INTRODUCTION
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ABSTRACT
Iron biomarkers were developed to define the size of iron stores and the adequacy of the iron supply required to meet functional needs. Approximately 80% of the iron delivered to tissues through the circulating plasma pool will be incorporated into hemoglobin. Consequently, with the exception of serum ferritin, iron biomarkers are measures of iron sufficiency for erythrocyte production. They have proven to be very valuable in the determination of the cause of anemia in the clinical setting in which additional information about factors that affect the patient’s health is available. However, all current biomarkers are affected by factors other than iron status, which limit their utility for the determination of the prevalence of iron deficiency in some populations, particularly in populations who live in developing countries. Furthermore, relations between iron status and functional outcomes such as neonatal and infant mortality; motor, cognitive, and emotional development in infants; and severe morbidity from malaria in young children are inadequately characterized. There is a need to identify and standardize biomarkers that have the highest predictive value for specific functional outcomes in each setting. The most appropriate biomarkers may vary with the setting and be influenced by age, sex, gestational stage of pregnancy, and environmental factors such as repeated or chronic infections. There is also an urgent need for improved technology to permit the use of specific biomarkers in field studies in resource-poor regions. Finally, more research is required to define the potential role of hepcidin and non–transferrin-bound iron assays. Am J Clin Nutr 2011;94(suppl):673S–8S.

INTRODUCTION
Iron balance is highly regulated by the control of absorption in humans. Deficiency and excess are both associated with significant morbidity and mortality. Iron deficiency is far more prevalent than iron overload. Iron deficiency disproportionately affects infants, young children, adolescents, and women of childbearing age, particularly individuals who live in developing countries because their diets provide insufficient bioavailable iron to meet their needs for growth, the replacement of menstrual losses, and pregnancy (1). This case study was limited to biomarkers for the detection of iron deficiency and determination of its severity.

Iron biomarkers are customarily used in the following settings:

1) Clinical evaluations.
   a. Identification of etiologic factors in patients who present with anemia and determination of iron status in patients who suffer from disorders that cause blood loss.
   b. Assessment of iron status in individuals at stages of the life cycle at which risk of iron deficiency is increased, particularly in children between 4 and 6 mo and 5 y of age, adolescents, and women of childbearing age. Such evaluations may be part of routine clinical surveillance or be indicated because of symptoms associated with iron deficiency.
2) Population surveys to determine the prevalence of nutritional iron deficiency.
3) Evaluation of the effect of nutrition interventions.

The specific utility of iron biomarkers that are currently available varies considerably depending on the setting in which they are applied. Appropriately selected biomarkers are both sensitive and specific when used in combination with relevant clinical data in individual patients who suffer from disordered iron homeostasis. Confounding factors limit both sensitivity and specificity when they are applied to populations.

Biomarkers for iron status were selected by physiologists who studied iron metabolism in animal models and humans. These biomarkers were chosen because of their specificity for the identification of functional aspects of iron storage, transport, and use and were focused on red blood cell production and turnover because the circulating red blood cells constitute the largest and most accessible iron containing compartment in the body. A clear appreciation of the utility and interpretation of iron biomarkers depends on an understanding of iron physiology. The following brief review of the salient aspects of iron transport and storage provides the background for the proposed approach for the selection of biomarkers.

Humans normally have 40–50 mg Fe/kg body weight, ≈75% of which is metabolically active (80% is in hemoglobin in circulating red cells). The rest constitutes a dynamic store that ensures an adequate supply for the immediate cellular needs despite short-term variations in requirements for rapid growth, pregnancy, and to replace iron lost through menstruation or pathologic bleeding. Most of the iron store is located in the spleen, liver, and bone marrow in the form of ferritin (2). Small quantities of ferritin [serum ferritin (SF)] are secreted into the circulating plasma by mechanisms that have been poorly elucidated (3). However, in the absence of inflammatory and neoplastic disorders and liver

1From the Department of Internal Medicine, Eastern Virginia Medical School, Norfolk, VA.
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3No funding was received for this study.
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A steady supply of iron is needed for cellular turnover. A transmembrane protein ferroportin exports iron from cellular storage sites into the circulation where it is rapidly bound to transferrin (4–6). The export rate is closely matched to tissue storage sites into the circulation where it is rapidly bound to transmembrane protein ferroportin exports iron from cellular storage sites. The export rate is closely matched to tissue storage sites into the circulation where it is rapidly bound to transferrin (4–6).

