Laboratory Diagnosis of Iron Deficiency

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INTRODUCTION

Until about 20 years ago, the diagnosis of iron deficiency was justifiably considered a simple matter. The focus of attention was then on hospitalized patients with a severe or moderate degree of anemia. When iron deficiency was suspected, the diagnosis could be substantiated by a decrease in serum iron, an elevation in the total iron-binding capacity (TIBC), and the typical changes of microcytosis, anisocytosis, and hypochromia on the blood smear. After the initiation of iron treatment, a rise in the reticulocyte count after 1 to 2 weeks and a slower, more gradual correction of the hemoglobin or hematocrit after about 2 months would confirm the diagnosis.

During the last 20 years, however, attention has shifted to the more common, milder cases of iron deficiency that are typically seen in an outpatient setting. Mild cases, in which the concentration of hemoglobin may be no more than 1 g/dl below the reference range, have proven to be an unexpectedly difficult diagnostic challenge. This is partly because textbook recommendations for diagnosis are often based on severe iron deficiency anemia and cannot be successfully extrapolated to the mild cases. The two types of patients require different diagnostic approaches.

In mild iron deficiency, the initial laboratory tests are less reliable in predicting a hemoglobin response than with severe iron deficiency because there is a substantial overlapping of results between iron-deficient and iron-sufficient populations (1–3). In contrast to severe iron deficiency, the blood smear cannot be distinguished from that of a normal individual (4). Furthermore, after treatment is initiated, the reticulocyte count does not usually rise sufficiently to allow a response to be detected.

In partial compensation for these inherent difficulties in diagnosing mild iron deficiency, there have been many technical improvements in established laboratory tests and a broader application of additional laboratory tests (5,6). Laboratory tests that have come into widespread use include the mean corpuscular volume (MCV), erythrocyte protoporphyrin (EP), and serum ferritin. Progress has also been made in automating and standardizing each of these laboratory tests as well as serum iron, TIBC, and hemoglobin analysis. The availability of more reproducible methods has led to increasingly reliable normative data for age and sex which are based on
healthy populations and which facilitate the interpretation of laboratory analyses, particularly in children (7).

Mild iron deficiency is very common, but it is not life threatening or even overtly symptomatic. Furthermore, in contrast to the adult man or elderly woman, iron deficiency in infants and children is usually of nutritional origin (7) and is rarely indicative of a serious occult disease, such as a peptic ulcer or intestinal carcinoma. It is therefore difficult to justify any routine for laboratory diagnosis unless it is safe, inexpensive, and simple for both the patient and the health worker. This means making a choice from among the many available laboratory tests, because using all of them is not a practical or realistic option. Fortunately, it is possible to select at least one regimen from among several simple combinations of laboratory tests that suit each clinical setting. In many situations, a therapeutic trial will be the most appropriate means of establishing a diagnosis.

In this review, we shall discuss both the therapeutic trial and the clinical application of the laboratory tests for mild and severe iron deficiency. A detailed discussion of the analytic procedures appeared in two recent reviews (5,6). Factors that influence the selection of laboratory tests include their local cost and availability, the technical difficulty of the analysis, the prevalence of iron deficiency, and the type of blood sample that can be obtained. However, it is necessary to emphasize at the outset that diagnostic routines are likely to change if the rapid pace of technical advances continues.

Groups That Are Most Likely to Develop Iron Deficiency

Because the clinical manifestations of iron deficiency are rarely apparent, the diagnosis is usually suspected on the basis of laboratory tests done at the time of a routine examination. Groups in whom laboratory screening for iron deficiency has the highest yield include infants, children, adolescents, and women between menarche and menopause (5,7,8).

Among infants, the prevalence of iron deficiency is highest between about 6 months (when neonatal iron stores become depleted) and 3 years of age. In the first few months after birth, the rapid rate of growth with the corresponding increase in red cell mass is the most important factor in the development of iron deficiency. Premature infants and twins who do not receive supplemental iron or iron-fortified formula are at particular risk because their neonatal iron stores are smaller and weight gain proportionately greater than in term infants; the former two groups may develop iron deficiency anemia as early as 2 or 3 months after birth (9). In term infants, whose postnatal weight gain has been greater than average, iron deficiency is also apt to develop by about 6 months because of the increased iron requirements imposed by rapid growth.

After neonatal iron stores have been mobilized for hemoglobin production, dietary factors gradually assume an increasingly important role. Infants whose diet consists of formulas without added iron or who are given whole cow's milk at an early age may develop iron deficiency by 6 months of age (10). Milk not only
contains very little iron, but can actually decrease the absorption of this mineral from other foods eaten in the same meal (see Cook and Bothwell, this volume). Furthermore, some infants who consume more than a liter of cow's milk a day develop occult intestinal blood loss, occasionally leading to severe iron deficiency anemia (11,12). The introduction of a weaning food that is based on cereal or legumes may predispose to iron deficiency (13). Although such foods may contain substantial amounts of iron, the percentage absorbed may be low (Bothwell and Cook, this volume) unless the diet also contains meat and/or ascorbic-acid-rich foods that enhance iron assimilation.

Between about 3 years of age and adolescence, iron deficiency in industrialized countries becomes far less common because the rate of body growth decreases while the diet becomes more diversified. However, in developing countries where hookworm infestation is common, the prevalence and severity of iron deficiency may be almost as high in this age group as in infants. Other parasitic infestations that are associated with blood loss, such as schistosomiasis, may also predispose to iron deficiency.

Adolescents constitute another high-risk group, but relatively little epidemiologic information is available about their iron status. Boys in industrialized countries gain an average of 10 kg/year at the peak of their growth spurt (14). At about the same age as the growth spurt, and concurrent with sexual maturation, the concentration of hemoglobin increases between 0.5 and 1.0 g/dl/year toward values that are characteristic of men (7). These changes require an increase of about 25% in total body iron during the year of peak growth. The iron needs of adolescent girls are similarly large but are more evenly spread out over several years. The average weight gain at the peak of the growth spurt—9 kg/year—is almost as great as in boys (14); however, the concentration of hemoglobin changes very little during this time (7). The onset of menses, which usually occurs well after the adolescent growth spurt, requires additional absorption of iron to balance the menstrual losses of iron. The caloric intake of females drops substantially below that of males in adolescence. Because the iron/1,000 kcal is similar for males and females, consumption of iron by females is substantially below that of males. One of the few detailed studies of adolescents dealt with menstruating high school girls in Helsinki, Finland that were randomly divided into a group receiving iron tablets and another group that was given a placebo (15). After 2 months, a significant response in hemoglobin had occurred in about 75% of the iron-treated group. This represented a surprisingly high prevalence of iron deficiency for a relatively affluent population.

In women, the last half of pregnancy is associated with the highest prevalence of iron deficiency (8). One can anticipate that the risk would be even greater among pregnant teenagers, since even at the beginning of pregnancy their iron stores are likely to be depleted by a recent growth spurt.

**Stages of Iron Deficiency**

When a person progresses from adequate iron balance to overt iron deficiency anemia, as in experimental protocols involving repeated phlebotomy, the deficiency...
may be considered to develop in a sequence of three stages (5). The first stage consists of a depletion of storage iron. This stage is characterized by a decrease in the concentration of serum ferritin, which reflects the declining concentration of iron stores in the liver, spleen, and bone marrow. An alternative to the analysis of serum ferritin is to obtain a bone marrow aspirate and stain it for iron to make a qualitative estimate of the amount present. Because it is much simpler to obtain a blood specimen, the analysis of serum ferritin is supplanting bone marrow aspiration for estimating the amount of storage iron.

A second stage of iron deficiency that is likely to be transient consists of a decrease in transport iron. This is characterized by a declining concentration of serum iron and an increase in the iron-binding capacity. These changes result in a decrease in the transferrin saturation, which is calculated from the ratio of the serum iron to the TIBC. The term "latent iron deficiency" is sometimes used to refer to these first two, preanemic stages of iron deficiency.

