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The rationale for selecting and standardizing iron status indicators

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Abstract

Both iron deficiency and iron excess have significant health consequences. A diet insufficient in bioavailable iron and blood loss are the major causes of iron deficiency worldwide. An improved intake of bioavailable iron can prevent the long-term consequences of nutritional iron deficiency. Iron indicators are needed to identify population groups at risk for nutritional iron deficiency and to monitor the impact of intervention strategies. Currently available iron indicators permit a specific diagnosis of iron deficiency and iron deficiency anaemia in the clinical setting where other patient-related information is available, but are more difficult to interpret in populations in developing countries because anaemia is multifactorial. Progress towards reducing the prevalence of nutritional anaemia worldwide will depend on improved selection and standardization of iron indicators in these settings. The predictive value of these indicators for significant functional outcomes could provide the basis for selection and standardization. The currently available indicators and a suggested approach are discussed in this brief review.

Introduction

Iron balance is regulated by the control of absorption in healthy human beings (*1*). A diet insufficient in bioavailable iron and blood loss are considered to be the leading causes of iron deficiency, although more research is needed to define the potential role of malabsorption due to disorders such as coeliac disease, tropical enteropathy and *Helicobacter* infections (*2*, *3*). Inherited iron malabsorption has also been described recently (*4*, *5*), but its prevalence is not known. Iron overload, on the other hand, is the result of genetic disorders that affect the control of iron absorption and haematological conditions which impair regulation by hepcidin (*6*). Both iron deficiency and iron overload have serious health consequences. This review focuses on the laboratory evaluation of iron deficiency because it is primarily a nutritional disorder and is prevalent among women and children in developing countries.

Review of indicators

Iron indicators are all laboratory measurements that are most often employed in the following settings:

- clinical diagnosis in individual patients:
  - evaluation of anaemia
  - assessment of iron status
  - evaluation of treatment
- population surveys:
  - prevalence of nutritional iron deficiency
  - adequacy of iron nutrition in infants and young children
  - adequacy of iron nutrition in women of childbearing age
  - adequacy of iron nutrition in pregnancy
- impact evaluation in populations:
  - field trials:
    - — fortification
    - — complementary food supplements
    - — supplementation
  - monitoring of iron status and programme evaluation.

Indicators of iron status that are in current use were selected by investigators studying iron metabolism in human beings and mammalian animal models. They were chosen for their specificity for identifying functional aspects of iron storage, transport, utilization and the status of the largest functional compartment, the circulating red blood cells. Human beings have 40–50 mg iron/kg body weight (*1*). Approximately 75% is metabolically active and most of this iron is in the haemoglobin of circulating red blood cells. The rest is a dynamic store that ensures an adequate supply of iron for immediate cellular needs despite variations in requirements for rapid growth, pregnancy and the replacement of iron lost through menstruation and pathological blood loss. Iron indicators are therefore measures of the size of the iron store, the adequacy of iron delivery to the bone marrow for red blood cell production and the status of this major functional pool (**Table A2.3.1**).

Uncomplicated nutritional iron deficiency has traditionally been classified by severity. The mildest form, storage iron depletion, is characterized by a reduced ("inadequate") iron store, but no evidence of impaired iron delivery to the functional compartment (low serum ferritin (SF)). The next stage, mild functional iron deficiency (also called iron-deficient erythropoiesis) is characterized by a disparity between the rate of delivery of absorbed iron and iron released from the stores and the cellular requirements (reduced serum iron (SI)), increased total iron

Table A2.3.1
Some iron indicators and their physiological basis

Indicator	Physiological basis
Serum ferritin	1 µg/L = ~ 8 mg storage iron (adult)
Serum iron/total iron binding capacity/transferrin saturation	Reflects balance between iron supply and demand
Transferrin receptor	Measures adequacy of iron supply; iron deficient cells express more transferrin receptors
Red cell zinc protoporphyrin/haem ratio	Measures adequacy of iron supply; zinc is substituted for iron in protoporphyrin when iron supply is inadequate
Haemoglobin/haematocrit/mean corpuscular volume/mean corpuscular haemoglobin	Measure of haemoglobin production

binding capacity (TIBC), reduced percentage saturation of transferrin (% Sat), increased plasma transferrin receptor concentration (TfR) and increased red blood cell zinc protoporphyrin, usually measured as the red blood cell zinc protoporphyrin/haem ratio (ZPP/H)). In the final and most severe stage there is evidence of a deficiency in the major functional compartment, the circulating red blood cell mass, established functional iron deficiency, also called iron deficiency anaemia (IDA). Iron-deficient red blood cells reveal evidence of inadequate haemoglobin synthesis (reduced red blood cell size measured as mean corpuscular volume (MCV) and haemoglobin content, measured as mean corpuscular haemoglobin (MCH)).

