiate mild iron deficiency from chronic infection, it is usually necessary to measure serum iron or ferritin or to demonstrate the status of iron stores in the bone marrow by special staining.

Films from patients with myelophthisic anemia (anemia produced by an invasion of the bone marrow with tumor cells or an increase in fibrotic tissue, e.g., myelofibrosis or tuberculous infection) will show a variety of red cell and

Table 10 - Normocytic anemia associated with low reticulocyte count

1. Renal failure
2. Infections, inflammation, malignancy
3. Protein malnutrition
4. Aplastic anemia
5. Marrow replacement
   a. tumor
   b. leukemia
   c. fibrosis
   d. infection (i.e., chronic granulomatous infection)
6. Toxins
7. Endocrine disorders (e.g., thyroid disease)
8. Ineffective erythropoiesis (dyserythropoiesis)

leukocyte changes. The red cells show moderate to marked variation in size (anisocytosis) and shape (poikilocytes), and teardrop cells are often seen. Nucleated red cells are frequently present in higher numbers than would be expected from the number of reticulocytes. The leukocyte count may be normal or elevated, and many of the earlier forms of the granulocyte series, including blasts, may be present. In these situations, bone marrow biopsy is indicated and is frequently diagnostic (see p. 34 and Table 11).The blood morphology is less helpful in the other conditions.

Hematologic findings in AIDS patients

There are many hematologic abnormalities associated with HIV-I infection culminating in AIDS. In the latent period, which ranges from months to years, there are no specific features, but thereafter lymphoid function deteriorates with resulting opportunistic infections and neoplasms. In early stages of the active disease the blood shows lymphopenia with or without reactive lymphocytes, and non-specific red cell changes. Neutropenia can sometimes follow. Thrombocytopenia occurs from production of abnormal antibodies. The bone marrow is often initially hypercellular with increased plasma cells and lymphocytes. Bizarre megakaryocytes are sometimes seen, as are dyspoietic normoblasts. Increased storage iron, characteristic of chronic inflammation, can occur. Evidence for secondary malignancies (Kaposi's sarcoma, B-cell lymphoma) and a wide range of secondary opportunistic infections (viral, bacterial, fungal, protozoal) can often be found.

Bone marrow

Bone marrow aspiration may be useful in some of these diseases, especially those associated with leukemia, myelophthisic anemia, aplastic anemia, myelofibrosis, or generalized granulomatous infection. If one fails to get marrow on the initial aspiration, if the material is scanty (suggesting hypocellularity), or if the information provided is inadequate, a needle biopsy should be considered (Table 11). This is, however, a more specialized investigation; processing of the material and interpretation usually require referral to a central laboratory.

Table 11 - Indications for bone marrow biopsy

1. Suspected aplastic anemia	5. Suspected miliary granulomatous
2. Suspected myelofibrosis infiltration	6. Suspected malignant lymphoma
3. Suspected aleukemic leukemia	7. Suspected myelodysplasia
4. Suspected metastatic tumor	