A third stage develops when the supply of transport iron decreases sufficiently to restrict hemoglobin production. This stage is characterized by an elevation of erythrocyte protoporphyrin and the gradual development of detectable anemia and microcytosis. An alternative definition of the third stage is in terms of a hemoglobin concentration that has decreased sufficiently to fulfill the laboratory definition of anemia. Iron therapy is usually directed at the correction or prevention of the anemia that characterizes the third stage of iron deficiency.

Iron Deficiency as a Steady State Condition

Although it is conceptually convenient to classify laboratory tests according to stages of iron deficiency, laboratory results do not consistently conform to this pattern among individual patients with mild iron deficiency (2,3). This is probably because the mild deficiency has developed very gradually, and is almost in equilibrium, due to conditions that have been present for many months and often for years. Under such circumstances of a mild iron deficiency that is virtually in steady state, the analytic and biologic variability of the laboratory tests may be relatively large in relation to the degree of deviation from normal. Thus, individual patients often prove to have an iron-responsive anemia (third stage) despite having a normal value for serum ferritin or transferrin saturation (3).

SCREENING TESTS

The laboratory tests that are used in the diagnosis of iron deficiency can be conveniently grouped into screening tests and confirmatory tests. Screening tests are most commonly used in the initial evaluation of those populations who are most likely to have iron deficiency. The usual goal is to identify persons with anemia whose hemoglobin concentration is likely to increase with administration of iron, an improved diet, or a combination of both.

Among term infants, laboratory testing to detect iron deficiency is commonly recommended at about 1 year of age; the corresponding age for preterm infants is
between 6 and 9 months (16). In many populations, these ages are characterized by the highest prevalence of iron deficiency. Other suitable ages for the laboratory detection of anemia are between 2 and 3 years, at about 5 years, and in adolescence. The most appropriate times will differ according to the population. At all ages, iron deficiency is far more common among lower socioeconomic groups than in affluent populations.

**Hemoglobin and Hematocrit**

The hemoglobin and hematocrit (5,6) are the most widely used tests to screen for anemia and iron deficiency. The concentration of hemoglobin is most reliably measured after accurate dilution of the blood specimen in a solution that converts hemoglobin to cyanmethemoglobin, which is then quantitated spectrophotometrically. The analysis is done either with a simple spectrophotometer or as part of a complete blood count by a more elaborate electronic counter in a centralized laboratory. Blood counts obtained by electronic counter usually include red cell indices, which provide valuable additional information for the differential diagnosis of anemia, as will be discussed below. The electronic counter will also provide a calculated hematocrit; however, this determination is not considered as reliable a means of diagnosing anemia as is the hemoglobin concentration.

In office and clinic laboratories, the hematocrit is often measured by centrifugation of a minute amount of blood that has been collected in a heparinized capillary tube. The hematocrit is then calculated by comparing the height of the column of packed red cells with the height of the entire column of red cells and plasma. The total volume of blood in the capillary tube is therefore not critical. An advantage of this method is its technical simplicity, particularly when applied to skin puncture blood specimens. On the average, the hematocrit is equivalent to the hemoglobin concentration multiplied by 2.9.

It is essential to interpret either the hemoglobin or hematocrit determination in relation to age-specific and sex-specific reference standards (7). Table 1 shows reference values for hemoglobin concentration after 6 months of age; laboratory testing for iron deficiency is rarely done before this age. The reference values from 6 months to 5 years of age are based on the 95% range in venous blood from white, nonindigent populations living at sea level and exclude those who have other laboratory evidence of iron deficiency or thalassemia minor. Between 6 months and 5 years, the lower limit of the 95% range for hemoglobin is 11.0 g/dl. Subsequently there is a gradual rise in hemoglobin values that continues throughout childhood. The use of developmental curves for hemoglobin (Fig. 1) during this period decreases the errors that are inherent in the abrupt stepwise increases of 0.5 to 1.0 g/dl from one age range to the next in the tabulated values. At puberty there is a further increase in concentration of hemoglobin in boys, and during adult life the hemoglobin concentration in men is maintained at an average of about 2.0 g/dl higher than in women.
TABLE 1. Estimated mean and lower limits of normal (95% range) for
hemoglobin, hematocrit, and mean corpuscular volume in Caucasians*

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Hemoglobin (g/dl)</th>
<th>Hematocrit (%)</th>
<th>Mean corpuscular volume (fl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Lower limit</td>
<td>Mean</td>
</tr>
<tr>
<td>0.5–2</td>
<td>12.5</td>
<td>11.0</td>
<td>36</td>
</tr>
<tr>
<td>3–5</td>
<td>12.5</td>
<td>11.0</td>
<td>36</td>
</tr>
<tr>
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<td>11.0</td>
<td>38</td>
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<td>11.5</td>
<td>39</td>
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<tr>
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<tr>
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</table>

*For ages 2 and above, data for hemoglobin and hematocrit are in accord with the
preliminary results of the United States Health and Nutrition Examination Survey II, 1976–
80 (N-HANES II). Data for ages 0.5 through 2 years and MCV values are from Dallman
et al. (7) and are in accord with the developmental curves shown on Figs. 1 and 2. The
lower limit of hemoglobin values from N-HANES II are somewhat lower than the corre-
sponding portions of the percentile curves between ages 6 and 11 years. The hemoglobin
data were provided through the courtesy of Clifford Johnson of the National Center for
Health Statistics.

The values in Table 1 for children and adults over the age of 3 years were derived
from the United States Health and Nutrition Examination Survey, 1976–80 (N-
HANES II) (manuscript in preparation). These values are from a randomly selected
population that excludes military personnel and institutionalized individuals. The
total of 27,801 subjects were selected according to census data. For purposes of
the tabulation, only values based on venipuncture in nonpregnant white individuals
were utilized and all subjects with MCV < 80 fl, Fe/TIBC < 16%, or EP greater
than 75 µg/dl packed red blood cells were excluded.

The laboratory definition of anemia is usually based on a hemoglobin value that
is below the 95% reference range for age and sex. This definition is used in most
clinical settings: An alternative operational definition of anemia is based on a rise
in hemoglobin (such as 1.0 g/dl or more) or hematocrit during a therapeutic trial
with iron. A hemoglobin response, despite an initial value within the normal 95% range,
is common in populations that have a high prevalence of iron deficiency and
indicates that the production of hemoglobin was restricted by a lack of iron. Of
course, anemia by this operational definition can only be identified retrospectively,
after a therapeutic trial. When serial hemoglobin values are available in the same
individual, a decline in concentration of 1 g/dl or more may represent anemia
because values normally remain relatively constant.
Hemoglobin, Percentiles

FIG. 1. Hemoglobin concentration in infants and children. The percentile curves were derived from nonindigent white children living at sea level or after exclusion of subjects with laboratory evidence of iron deficiency, thalassemia minor, and/or hemoglobinopathy. (From Dallman et al., ref 7.)

Anemia in Blacks

Numerous surveys have shown that hemoglobin concentrations in blacks are 0.3 to 1.0 g/dl lower than in white or other races. Although socioeconomic status and higher prevalence of iron deficiency may play a role, these factors cannot entirely
explain the difference. In the United States, iron deficiency anemia appears to be more prevalent among black infants, children, and women during the childbearing years than among whites. The results of one study in infants indicated that the effect of a slight but significant inherent tendency to lower hemoglobin values in blacks was counterbalanced by a substantially higher prevalence of iron deficiency anemia (17). From the results of a therapeutic trial with iron, the use of uniform hemoglobin screening criteria seemed justified in this population. However, it may not be possible to extrapolate this conclusion to populations of blacks that are at lower risk of iron deficiency, such as black adult males in the United States.