The sensitivity and specificity of these iron indicators vary considerably, depending on the setting in which they are applied. Details of a patient's medical history are available to the health professional in medical clinics. Specific conclusions about iron status can usually be drawn. Unidentified confounding factors limit both sensitivity and specificity in screening surveys and impact evaluation in populations. There is therefore less agreement about the optimal approach.

Haemoglobin

The prevalence of iron deficiency has traditionally been calculated from surveys of anaemia prevalence (*7*). IDA is assumed to represent 50% of all anaemias. However, the prevalence of iron deficiency without anaemia is considered to be equal to that of IDA (*8*). Consequently, the overall prevalence of iron deficiency is believed to be equal to that of anaemia from all causes. Anaemia has been used as a proxy for iron deficiency because haemoglobin is the only indicator that is measured in most developing countries. The technology for haemoglobin assays is available and affordable. However, anaemia is a poor proxy for iron deficiency. It lacks both sensitivity and specificity. Sensitivity is low because the distribution of haemoglobin levels in iron-sufficient individuals overlaps that of those who are iron deficient, especially if cut-off values used to identify anaemia are not adjusted for age, gender, pregnancy, ethnicity, smoking and altitude (*9*, *10*). Specificity is poor because there are many other causes of anaemia. Endemic infections, particularly malaria, human immunodeficiency virus (HIV) disease and tuberculosis, and vitamin A deficiency are important contributing factors in developing countries. The red blood cell indices (MCV, MCH) are reduced in iron deficiency. They can therefore be helpful in distinguishing IDA from some other causes. However, once again the feature is not specific to iron deficiency. Red blood cell indices are also reduced in the thalassaemic syndromes, which are common in many developing countries, and to some extent in the anaemia of infection and inflammation.

Serum ferritin

Serum ferritin is the specific iron status indicator that has gained widest acceptance. It reflects the size of the iron store. It has proven very useful in populations where the prevalence of infectious and inflammatory disorders is low. Plasma ferritin is, however, an acute phase protein. Values may not reflect iron status accurately in the presence of infection. Its utility is therefore more limited in developing countries where malaria, HIV disease and tuberculosis are prevalent. The value of serum ferritin assays is also questionable for stages of the lifecycle during which depleted iron stores are physiologically appropriate (second and third trimesters of pregnancy and infancy between 6 and 12 months).

Serum iron, total iron binding capacity and percentage saturation of transferrin

Percentage saturation of transferrin is the element of these inter-related indicators that has been employed most often in the evaluation of iron status in the past (*11*). Its utility is limited by physiological and diurnal variability. Furthermore transferrin saturation is characteristically low in both iron deficiency and the anaemia of inflammation (chronic disease). Finally, assays require access to sophisticated laboratories. It is unlikely that SI/TIBC/% Sat will be suitable for assessing iron status in developing countries.

Red blood cell zinc protoporphyrin

Red blood cell protoporphyrin is now most often reported as ZPP/H because of the availability of the direct reading haematofluorometer. Attractive features of this assay are its applicability to capillary blood samples, minimal sample processing and the immediate availability of the result. The major obstacles to its widespread use in developing countries are the need to improve instrument technology and better assay standardization and quality control. It is also important to emphasize that ZPP/H is a measure of the adequacy of the iron supply to the bone marrow for red blood cell production. It is therefore not specific for iron deficiency. Values are also above the normal range when iron absorption and its release from stores are restricted by infection or inflammation, in thalassaemic syndromes and after chronic exposure to environmental lead.