In the simplest scenario, an individual with a normal iron status in the absence of liver disease and infectious and inflammatory disorders; 1 μg SF/L is indicative of 8–10 mg storage iron in an adult. Stainable bone marrow iron is a semiquantitative measure of iron stores, but it requires bone marrow sampling, which is a highly invasive procedure. This sampling is used rarely, in complex clinical settings or for research purposes.

**EARLY FUNCTIONAL IRON DEFICIENCY**

A discrepancy between iron supply and requirements is considered to be evidence of early functional iron deficiency. When insufficient iron enters the plasma pool to fully meet requirements for optimal red cell production, the plasma iron concentration (serum iron) and transferrin iron saturation (percentage saturation) decline; hemoglobin values are still within the normal range. A percentage saturation of transferrin <16% is the most reliable criterion of a functional deficit (22). The sTfR concentration is increased because more TIRs are expressed on the surfaces of cells that require additional iron. Red blood cell zinc protoporphyrin (ZPP; also referred to as free erythrocyte protoporphyrin) concentrations rise when there is insufficient iron for optimal heme synthesis because zinc replaces iron in a small proportion of the erythrocyte protoporphyrin. Finally, inadequate heme synthesis may be shown in reticulocytes and young red blood cells produced when the iron supply is suboptimal (reticulocyte hemoglobin concentration and percentage hypochromic erythrocytes), before there is a detectable decrease in hemoglobin concentration or mean red cell volume.

**ESTABLISHED FUNCTIONAL IRON DEFICIENCY**

Established functional iron deficiency is also often referred to as IDA and is defined by hemoglobin or hematocrit values that fall below the normal range. Hemoglobin synthesis is suboptimal in iron-deficient red blood cells that are characteristically smaller with a lower hemoglobin content (a reduced mean cell volume and mean cell hemoglobin).

All of the biomarkers of functional iron deficiency were chosen for their effects on red cell production. However, the supply to other cells may be impaired before restricted hemoglobin synthesis is detected. Inadequate iron acquisition by the brain may have the most important functional consequences (23, 24). Detrimental long-term effects on brain development and function were postulated to result from iron deficiency in utero and in early infancy (25).

**SELECTION OF IRON BIOMARKERS**

Clinical applications

1) Identification of the etiologic factors in patients presenting with anemia (characteristically microcytic anemia) and the determination of iron status in those suffering from disorders causing blood loss. It is usually possible to make a definitive determination of the contribution of iron deficiency to the anemia in these patients (26, 27). The availability of additional clinical information makes the individual selection of the most appropriate biomarkers possible.

2) Assessment of iron status in individuals at stages of the life cycle where risk of iron deficiency is increased. Again, the currently available biomarkers provide important information that permits the correct treatment to be offered to patients for whom the relevant clinical information is available. However, the definition of normal iron status and the application of biomarkers to prevent long-term
consequences of iron deficiency at critical stages of the life cycle need to be evaluated more carefully. Cook defined normal iron status as “a normal hemoglobin, serum ferritin and transferrin receptor concentration” (28). This definition may be appropriate at many stages of the life cycle. However, it implies that a significant iron store is not a necessary component of normal iron status and, therefore, may not be adequate at some important critical times in the life cycle. The following are 2 examples:

a. Pregnancy. In the past, the assessment of nutritional adequacy for iron in pregnancy has been concentrated on the iron status of the mother and the prevention of maternal anemia. The infant was considered to be protected because iron would be preferentially transferred to the fetus at the expense of maternal requirements for erythropoiesis. However, there is an accumulating body of evidence that suggests that the focus on maternal health may be too restrictive. Iron supplementation during pregnancy has been reported to reduce the prevalence of prematurity, low birth weight, the infant mortality rate, and the likelihood that the infant will be iron deficient after 3 mo of life (29, 30). The benefit for birth weight was observed (31) even in mothers who were not anemic at the first prenatal visit. Consideration must also be given to the gestational age of the pregnancy at which the measurements are made.

b. Infancy (0–6 mo of age). During this period, a dynamic switch in hemoglobin type and synthesis occurs. Iron is conserved by temporary transfer to stores. An evaluation on the basis of iron stores alone may be misleading unless some adjustment is made for future requirements.

Population surveys

There is far less agreement about the best approach to population screening. Anemia is still used as a proxy for iron deficiency. However it lacks sensitivity and specificity as an indicator of iron status. Sensitivity is low because the distributions of hemoglobin concentrations in iron-sufficient and iron-deficient individuals overlap. In addition, cutoffs used to identify anemia may not be appropriately adjusted for age, sex, pregnancy, ethnicity, smoking, and altitude. Specificity is poor because individuals with IDA are a subgroup of all anemic individuals in a population. A multitude of disorders other than iron deficiency can produce anemia, including other nutritional causes (such as deficiencies of vitamins A and B-12 and folic acid), infection (notably malaria, HIV disease, and tuberculosis), inflammation, and acquired and inherited red cell disorders (such as thalassemic syndromes and hemoglobinopathies). Considerable variability is present from population to population in proportions of individuals with anemia who have iron deficiency and in proportions of individuals with iron deficiency who have IDA. In resource-poor settings with a high prevalence of malaria and other infectious diseases, anemia as a single indicator of iron status is of limited usefulness.