**Mean Corpuscular Volume**

Electronic counters have made the MCV an accurate and practical laboratory test. The manual determination of the MCV was a time-consuming and poorly reproducible procedure because it was derived from the ratio of the hematocrit to a red blood cell count that was obtained microscopically with a counting chamber. The electronic determination of red cell volume is highly reproducible and is actually less subject to sampling error in skin puncture blood than the hemoglobin determination because dilution by tissue fluid does not affect red cell size. When a blood count is obtained by electronic counter, it is important to give full attention to the result of the MCV because this provides valuable help in the differential diagnosis. A low MCV with anemia favors the diagnosis of iron deficiency. Small red blood cells are also characteristic of thalassemia minor (8) and may sometimes be found with the anemia of infection and chronic disease. However, most other anemias are characterized by a normal or elevated MCV.

As in the case of hemoglobin and hematocrit determinations, the MCV normally changes during development, making it important to refer to age-specific reference standards (7) (Table 1) (Fig. 2). Red cells are normally larger at birth than in adulthood, but red cell size decreases rapidly during the first 6 months of life. Cells are smallest during the remainder of infancy and gradually increase in size during childhood. There is little difference in red cell volume between sexes, and values increase only to a minor degree in adult life.

Other red cell indices obtained by electronic counter include mean corpuscular hemoglobin (MCH), which is derived by dividing the hemoglobin concentration by the red cell number and which undergoes similar changes in iron deficiency as the MCV, and the mean corpuscular hemoglobin concentration (MCHC), which is the least directly measured and least useful of the indices obtained by electronic counter. Because the MCHC can be calculated by dividing hemoglobin by hematocrit, it is the only red cell index that is readily obtained without electronic counters. However, it is the last of the indices to become abnormal during the progression of iron deficiency (9).

**Abnormal Findings in Screening Tests**

When the hemoglobin concentration (or the hematocrit) is below the lower limit of normal, the diagnosis of an iron-responsive anemia should be considered. If
FIG. 2. Mean corpuscular volume (MCV) in infants and children. The percentile curves were derived from a portion of the same population as in Fig. 1. (From Dallman et al., ref. 7.)

blood specimens have been analyzed by an electronic counter, the presence of a low or low-normal MCV in conjunction with anemia increases the likelihood that the anemia is due to iron deficiency (20). If the anemia is mild (within 1 g/dl of the reference range), and iron deficiency is suspected but not yet confirmed, there are two choices: either additional laboratory tests should be obtained or, alternatively, a therapeutic trial with iron may be initiated. Our own practice is not to proceed with additional laboratory tests on the basis of a borderline hemoglobin value on skin puncture blood. We would first confirm the result on venous blood
because of the relatively poor reproducibility of hemoglobin values obtained from skin puncture blood (see section entitled Skin Puncture and Venous Blood Sampling).

**Relationship Between Prevalence and Diagnostic Criteria**

Knowing the approximate prevalence of iron deficiency among the group of patients being screened will influence the selection of a cutoff value below which iron deficiency should be suspected. The importance of taking prevalence into account is familiar to epidemiologists, and it would scarcely warrant discussion here if it were not for the fact that prevalence is rarely considered in the clinical interpretation of laboratory tests. The mathematical methods for dealing with prevalence have been recently reviewed (21). A screening limit for anemia that is set too low will result in a high percentage of individuals with iron deficiency anemia as demonstrated by a high rate of response to a therapeutic trial. This superficially satisfactory state of affairs will provide no indication that a very large percentage of individuals with iron-responsive anemia is being missed by being classified as screen-negative. Conversely, when the screening limit is set too high, the proportion of individuals with iron deficiency anemia as demonstrated by response to treatment becomes unacceptably low because too many normal individuals are considered screen-positive.

For the purpose of this discussion, the two hypothetical examples in Fig. 3 may be helpful. The left portion of the figure represents a population of infants in whom about 15% would have a significant hemoglobin response if they were treated with iron. This responsive group is represented by the gray area under the dashed curve. The nonresponsive 85% of the population is represented by the larger open area.

![Figure 3](image_url)

**FIG. 3.** The role of prevalence in the selection of screening criteria. The left side of the figure shows the frequency distribution of hemoglobin in a hypothetical population of infants in which 15% would have a hemoglobin response to iron treatment (represented by the gray area under the dashed curve) and 85% would not respond (the open area under the solid curve). The vertical line at 11.0 g/dl indicates the lower limit of the 95% range of normal. The right side of the figure illustrates a population in which only 2% would have a hemoglobin response to therapy. In the population with a high prevalence of iron deficiency shown on the left, the use of a screening criterion higher than the usual limit of 11.0 g/dl would be justified.
under the solid curve. These conditions are analogous to those described in a later section entitled Diagnosis of Iron Deficiency in Healthy Infants in the United States, in which only about half of those subjects with a hemoglobin response of at least 1 g/dl would have been detected by the traditional approach of treating only those infants with a hemoglobin value below the lower limit of the 95% range of normal (shown by a vertical line) (22). Consequently, it could be argued that raising the hemoglobin criteria for further laboratory evaluation or for treatment by about 0.5 g/dl would be worthwhile in screening this population for iron deficiency anemia. However, this degree of relaxation of the treatment criteria would be totally inappropriate for a population with a 2% prevalence of iron deficiency. This population might correspond to infants who have received an adequate supply of dietary iron. In this group, the use of the traditional 95% reference range would be more appropriate. If a higher hemoglobin cutoff value were used, too many normal infants would be considered screen-positive for every one that is iron-deficient. Thus, laboratory cutoff values used in screening have to be adjusted to suit local conditions. In the case of hemoglobin, the usual convention of using the lower limit of the 95% range will be appropriate where iron-responsive anemia is relatively rare, but a somewhat higher value can be justified when iron deficiency is common.

**DIAGNOSIS BY THERAPEUTIC TRIAL**

Dietary iron deficiency is by far the most common cause of anemia in otherwise healthy infants and children. A relatively brief therapeutic trial of iron for about 1 month is therefore often justified on the basis of anemia alone. An iron dose of 3 mg/kg/day as ferrous sulfate is well tolerated by infants as a single before-breakfast dose (22). This is equivalent to about 30 mg/day in a representative 1-year-old infant. In older children, the 3 mg/kg/day may be divided into two or three doses. The medication is far better absorbed between or before meals than with meals (23). In the relatively rare case of gastrointestinal intolerance, however, symptoms may resolve when the dose is given with meals. It should be emphasized that a therapeutic trial should rarely continue for more than 1 month.

In an otherwise healthy individual, the recovery from anemia is about two-thirds complete after 1 month (24). If the concentration of hemoglobin has increased significantly during the month, as will be the case with most anemic subjects, an expensive workup will have been avoided. Medication can then be continued for 3 to 5 months to replenish iron reserves. More prolonged treatment will provide no additional benefit and might lead to iron overload in individuals with thalassemia.

If there has been no hemoglobin response after 1 month, the decision on how to proceed will depend on the posttreatment hemoglobin value in relation to the 95% reference range for age and sex. If anemia persists, a more extensive diagnostic workup is probably indicated, especially if the value remains more than 0.5 g/dl below the reference range. The 1-month delay in diagnosis in otherwise healthy infants and children with mild anemia is unlikely to be harmful and is outweighed by the substantial initial savings in additional laboratory tests.
LABORATORY DIAGNOSIS

If a borderline anemia persists after a therapeutic trial (a value within 0.5 g/dl of the reference range), there is a possibility that the individual's hemoglobin is normally in the lowest part of the frequency distribution curve. On the other hand, there may be a condition, such as thalassemia minor or hereditary spherocytosis, which would be detected by further laboratory tests. Decisions on how to proceed in this situation are difficult and will be swayed by the characteristics of the population, the laboratory resources that are available, and the history of the individual child. Whatever the specific situation, it is important to be alert to causes of anemia other than iron deficiency while also avoiding the extreme of an expensive and elaborate workup for every child with a borderline anemia.

If the posttreatment hemoglobin value is within the low-normal range, it is probable that the individual did not have an iron-responsive anemia and that no further treatment or diagnostic workup is indicated.