Plasma transferrin receptor concentration

Raised plasma transferrin receptor concentration is potentially the most useful indicator of a functionally significant iron deficit. As with SI/TIBC/% Sat and ZPP/H, it is a measure of the discrepancy between iron supply (from stores and absorption) and requirements (primarily for haemoglobin production). It is therefore not a specific indicator of iron deficiency, since levels are raised above normal if the iron supply is interrupted by diminished absorption and release from stores and when requirements are increased. It is, however, less affected than SF by inflammation and infection, possibly because the reduced iron supply is to some extent offset by a diminished requirement resulting from suppressed erythropoiesis. The most important confounding factor appears to be increased erythropoiesis due to haemolysis in conditions such as malaria.

Other potential indicators that require further research

Reticulocyte haemoglobin and *percentage hypochromic erythrocytes* are indicators of recent iron delivery to the bone marrow. They require special instrumentation and are unlikely to prove useful for the evaluation of nutritional iron deficiency. *Hepcidin*, a recently discovered peptide hormone, is the principal regulator of systemic iron homeostasis (*12*). Plasma and urinary assays are available and have been shown to provide information about iron status and

metabolism. There is considerable enthusiasm for its potential role as an iron status indicator. However, more research is needed to determine its possible utility. Finally, there has been considerable interest in the possible role of *non-transferrin bound iron* (NTBI) as a mediator of the putative adverse effects of iron supplementation observed among young children exposed to *P. falciparum* malaria (*13*). Assays of NTBI can, however, only be considered a research tool at the present time.

Discussion

Population surveys

The selection of indicators of nutritional iron deficiency has traditionally been tied to the presence or absence of anaemia (*14*). Cook et al. pioneered the use of a combination of three biomarkers (SF, % Sat and red cell protoporphyrin) for estimating the prevalence of nutritional iron deficiency in the USA (*15*). The prevalence of anaemia in a sample of 1564 volunteers living in northwestern USA was just slightly greater (10.9%) than that in the entire sample if only one parameter was abnormal. It increased to 28% with two or more abnormal parameters and to 63% when they were all abnormal. The investigators selected two of three abnormal indicators to define iron deficiency in population studies. This definition was employed in various National Health and Nutrition Examination Surveys (NHANES) in the USA (*16*). These indicators have also been employed in surveys and nutritional studies in other countries, but there has been little consistency in the way they were applied. Three indicators were not always measured. When two were measured, iron deficiency was often defined as an abnormal result for either indicator.

The TfR/SF ratio (*17, 18*) has replaced the multiple indicator method for iron status evaluations in NHANES evaluations (*19*). The method has several important advantages. It is the only method that has been calibrated against experimentally measured iron status, although it must be conceded that the observations involved only 14 adult volunteers; it provides a quantitative estimate of the iron store or iron deficit through the full iron status spectrum from deficiency to excess; haemoglobin measurements are not required to determine the severity of iron deficiency; the calculated iron store is not dependent on the selection of cut-off values; the assay methods can be automated and standardized and are potentially suitable for surveys in developing countries. There was reasonably good agreement between the prevalence of iron deficiency by the TfR/SF ratio and the former multiple indicator index in preschool children and women of childbearing age in samples drawn from NHANES 2003–2006 (*19*). The major current obstacles to the implementation of the TfR/SF ratio method as the standard approach in countries with a low prevalence of malaria and other infectious disease are incomplete standardization of the TfR assay (an international standard is available to calibrate SF assays) and the expense of the reagents required. More research is needed to determine whether the SF/TfR ratio method could also be widely applied in developing countries where malaria, HIV disease, tuberculosis and other infections are endemic. SF is less reliable as a measure of iron status in these settings because it is an acute phase protein and therefore responsive to infectious and inflammatory stimuli. However, considerable progress towards developing correction factors for this effect has been made (*20*). TfR is less affected by inflammation, but results may be confounded by changes in erythropoietic activity. Haemolysis induced by malaria is particularly important.