A low plasma ferritin concentration is the iron status indicator most often used to define iron deficiency or IDA. It has proven very useful in populations where the prevalence of infection is low, but plasma ferritin is an acute phase protein. Therefore, SF values may not reflect the iron status accurately in the presence of infection or inflammation. Currently, the best approach to this problem is to measure one or more other acute phase proteins concurrently [usually C-reactive protein (CRP) or α1-acid glycoprotein (AGP)] and to exclude or correct SF in samples with a raised CRP or AGP concentration (32). However, SF used alone has another important limitation. SF cannot be used as a measure of the extent of an iron deficit because values remain stable once the iron store is exhausted (33). Therefore, the utility of SF is limited at stages of the life cycle during which there is little storage iron because of high requirements. The second and third trimesters of pregnancy and young children between the ages of 6 and 12 mo are the best examples. At these times, absorption is fully up-regulated only after stores have been exhausted. The iron supply for functional needs may or may not be adequate. Measurements of a discrepancy between the iron supply and functional requirements (early functional iron deficiency) are more likely to be predictive of functional outcomes.

Low percentage transferrin saturation, raised red blood cell ZPP, and raised plasma TIR concentration are early measures of a suboptimal iron supply. Low percentage transferrin saturation was widely used. However, there is a diurnal variation in values, results are affected by inflammation, and the method is not suitable for field trials. Rised red blood cell zinc protoporphyrin (most frequently reported as the ZPP:heme ratio) has been used extensively, particularly in studies that involved children, and remains a potentially valuable tool, but values are also affected by inflammation and may be unreliable in the presence of high environmental exposure to lead. An attractive feature of the assay method is the ability to perform immediate point-of-contact assays in the field, but there is a need for improved technology and standardization (34). A raised plasma TIR concentration is the newest and potentially most useful indicator of a functional deficit available. Plasma TIR is less affected by inflammation than is SF, but plasma TIR may be raised when the erythropoietic rate is high even if iron stores are present (35). sTfR is suitable for field assays and an automated methodology but requires further standardization. When used in conjunction with SF, the sTfR:SF ratio can be used to provide an estimate of the complete distribution of iron-status values in a population sample that is not dependent on hemoglobin measurements (33, 36).

Other potential indicators include the following:

1) The reticulocyte hemoglobin concentration and percentage of hypochromic erythrocytes. Measurement requires special instrumentation. These biomarkers are unlikely to have a useful role as indicators of nutritional iron deficiency in population studies in the near future.

2) Red cell indexes, particularly the mean red cell volume and mean red cell hemoglobin. Both indexes are abnormal in thalassemic syndromes, which limits their potential usefulness.

3) Plasma and urinary hepcidin concentrations (16). There is considerable enthusiasm for a potential role for this indicator, but further research is needed to define its utility.

RATIONALE FOR THE SELECTION AND STANDARDIZATION OF BIOMARKERS

The selection of biomarkers for the estimation of the prevalence of nutritional iron deficiency in populations has traditionally been tied to the prevalence of anemia. Cook et al (37) pioneered the use of a combination of 3 biomarkers (SF; transferrin saturation,
and red cell protoporphyrin) for estimating the prevalence of nutritional iron deficiency in the United States. Iron deficiency was defined by an abnormal value for 2 of the 3 results. Similar combinations of biomarkers have been used in surveys in other countries. However, there has been little consistency in the way that they are applied. Three biomarkers are not always measured. When 2 biomarkers are measured, iron deficiency is often defined by an abnormal result for either indicator. The sTfR:SF ratio is an alternative method that provides a quantitative estimate of iron status in individuals with iron deficiency, a normal iron balance, and increased iron stores. The result is expressed as the estimated size of the iron store or, in the case of iron deficiency, the quantity of iron that would be needed to replace the functional deficit. The sTfR:SF ratio has several advantages. It does not rely on parameter cutoffs, it yields a measure of the size of the iron deficit that is independent of the hemoglobin concentration, it is the only method that is based on actual experimental observations, and the assay methods are readily automated. Furthermore, there was reasonably good agreement between the estimated prevalence of iron deficiency by the sTfR:SF ratio and the previously described multiple indicator index in preschool children and women of childbearing age by using samples from the US NHANES 2003–2006 (38).