Regression to the mean is an important statistical phenomenon that affects the interpretation of the results of a therapeutic trial (21), particularly if it has been based on a hemoglobin value from skin puncture blood. If an individual is singled out on the basis of a low laboratory value, simply repeating that laboratory test is likely to yield a result that is closer to the normal mean for that test. This so-called regression to the mean is due to a composite of random factors, such as sampling and laboratory errors and biologic variations, that could have been partly responsible for the initial outlying value. Thus a therapeutic trial might seem successful when it actually resulted from random events. In the case of a venous hemoglobin, both biologic variations and analytic variations are relatively low with coefficients of variation under 3%. However, sampling error is very large when skin puncture blood is used (see section entitled Skin Puncture and Venous Blood Sampling).

The alternative to a therapeutic trial is a more extensive initial laboratory workup to strengthen the presumptive diagnosis of iron deficiency. The confirmatory tests that are most commonly used are the EP, serum ferritin, and serum iron/TIBC. Each of these tests has certain advantages and disadvantages to be discussed below.

CONFIRMATORY TESTS FOR IRON DEFICIENCY

Serum Ferritin

Ferritin is normally present in the serum but in such small quantities that it was undetectable prior to the recent development of a radioimmunoassay. Under most circumstances, the concentration of serum ferritin is roughly proportional to the abundance of storage iron (25). Thus, the serum ferritin is the only blood determination that is helpful in allowing an evaluation of iron status within the normal range, as well as in the diagnosis of iron deficiency. For example, the developmental changes in serum ferritin levels reflect normal changes in iron stores with increasing age (7).

High values in newborn infants reflect the abundant iron stores that exist at birth. Values fall rapidly during early infancy and remain low throughout later infancy and childhood. The serum ferritin remains low in women of childbearing age but rises after menopause. In men, a rise in serum ferritin occurs after adolescence.
and continues at a more gradual rate throughout adult life. At all ages, a serum ferritin value of <10 or 12 μg/liter (or ng/ml) indicates depletion of iron stores.

A low concentration of serum ferritin is indicative of depleted iron stores. However, values may be in the normal range despite the presence of iron deficiency, particularly in association with infection or inflammatory disease. Even mild upper-respiratory infections are associated with an elevation in serum ferritin (26). With severe infections, the serum ferritin elevation often persists for several weeks beyond the symptomatic period (7). It is possible that enteritis and parasitic infestation, which are common in many parts of the developing world, may involve sufficient inflammation to modify serum ferritin values. Liver disease, even if it is mild, can result in major elevations of serum ferritin (28). Consequently, the serum ferritin is of little use in diagnosing iron deficiency in patients with suspected or proven liver disease.

The serum ferritin determination may be useful in diagnosing iron deficiency anemia in the presence of inflammatory diseases that do not affect the liver if a lower limit of 25 or 50 μg/liter is used. For example, anemia in patients with rheumatoid arthritis cannot be assumed to be due to their chronic disease unless iron deficiency has been excluded as a contributing factor (29). Iron deficiency anemia due to blood loss is common among patients with rheumatoid arthritis who are chronic users of aspirin. A serum ferritin value below 25 μg/liter in such persons makes it very likely that there will be a hemoglobin response to iron therapy. However, a higher value is less helpful because it does not exclude the possibility of response.

In patients with chronic renal disease who are on hemodialysis regimens, the blood loss that occurs at the time of each dialysis makes the eventual development of iron deficiency virtually inevitable. In such patients, serum ferritin determinations obtained at approximately 3-month intervals will show a gradual decline in values. When the serum ferritin concentration falls below about 50 μg/liter, the initiation of iron prophylaxis is appropriate. However, when values are higher than 100 μg/liter, iron treatment is unnecessary.

**Serum Iron and Iron-Binding Capacity**

Almost all of the iron in the serum is bound to the iron-binding protein, transferrin (5,6). Serum iron and TIBC are generally measured by spectrophotometric techniques. The assay, when done manually, is time consuming and subject to errors due to contamination by iron from the environment. Automated techniques make it possible not only to obtain results more rapidly but to achieve greater reproducibility. A disadvantage of the serum iron is its large biologic variability compared to the other laboratory tests. One component of this variability is a pronounced diurnal fluctuation, after about 3 years of age (30), usually with high values in the morning and low values at night (31). It is therefore easiest to interpret results from blood specimens drawn in the morning or early afternoon because a low value (less than 30 μg/dl or 5.4 μmoles/liter) is most likely to represent iron deficiency at this time of day.

The TIBC is less subject to biologic variations than the serum iron, but its analytic error is greater than that of serum iron. The normal range for the TIBC
is 250 to 400 μg/dl (or 45–72 μmoles/liter). Because the serum iron decreases whereas the TIBC is likely to increase in iron deficiency, the transferrin saturation, which is the ratio of the two values, is more consistently helpful than either value alone. The transferrin saturation is calculated by dividing the concentration of serum iron by the TIBC and multiplying by 100 to express the results as a percentage. Thus, transferrin saturation will reflect the biologic variability and laboratory errors of both the serum iron and TIBC. In adults, a transferrin saturation below 16% is considered indicative of iron deficiency (5). In infants and children, the corresponding value is about 10% (32,33).

Transferrin saturation may decrease in inflammatory disease (34) as well as in iron deficiency. In some instances the TIBC is useful in distinguishing between the two conditions. A TIBC of more than 400 μg/dl strongly suggests iron deficiency, whereas a value below 200 μg/dl is characteristic of inflammatory disease. Unfortunately, the overlapping of laboratory values between these two conditions is considerable, and most values will be in the intermediate range between 200 and 400 μg/dl.

**Erythrocyte Protoporphyrin**

There is an accumulation of protoporphyrin in red blood cells when insufficient iron is available to combine with protoporphyrin to form heme. The recent interest in the diagnostic use of this test can be largely attributed to the availability of a simplified method for extracting EP from small samples of blood and measuring it fluorometrically (35). In addition, specialized fluorometers have been developed that provide a result directly from a thin film of whole blood (36). The use of such instruments requires little technician time or training and makes it practical to diagnose and initiate treatment for iron deficiency on a single clinic visit. The EP is elevated in both iron deficiency and lead poisoning and is therefore used to screen infants and young children in urban, low-income areas where both conditions are common. In such settings, an elevated EP value will warrant a follow-up analysis for blood lead before it can be attributed to possible iron deficiency. The EP is also increased in infection and inflammatory disease. An advantage of the EP is that it is unaffected by recent iron medication, in contradistinction to serum iron and, in the case of very high iron doses, serum ferritin (37).

There is a strong association between lead toxicity and iron deficiency that appears to be due to a shared mechanism for the intestinal absorption of lead and iron (38). In iron-deficient individuals, the homeostatic increase in absorption of iron is accompanied by a similar increase in absorption of ingested lead. Thus, iron deficiency appears to predispose to lead toxicity. Indeed, anemia in the presence of lead intoxication may be primarily or entirely attributable to iron deficiency.

Marked elevations in EP are most likely to be due to lead exposure, whereas moderate increases can be associated with iron deficiency, lead exposure, or both. The upper limit of normal for EP is considered to be about 3 μg/g Hgb (or 30
μg/dl whole blood or 100 μg/dl red cells). In mild iron deficiency, the EP is rarely more than two times higher than these upper limits. In infants, EP is normally somewhat higher than in adults, but normative developmental data remain to be established.

**SELECTION OF LABORATORY TESTS**

Factors that will influence the selection of laboratory tests include the type of blood sample obtained, the experience of laboratory personnel, the availability of equipment, the proximity of laboratory facilities, and the rapidity with which results can be provided. Another important consideration is the prevalence of conditions that may complicate the diagnosis, such as thalassemia minor, acute infections, chronic inflammatory disease, and nutritional deficiencies other than iron lack.