Haemoglobin is likely to continue to be used to screen for iron deficiency. It is therefore important to define the relationship between anaemia and iron deficiency as clearly as possible. Although it lacks sensitivity, anaemia is a useful screen for iron deficiency in women and children in Western societies because iron deficiency is the predominant cause of anaemia in these populations. It is less useful in developing countries where anaemia is multifactorial. The

general assumption is that approximately 50% of the anaemia is due to iron deficiency and that the other predominant cause is infection. It is noteworthy that the haemoglobin response to iron interventions is smaller in young children living in malarious regions when compared with regions with a low prevalence of malaria. Furthermore between 37.9% and 62.3% of baseline anaemia (haemoglobin <11 g/dL) was responsive to iron supplementation among children under 6 years of age in malarial non-hyperendemic regions; the corresponding range for malarial hyperendemic regions was lower and more variable (5.8% to 31.8%) (*21*). These differences are usually attributed to infection as a cause of anaemia. However, the possible role of α-thalassaemia carrier status, which is prevalent in these regions, should be re-evaluated.

Anaemia is not a functional outcome although correlations between anaemia and functional outcomes such as maternal mortality in pregnancy have been published (*22*). There is an urgent need to define iron status criteria that have predictive value for true functional outcomes. Possible outcomes that could be used to develop criteria for iron sufficiency are listed in **Table A2.3.2**. The first three are the most likely to prove useful.

Table A2.3.2
Iron deficiency: functional outcomes

Pregnancy outcome: increased risk of prematurity and low-birth-weight infants, and higher early neonatal mortality
Motor and cognitive developmental delays in infancy; effects on emotional maturation and later academic achievement at school
Increased risk of severe morbidity and death from malaria in young children
Impaired physical performance and reduced earning capacity
Increased prevalence and duration of upper respiratory infections in young children
Suboptimal response to iodine in populations with endemic goitre and increased risk for suboptimal thyroid function during pregnancy in iodine-deficient populations
Increased risk of chronic lead poisoning in high-lead environments
"Restless legs" syndrome

It will also be important to reassess the criteria for selecting a particular indicator or group of indicators for periods of the lifecycle during which the requirement for absorbed iron is high. The second and third trimesters of pregnancy and infancy between 6 and 12 months of age are two important examples. At these times the high functional requirements necessitate the depletion of iron stores (iron stores are consumed before absorption is optimally up-regulated; high absorption rates can only be maintained while there is little storage iron because the size of the iron store regulates the rate of absorption). Since stores will always be low, an indicator of supply such as ZPP/H or TfR may have greater utility than SF during these periods in the human lifecycle.

Impact evaluation

Mei et al. (*23*) analysed data from nine randomized, placebo-controlled iron intervention trials considered very likely to have an impact on iron status, to determine which of the following indicators showed the largest response: haemoglobin, SF, TfR, ZPP, MCV, % Sat and total body iron store calculated from the TfR/SF ratio. They concluded that haemoglobin and SF were the most efficient indicators of impact. Yang et al. (*24*) compared SF and TfR/SF ratio using data from four intervention trials. SF was adequate as an indicator although the effect sizes for the changes in iron status were significantly greater for the TfR/SF ratio in three studies. An earlier study in pregnant women also suggested greater sensitivity for the ratio method (*18*). There is,

however, good reason to be cautious before endorsing the measurement of SF as the sole indicator for impact. Changes in SF are biased towards the more iron-sufficient individuals with higher SF values (*24*). It is, however, more important to ensure that those who are most iron deficient derive the greatest benefit. Moreover, Moretti et al. (*25*) reported that the absorption of ferric pyrophosphate (FePP) is poorly up-regulated in iron-deficient volunteers. As a result absorption of iron from ferrous sulfate and FePP was approximately the same in individuals with a serum ferritin of about 50 µg/L, but three times higher from ferrous sulfate when serum ferritin levels were below 10 µg/L. Impact evaluation of a trial employing FePP could be misleading if based on SF alone. Moretti and co-investigators suggested that other water-insoluble forms of fortification iron may behave like FePP.

Conclusion

There is an urgent need for improved selection and standardization of iron status indicators. A rational approach based on the analysis of observed outcome data should be applied. The current focus on anaemia prevention in developing countries, particularly those where malaria is endemic, merits more rigorous review. More research is needed to define the relationship between functionally significant iron deficiency and anaemia in these settings. The indicators with the greatest potential at the present time are SF, TfR and ZPP/H. More research is required to define their specific applications and to develop assay methods that will allow their use in populations in developing countries and to make them affordable.

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