Iron biomarkers should be predictive of risk. The prevalence of anemia has been a useful surrogate for risk of impaired function in Western societies because iron deficiency is the primary cause in women of childbearing age and in young children. It is less useful in countries where anemia is multifactorial. The use of hemoglobin cutoffs is further complicated by the frequent failure of investigators to adjust for factors known to influence hemoglobin. In addition, their cutoffs may differ from World Health Organization values (39), and the severity of anemia necessary to impair a functional outcome is poorly defined. An alternative approach could be to collect data on specific iron-status indicators that would be related to immediate or future functional outcomes. Functional consequences of iron deficiency that could be used to develop such indications are listed in Table 1. Those most likely to prove useful are summarized in Table 2. Although only a few functional outcomes are listed, they do address the following 2 periods when an optimal iron supply appears to be of critical importance: pregnancy and early childhood.

An attempt should also be made to select and standardize the biomarker(s) most likely to have predictive value for functional outcomes at the stage of the life cycle being addressed. For example, SF may provide useful information during the first trimester of pregnancy but is of limited value in the third trimester when iron stores are virtually always depleted. My suggestions for the selection of biomarkers for individuals and population samples during various stages of the life cycle are

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Functional consequences of iron deficiency</th>
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<tbody>
<tr>
<td><strong>Pregnancy</strong></td>
<td>Increased risk of anemia and maternal morbidity</td>
</tr>
<tr>
<td></td>
<td>Increased risk of prematurity and lower birth weight</td>
</tr>
<tr>
<td></td>
<td>Higher infant mortality</td>
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<tr>
<td><strong>Infants and young children</strong></td>
<td>Motor and cognitive developmental delays in infancy</td>
</tr>
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<td></td>
<td>Effects on emotional maturation</td>
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<td></td>
<td>Poorer academic achievement in school-age children who were iron deficient in early childhood</td>
</tr>
<tr>
<td></td>
<td>Increased risk of severe morbidity from malaria in children &lt;5 y of age</td>
</tr>
<tr>
<td></td>
<td>Increased prevalence and duration of upper respiratory tract infections in children</td>
</tr>
<tr>
<td><strong>All ages</strong></td>
<td>Impaired physical performance and earning capacity</td>
</tr>
<tr>
<td></td>
<td>Suboptimal response to iodine in populations with endemic goiter and increased risk of impaired thyroid function in the presence of iodine deficiency</td>
</tr>
<tr>
<td></td>
<td>Increased risk of chronic lead poisoning in high-lead environments</td>
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<tr>
<td></td>
<td>Increased risk of “restless legs syndrome”</td>
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</tbody>
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<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Potential functional outcomes for selecting and standardizing iron-status biomarkers</th>
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<tbody>
<tr>
<td><strong>Infants and children</strong></td>
<td>Motor, mental, and emotional maturation</td>
</tr>
<tr>
<td></td>
<td>School performance</td>
</tr>
<tr>
<td></td>
<td>Severe malarial morbidity</td>
</tr>
<tr>
<td><strong>Pregnancy</strong></td>
<td>Maternal morbidity/mortality</td>
</tr>
<tr>
<td></td>
<td>Birth outcome: gestational age, birth weight, infant mortality, early childhood mortality (more research needed), and iron status of infants after 4 mo of age</td>
</tr>
</tbody>
</table>

1 Data from reference 1.
The latter approach has proven to be very successful in the different strategies may be improved by using the sTR:Sf ratio sensitivity for detecting quantitative differences between different causes of inflammation. More research is needed to improve the correction of SF for the acute-phase response. With improved technology, the ZPP:heme ratio could become a specific tool for the identification of children who live in malarious regions and are likely to benefit from iron supplements.

RESEARCH PRIORITIES

The most urgent research priorities are as follows:

1) The development and standardization of methods for identifying iron deficiency in populations exposed to malaria, HIV disease, tuberculosis and other infections.

2) An improved understanding of links between iron-status indicators and functional outcomes.

3) A better appreciation of the relation between iron deficiency and mild anemia in tropical countries because of the multiplicity of factors that may affect the hemoglobin values of people living in these regions.

4) An evaluation of the potential role for the ZPP:heme ratio in detecting iron deficiency in young children in regions where malaria is prevalent. There is an urgent need for improved technology for ZPP:heme ratio assays.

5) An evaluation of the potential role of new indicators of iron status and the potential iron toxicity (eg, hepcidin), nontransferrin-bound iron.

The author had no conflicts of interest.

REFERENCES