**Skin Puncture and Venous Blood Sampling**

It is technically much simpler to obtain skin puncture blood from a fingertip, particularly during infancy, but also throughout childhood. However, the use of skin puncture blood substantially decreases the diagnostic reliability of some laboratory tests. Probably the most important problem is the variability of skin puncture hemoglobin and hematocrit values. In the case of hemoglobin, sequential venous values in the same individual usually remain within 0.6 g/dl (39). In contrast, the discrepancy between skin puncture and venous values is often 0.5 to 1.0 g/dl (20). Such a sampling discrepancy that approaches 10% of the hemoglobin concentration is a serious problem when the degree of anemia in the population rarely involves more than a 10% depression below the normal range. The difficulties created by skin puncture sampling was recently highlighted for us by a study in which 1-year-old infants were considered potentially iron-responsive if their capillary hemoglobin value was less than 11.5 g/dl (20). When the determination in these 122 infants was repeated on venous blood within a 2-week period, the finding was confirmed in only 53%. We concluded that the diagnosis of mild anemia on the basis of a skin puncture sample is more tentative than with a venous sample.

In the case of other laboratory tests, the consequences of a sampling error approaching 10% would be less serious than with hemoglobin. This is particularly true of the MCV, since red cell size is unaffected if the sample is diluted by tissue fluid. In the case of serum ferritin and EP, skin puncture sampling appears to pose no major problems because only a few drops of blood are required, and the clinically significant deviations from the normal range usually exceed 10%. Serum iron and TIBC are rarely determined in capillary blood because of the larger volume of blood required.

To obtain the best possible skin puncture sample, it is important to warm the extremity in order to facilitate a free flow of blood and to avoid any squeezing of the finger so that contamination of blood with tissue fluid is minimized. Samples of blood can be obtained in larger volume from an antecubital vein and with no more pain than skin puncture samples from a fingertip, where there is a denser
network of nerve endings. However, venipuncture in infants and small children has
the distinct disadvantage of requiring a second person to immobilize the extremity.
Health workers with limited experience will also have more difficulty in placing a
needle into the vein, particularly in children between 6 months and 2 years of age
whose veins are likely to be obscured by subcutaneous fat. In this age group, the
external jugular vein may be the easiest site for venous blood sampling. However,
use of the external jugular may arouse more parental anxiety than venipuncture of
an antecubital vein and may therefore be impractical for screening purposes.

Time Required for Analysis

In the outpatient management of iron deficiency, the eventual goal is to establish
the diagnosis and to start treatment in a single, brief visit. This requires laboratory
tests that can be performed quickly and in close proximity to where the patient is
seen. In the past few years, technical improvements have made it possible to have
the result of any of the tests that have been discussed within a few hours. Of the
established and generally available methods, those that can be done most rapidly
and with the least expensive equipment include the hemoglobin, hematocrit, and
the EP. In addition, cheaper electronic counters for MCV and automated methods
for serum iron may make these assays more attractive in the future for use in small
clinic laboratories. Even the serum ferritin assay, which until recently was perhaps
the most expensive and time-consuming test, can be simplified to serve as a 90-
min screening test (40).

DIFFERENTIAL DIAGNOSIS

Problems of differential diagnosis fall into two categories. The simpler of the
two involves distinguishing among different conditions that may result in similar
laboratory findings, e.g., the anemia and microcytosis that characterize both iron
deficiency and thalassemia minor. The second and more difficult type of problem
is the detection of iron deficiency in the presence of another condition which may
confuse the interpretation of laboratory results, e.g., iron deficiency in the presence
of acute infection or chronic inflammatory disease. We will discuss both types of
situations with an emphasis on the most common problems in differential diagnosis.

Chronic Inflammatory Diseases

Chronic inflammation is commonly associated with a mild anemia that can either
mimic or be partially caused by iron deficiency (34). Factors that predispose to
the anemia include a decreased red blood cell survival and a decreased erythro-
poietic response to anemia. In addition, there are major changes in iron metabolism
that include a diminished reutilization of iron from senescent red cells and decreased
intestinal absorption of iron. Both of these changes result in a redistribution of iron
from hemoglobin to iron stores and help to account for a decline in serum iron.

In some instances, medications can aggravate the anemia of chronic inflammatory
disease. An example is aspirin treatment of polyarticular rheumatoid arthritis.
Chronic aspirin use often results in iron deficiency due to prolonged occult loss of blood from the intestine. However, the diagnosis of iron deficiency is likely to be obscured by the effects of chronic inflammation. Both iron deficiency and inflammation are often characterized by mild anemia, an elevated EP, and a low serum iron. The MCV is only occasionally decreased with inflammatory conditions.

The two laboratory tests that may sometimes, but not always, show divergent results in chronic inflammatory disease and iron deficiency are the TIBC and the serum ferritin. In chronic disease, the TIBC is often depressed, whereas in iron deficiency it is frequently elevated. Conversely, a depressed serum ferritin (below 10 to 12 µg/liter) is diagnostic of iron deficiency, whereas infection and chronic disease are associated with normal or elevated values. A serum ferritin below 25 µg/liter in anemic patients with rheumatoid arthritis (29), chronic renal disease, or other chronic disorder suggests coexisting iron deficiency anemia. A therapeutic trial of iron is likely to decrease the degree of anemia in such cases. However, some patients will also have a hemoglobin response to iron therapy despite a serum ferritin value of greater than 50 µg/liter. This seems to be in part due to impaired mobilization of iron stores with inflammatory disease but also due to a serum ferritin that is disproportionately elevated in relation to iron stores. Because of the often confusing pattern of laboratory results, a 1-month therapeutic trial of iron may prove to be more worthwhile than an extensive laboratory workup for mild anemia accompanying chronic disease.

**Acute Infection or Inflammation**

These conditions are common among infants and children and complicate the diagnosis of iron deficiency. Indeed, the patient is frequently brought to medical attention for the acute illness, and the anemia is merely an incidental finding. In some instances, the anemia proves to be related to the infection or inflammation, whereas in others there is a coexisting iron deficiency. One of us (J.D.R.) recently did a prospective study of 9 children who were hospitalized for an acute infection or inflammatory illness. During the period of active illness, which averaged 5 days, there was a mean decline in hemoglobin concentration of 1.7 g/dl. This was far in excess of what could be explained by changes in hydration or removal of blood for laboratory studies. Subsequently, during recovery there was a mean rise in hemoglobin concentration of 2.4 g/dl over a period of follow-up that averaged 4 weeks.

The changes in laboratory results in acute infection are similar to those in chronic inflammatory disease. However, it is noteworthy that at least some of the abnormalities persist for several weeks after the disappearance of the clinical manifestations. This is the case with elevated serum ferritin (41). If the elevation of serum ferritin that occurs with mild infections (26) proves to be similarly persistent, it might explain the fact that infants often have an iron-responsive anemia despite having had a normal serum ferritin (42).
Thalassemia Minor

Aside from iron deficiency, thalassemia minor is the most common cause of mild anemia accompanied by microcytosis. In thalassemia minor, the MCV is usually well below the normal range, even when the concentration of hemoglobin is in the low-normal range or only slightly depressed (18). Thus, a disproportionately low MCV suggests thalassemia minor. However, the presence of one condition does not exclude the other; individuals with thalassemia minor are just as likely to be iron-deficient as nonthalassemic individuals.

If an individual is anemic, the ratio of the MCV to the red blood cell (RBC) count in millions (the Mentzer Index) is helpful in distinguishing iron deficiency from thalassemia minor (43). In thalassemia minor, the RBC count tends to be high for the degree of anemia. Thus an MCV/RBC of 13 or less is found in about 85% of subjects with thalassemia minor, whereas a similar percentage of patients with iron deficiency anemia have higher values. The Mentzer Index has been proposed only for use in differential diagnosis of anemia. Its effectiveness is uncertain when the hemoglobin is in the low-normal range.

In beta-thalassemia minor, hemoglobin electrophoresis reveals an elevation in hemoglobin $A_2$. Alpha-thalassemia minor is suspected if one parent also has microcytosis. In thalassemia minor without iron deficiency, serum iron, TIBC, serum ferritin, and EP are normal.

Combined Nutritional Deficiencies

Iron deficiency is often found in combination with other nutrient deficiencies, particularly in developing countries. Combinations that are common and that have been well studied include iron lack with either protein-calorie or folate deficiency. Each of these deficiencies not only results in anemia but also obscures the diagnosis when found in combination with iron deficiency.

Protein-Calorie Malnutrition

Anemia is common in severe protein-calorie malnutrition. The concentration of hemoglobin is often of the order of 10 g/dl in infants between the ages of 1 and 3. In Thailand (Fig. 4), groups of infants and young children who were treated with a regimen designed to correct protein-calorie malnutrition had an encouraging reticulocyte response, but their hemoglobin concentration reached a plateau well before the anemia was corrected (44). Bone marrow iron became depleted as the concentration of hemoglobin reached the plateau. When iron was added to the rehabilitation regimen at that time, there was a prompt second reticulocytosis and a further rise in the concentration of hemoglobin. The diagnosis of iron deficiency was not initially evident by laboratory studies. Indeed, the infants did not become overtly iron-deficient until their rapid growth and hemoglobin production outstripped their limited supply of storage iron. On the basis of such findings, many
Correction of anemia with treatment of protein-calorie malnutrition (44). One group of patients initially received supplemental iron with its treatment regimen (closed circles and solid bars). A second group received supplemental iron only after 6 weeks (open circles and striped bars). A complete response in hemoglobin was obtained only after addition of iron to the regimen (arrows). The fall in concentration of hemoglobin during the first few days of treatment was due to an expansion of the blood volume.

Laboratory evaluation of iron status is also more reliable after general malnutrition has been treated for about 2 weeks and when infection, dehydration, and other acute and life-threatening problems have been resolved.

Folate Deficiency

Folate deficiency combined with iron deficiency may occur in preterm infants who are fed unsupplemented formulas based on evaporated milk and among infants who are fed goat's milk. Infants with diarrhea, malnutrition, infection (45), and hemolytic anemia (46) are at increased risk of developing folate deficiency. Combined folate and iron deficiencies are also common in women during the last half of pregnancy.
Mild folate deficiency is often overshadowed by a more dominant and severe iron deficiency. In this situation, the detection of hypersegmentation of the neutrophils is diagnostically useful. Hypersegmentation of the neutrophils is a familiar characteristic of vitamin B$_{12}$ and folate deficiency, but it was previously believed that it could also occur with simple iron deficiency. However, more recent studies showed evidence of covert folate deficiency among most patients in whom an apparently uncomplicated iron deficiency was associated with hypersegmentation (47). The importance of detecting these subtle manifestations of folate deficiency was underscored by the development of megaloblastic bone marrow changes in some of these patients when they were treated with iron alone. The appearance of more overt folate deficiency after treatment of iron deficiency suggested that the patient's body pool of folate was marginally adequate to sustain the lower red cell mass present during iron deficiency but not adequate for the increased erythropoiesis that occurred in response to iron treatment.

Other Nutrient Deficiencies

Vitamin B$_{12}$ deficiency in strict vegetarians and in patients with malabsorption of the vitamin (pernicious anemia) may result in megaloblastic anemia. The interaction of vitamin B$_{12}$ deficiency with iron deficiency is similar to that of folic acid in that the milder of the two deficiencies comes to prominence only after the more severe one is treated.

Other nutrients that have been associated with anemia in humans and experimental animals include vitamins A, E, and B$_{1}$ (thiamine). The role of each of these nutrients in the anemia that is prevalent in various developing countries is not yet clear. Possibly, a lack of one or more of these or other nutrients will explain the fact that treatment with iron sometimes results in a plateau of hemoglobin concentration somewhat below what is considered normal in industrialized countries.

CLINICAL STUDIES

Diagnosis of Iron Deficiency in Healthy Infants in the United States

At the present time, 1-year-old infants are probably at a higher risk of developing iron deficiency than any other group in the United States (3,20,22). For this reason, screening for anemia at about 1 year of age has become routine practice. About 4 years ago we decided to examine various laboratory tests and screening criteria for iron deficiency in terms of their value in predicting a hemoglobin response to treatment with iron. We studied a group of 1-year-old infants who were dependents of military personnel and who were being screened at Travis Air Force Base. The experimental protocol was designed to fit as closely as possible into routine screening procedures. The data described below pertain to 1,128 infants who had a well-baby visit at 1 year of age between 1978 and 1980.
Skin puncture blood was initially obtained for Coulter counter determination of hemoglobin and red cell volume. Any infant who had a hemoglobin below 11.5 g/dl or a red cell volume below 72 fl (criteria that intentionally included the lower portion of the normal ranges) was considered screen-positive and was asked to return for a venipuncture. Hemoglobin and MCV determinations were then repeated on venous blood and, in addition, serum ferritin, EP, serum iron, iron-binding capacity, and hemoglobin electrophoresis were done. All screen-positive infants were then treated with a 3-month course of 3 mg/kg/day of iron as ferrous sulfate given orally before breakfast. Three months later, they were seen again for a repeat determination of the hematologic and biochemical measures on venous blood.

This population was probably fairly representative of infants in the United States, except that extremes of affluence and poverty were not included. Mild anemia was quite common, but severe anemia was rare. Twenty-five percent of the infants had a capillary hemoglobin value below 11.5 g/dl, the estimated 10th percentile of a healthy, iron-sufficient population of infants. Eleven percent had concentrations below 11.0 g/dl, the lower limit of the normal 95% range, and could therefore be considered anemic. Only 2% had a hemoglobin concentration below 10.0 g/dl, however.

All of the laboratory tests for iron deficiency were reasonably accurate in identifying those few infants who were most severely iron-deficient. The laboratory values for those infants who had initial hemoglobin values below 10 g/dl and who responded to iron therapy with more than a 2.0-g/dl rise in venous hemoglobin are shown in Table 2. Since these infants had values that were at least 1 g/dl below the lower limit of the reference range, they were easily identified as anemic. In addition, the results of all of the four additional laboratory tests (MCV, EP, transferrin saturation, and serum ferritin) were abnormal in all but one of these most anemic infants. Thus, the laboratory diagnosis of moderate to severe iron deficiency seemed relatively straightforward in that any single one of the confirmatory tests for iron deficiency would have been diagnostically useful.

The situation became more complicated when the hemoglobin concentration was within 1.0 g/dl of the reference range and the definition of response to iron therapy was broadened to include all infants with a rise in venous hemoglobin equal to or greater than 1.0 g/dl. This degree of response is significant from the public health point of view and would also be considered clinically meaningful in individual patients. About half of the infants who were anemic (hemoglobin below 11.0 g/dl) had at least a 1.0-g/dl response. The rate of response would probably have been even greater if a smaller increase in hemoglobin concentration had also been considered a response or if compliance had been more complete.

Perhaps of greater interest than the response of the anemic group was the high rate of response in infants whose hemoglobin values were in the lower part of the 95% reference range, between 11.0 and 11.5 g/dl, and who might therefore have been classified as normal. As would be expected, the rate of response in this group was lower than in the anemic infants; slightly less than one-third of this group had an increase in venous hemoglobin of at least 1.0 g/dl. Although the average response
### TABLE 2. Diagnosis of moderate and severe iron deficiency

<table>
<thead>
<tr>
<th>Patient identification number</th>
<th>Pretreatment Hgb (g/dl)</th>
<th>Posttreatment Hgb (g/dl)</th>
<th>Pretreatment values</th>
<th>Serum ferritin (mg/liter)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>MCV (fl)</td>
<td>Protoporphyrin (µg/g Hgb)</td>
</tr>
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<td>75</td>
<td>6.3</td>
<td>13.2</td>
<td>48</td>
<td>19.4</td>
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<td>86</td>
<td>9.5</td>
<td>11.8</td>
<td>64</td>
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</tr>
<tr>
<td>104</td>
<td>8.0</td>
<td>12.0</td>
<td>48</td>
<td>—</td>
</tr>
<tr>
<td>122</td>
<td>6.0</td>
<td>14.1</td>
<td>48</td>
<td>19.3</td>
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<tr>
<td>177</td>
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<td>12.9</td>
<td>59</td>
<td>16.7</td>
</tr>
<tr>
<td>203</td>
<td>7.7</td>
<td>11.1</td>
<td>54</td>
<td>18.8</td>
</tr>
<tr>
<td>204</td>
<td>9.7</td>
<td>11.8</td>
<td>62</td>
<td>—</td>
</tr>
<tr>
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<td>6.4</td>
<td>13.1</td>
<td>46</td>
<td>45.9</td>
</tr>
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<td>54</td>
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<td>Normal cutoff</td>
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<td>≥11.0</td>
<td>≥70</td>
<td>≥3.0</td>
</tr>
</tbody>
</table>

*With a single exception (transferrin saturation, 24.7%), all laboratory tests were abnormal in the 8 infants with an initial hemoglobin concentration of less than 10 g/dl who subsequently had a hemoglobin rise of at least 2 g/dl after treatment with iron for 3 months.*
was 1.6 g/dl compared to 2.3 g/dl in the anemic group, the absolute number of responses was equal to that in the anemic group. Thus, by restricting treatment only to the anemic infants, we would have missed treating half of the potential responders. These results illustrate the clinical dilemma posed by the overlapping of hemoglobin values in normal and mildly iron-deficient individuals.

The same problem of overlapping values was also evident with the other laboratory tests of iron nutrition, as illustrated in Fig. 5. The hatched bars designate infants who had a hemoglobin response equal to or greater than 1.0 g/dl and the open bars indicate infants whose response, if any, was less than 1.0 g/dl. With each of the tests, an abnormal value (to the left of the diamonds) was associated with a rate of response greater than 50%, a result that is clinically acceptable, although far from ideal. What is of far greater concern is that over half of the potentially responsive infants would have been falsely classified as normal by any one of the tests. This problem of overlapping laboratory values in infants with mild iron deficiency is likely to be representative of other populations in which iron deficiency is common but most cases are mild.

Because of the difficulties in applying the standard hematologic or biochemical tests to individual infants with mild iron deficiency, we concluded that the diagnosis of iron deficiency could be established much more inexpensively and conclusively by doing a therapeutic trial based on the initial measurement of hemoglobin (or hematocrit) with or without the MCV. This applied particularly to the vast majority of individual infants whose values were in the low-normal range or no more than 1 g/dl below normal. On the other hand, all of the laboratory tests were useful in characterizing the iron status of the infants as a group. Mean values of MCV, EP, transferrin saturation, and serum ferritin were all substantially and significantly different in the iron-responsive and nonresponsive groups.

Evaluating the Iron Status of Chilean Infants in Response to a Fortification Program

In January 1982, Chile embarked on a national program of iron fortification using as a vehicle the powdered milk that is distributed to most of the infants in the country. During one of the preparatory pilot studies, laboratory tests of iron status were used to monitor the effectiveness of this type of program in the prevention of iron deficiency, as described elsewhere (Stekel, this volume). In one phase of these studies, 280 infants received an iron-fortified milk and 278 in a control group received the regular unfortified milk. Acceptance of the milk was very good, with about 90% of infants still taking it at 15 months of age. Analysis of laboratory data showed significant and substantial differences between the two groups in hemoglobin, serum iron, TIBC, transferrin saturation, free EP, and serum ferritin at 15 months of age. Anemia was present in only 1.6% of infants receiving the fortified milk. The corresponding figure in the control group was 27.8%. Only 6.4% of infants in the fortified group had a transferrin saturation below 9% versus...
35.2% in the control group. Erythrocyte protoporphyrin values over 100 μg/dl whole blood were present in 22.4 and 4.6% of the cases, respectively. Thus, all three laboratory tests were helpful in showing a marked difference between the groups that would be ascribed to iron fortification.

In another of the pilot studies, infants who had received either unfortified or fortified milk were given iron medication under the supervision of a health aide for 75 days. The purpose was to determine the degree of iron-responsive anemia present in the two groups. The dose was 45 mg of iron as ferrous sulfate per day. The results showed very little hemoglobin response in the group that had received the iron-fortified milk; of the total of 43 infants, none had a concentration of hemoglobin below the lower limit of the normal 95% reference range (11.0 g/dl), and only 5 infants had an increase equal to or greater than 1.0 g/dl in hemoglobin concentration. In contrast, in the unfortified group of 43 infants, 12 had a hemoglobin concentration below 11.0 g/dl and 28 had an increase of at least 1.0 g/dl in hemoglobin concentration. These findings were impressive in verifying the effectiveness of iron fortification. They also provided additional data for the interpretation of hemoglobin values in infants. As in the Travis Air Force Base study, there was a clear demonstration that hemoglobin values in individual iron-responsive and unresponsive infants overlap to a marked degree. Among the 43 infants that received the unfortified milk, 13 had a hemoglobin response despite an initial hemoglobin value that was within the normal reference range.

Estimating Prevalence of Anemia and Iron Deficiency Among Children

In a healthy, well-nourished population, hemoglobin values approximate a Gaussian distribution. When there is a high prevalence of anemia, some estimate of the prevalence and severity of the anemia is provided by the extent to which a frequency distribution curve for hemoglobin is skewed to the left. The degree of skew is easiest to appreciate when the familiar frequency distribution plot is converted to a plot of the cumulative distribution of values on a probability scale. This scale results in a linear plot when values show a Gaussian distribution, but when there is a high prevalence of anemia, the values show a deviation to the left of linearity in the lower portion of the curve. This approach, which has been termed "distribution analysis," can provide a relative estimate of the prevalence of anemia. Unfortunately, the results are unreliable when the size of the population is under about 100, when the prevalence of iron deficiency is low, or when children within a broad age range are being evaluated.

**FIG. 5.** Distribution of MCV, ER transferrin saturation, and serum ferritin values in infants with a skin puncture hemoglobin concentration below 11.5 g/dl who subsequently had at least a 1-g/dl rise in venous hemoglobin in response to iron therapy (hatched bars). Values for infants who had no response or a less than 1-g/dl response are shown by open bars. The estimated limits of the normal range are indicated on the horizontal axes (diamond symbol); values to the left of the symbol are considered indicative of iron deficiency. FL = femtoliters. (From Dallman et al., ref. 3.)
MEAN CORPUSCULAR VOLUME

TRANSFERRIN SATURATION

ERYTHROCYTE PROTOPORPHYRIN

SERUM FERRITIN
Black and White Infants at Travis Air Force Base

Some of the advantages and limitations of distribution analysis are illustrated in Fig. 6. The left portion of the figure shows the frequency distributions of hemoglobin for 159 black and 800 white infants. The same data are shown as probability plots on the right side of the figure. Values for white infants were distributed in a linear fashion consistent with a Gaussian distribution. Values for blacks were parallel in the middle and upper parts of the distribution, with a median value that was 0.3 g/dl lower than that for whites. The identical difference of 0.3 g/dl was found when the groups were matched socioeconomically by comparing values from only those infants whose paternal military rank was in the middle range. The hemoglobin in the middle and upper portions of the distribution is in accord with an inherently, slightly lower hemoglobin concentration in blacks than in whites but was of smaller magnitude than the 0.5 to 1.0 g/dl in previous reports (17).

The other point illustrated by Fig. 6 is that hemoglobin values in blacks showed a substantial skew from a normal, Gaussian distribution in the lower portion of the hemoglobin range, a finding that was not evident in the data for whites. This marked deviation from linearity at the lower end of the curve in blacks indicated a higher prevalence of anemia that was substantiated by therapeutic trial.

The limitations of distribution analysis become evident on considering that more than a third of the 22% of white infants with a hemoglobin concentration below 11.5 g/dl had a venous hemoglobin response of at least 1.0 g/dl with iron treatment.

![Fig. 6. Distribution analysis. The left side of the figure shows the frequency distribution of hemoglobin values in black (dashed line) and white (solid line) infants at Travis Air Force Base. On the right, the same data are plotted as cumulative distribution curves on a probability scale. The median hemoglobin concentration was 0.3 g/dl lower in blacks than in whites. The linearity of the plot for whites indicates a Gaussian distribution. The deviation to the left of the lower portion of the plot for black infants shows a skew of the distribution toward anemic values. (From Reeves et al., ref. 17.)](image-url)
Thus, an 8% prevalence of iron-responsive anemia would have been missed on the basis of finding a Gaussian distribution for hemoglobin concentration by distribution analysis.

**Eskimo Children in Alaska**

Margolis et al. (48) recently evaluated the effect of iron therapy on the distribution of hemoglobin values among a group of Alaskan Eskimo children whose ages ranged from 2 to 16 years. Although the total population of 533 children in the pilot study was large, conventional analysis of the data was hampered by the fact that there are major changes in normal hemoglobin concentration within the age span of the group, including the divergence of male and female values after 10 years of age. If the population had been divided into smaller age groups to correspond to tabulations of hemoglobin concentration by age and sex, many of the advantages of a large study group would have been lost.

A high prevalence of mild anemia that affected all ages and both sexes to a similar degree became evident when hemoglobin concentrations from individual children were plotted on percentile curves (Fig. 7). Twenty-one percent of the group had hemoglobin concentrations below the third percentile of normal. After 277 of the children had been treated with iron for a 3-month period, 43% had an increase in venous hemoglobin of 1.0 g/dl or more, whereas only 31% had a rise of less than 0.5 g/dl increase. The change in hemoglobin distribution could be best appreciated by using an approach that would take into account normal developmental changes in the concentration of hemoglobin. This was done by converting the hemoglobin concentrations to standard deviation scores for age and sex, based on the percentile curves for hemoglobin. The standard deviation (SD) scores allowed an evaluation of the change in distribution of age-normalized hemoglobin values for the group as a whole, as well as a comparison with an ideal Gaussian distribution (Fig. 8). There was a marked skew toward low hemoglobin values among the 533 children in the pilot study and the 277 children in the treatment group prior to iron administration. After treatment, values approximated a Gaussian distribution for hemoglobin concentration in a healthy population.

Several conclusions emerged from Fig. 8. First, in accord with other laboratory data, iron lack was the major cause of anemia since only 5% of the children had hemoglobin values below –2 SD for the reference population after they had been treated with iron. Second, there was an impressive overall shift in the distribution curve. Before treatment, very few of the individual SD scores were above the reference mean, and 42% were below the normal distribution curve, whereas after treatment the number of values above the reference mean was close to normal. The change in distribution indicated not only that the pretreatment group had a high prevalence of anemia but that marginal iron reserves were a virtually universal finding. It would be conceptually misleading to conceive of this group as being composed of distinctly iron-deficient or iron-sufficient individuals. Instead, the
population appeared to consist of varying gradations from mild iron deficiency anemia to marginal iron sufficiency. This is probably a common characteristic of groups in which there is a high prevalence of iron deficiency anemia.
Mexican School Children

The results of a treatment trial with iron- and copper-supplemented milk in a group of Mexican school children between 6 and 15 years of age were depicted in a different manner (49). The study was designed to include a placebo group and two iron-treatment groups, each receiving different forms of supplemental iron. Change in concentration of hemoglobin is depicted in a bar graph diagram (Fig. 9). This method of presenting the data also lends itself to studies involving children of a broad age range. Both iron groups showed a response that was highly significant in relation to the placebo group. However, supplementation with iron as ferrous chloride was much more effective in raising the hemoglobin concentration than ferric lactobionate, a more stable iron chelate. As in the Alaskan Eskimo population,
CONCLUSIONS

The initial step in the diagnosis of iron deficiency is a determination of the concentration of hemoglobin or, as a second choice, the hematocrit. Venipuncture
samples are preferable, but when circumstances allow only skin puncture sampling, it is important to obtain a free flow of blood from a warm extremity. If the patient is found to be anemic (the hemoglobin or hematocrit is below the 95% range for a healthy population), the next steps to be taken are relatively straightforward. If the anemia is severe (the hemoglobin is more than 1 g/dl below the lower limit of normal), one or two further diagnostic tests will usually be indicated, and in most cases they will help either to establish or to exclude a diagnosis of iron deficiency. These additional tests could include MCV, serum iron/TIBC, EP, or serum ferritin. Which of these tests are selected will depend on the differential diagnosis and the specific clinical setting. When severe iron deficiency is diagnosed among low-risk populations (e.g., adult males or postmenopausal females), causes of pathologic intestinal blood loss must be investigated. This is also true when the anemia in infants and children is so severe that it becomes difficult to attribute the iron deficiency to dietary factors alone.

Patients with mild anemia present a more difficult problem because they tend to be either passed off as normal or evaluated with an excessive number of laboratory tests. When mild anemia is diagnosed on the basis of a skin puncture sample, it is helpful if the diagnosis of anemia can be confirmed by repeating the hemoglobin analysis on the venous blood. The next steps to be taken should then be influenced by factors such as the age, sex, and socioeconomic group of the patient. When such factors suggest a greater likelihood of iron deficiency, as in infants and adolescents from a poverty group, a therapeutic trial of iron may be more appropriate than further laboratory studies, especially if previous experience in the same population has shown the anemia to be due primarily to nutritional iron deficiency. A more extensive workup can then be reserved for the relatively small number of patients whose anemia is unresponsive to a 1-month course of iron therapy. However, further laboratory tests would be much more useful among patients in whom anemia is normally rare (e.g., men in industrialized countries). The development of mild iron deficiency anemia in such groups often signals a serious underlying disease that requires prompt attention.

The most difficult problem is the detection of the relatively mild cases of iron-responsive anemia in individuals who have a screening hemoglobin that is in the lower part of the normal range. In populations that rarely develop iron deficiency, such patients are so vastly outnumbered by healthy individuals that it is impractical to seek them out unless the history or physical examination suggests a reason to do so. In contrast, in a population known to have a high prevalence of iron deficiency (e.g., 1-year-old infants), a therapeutic trial of iron may be justified because there may be about as many iron-responsive infants with low-normal hemoglobin values as there are anemic ones. Because other laboratory tests were not of much value in distinguishing iron-responsive from unresponsive individuals in the low-normal hemoglobin group, a therapeutic trial would be a more cost-effective means of identifying iron-responsive subjects.

When populations are monitored for iron deficiency for the purpose of guiding health policy or planning fortification programs, it is essential to rely on more than one laboratory test. In addition to the hemoglobin determinations, the MCV will
be helpful because it can be obtained at little extra cost if an electronic counter is available. Of the other tests, the serum ferritin has the clear advantage of allowing an estimate of iron reserves, as well as identification of iron deficiency. However, serum iron/TIBC and EP also provide information that may be helpful and may offer advantages in certain settings. If funds permit, the most reliable information is obtained by obtaining a broad battery of these tests on an appropriately selected sample population. Thus, multiple tests should be emphasized in the evaluation of populations, whereas a simpler approach of few tests and reliance of a brief therapeutic trial can be the basis for management of individual patients.

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DISCUSSION

Dr. Finch: One variation that we tend to overlook is that plasma volume may change rapidly. If the venipuncture is painful, hemoglobin concentration can increase by 1 g as blood is drawn. If you then leave the needle in the vein for 5 or 10 min, you may get a hemoglobin concentration change due to the positioning of the individual, whether standing or lying down.