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Laboratory methodologies for indicators of iron status: strengths, limitations and analytical challenges[1,,2]

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Abstract

Biochemical assessment of iron status relies on serum-based indicators, such as serum ferritin (SF), transferrin saturation, and soluble transferrin receptor (sTfR), as well as erythrocyte protoporphyrin (EP). These indicators present challenges for clinical practice and national nutrition surveys, and often iron status interpretation is based on the combination of several indicators. The diagnosis of iron deficiency (ID) through SF concentration, the most commonly used indicator, is complicated by concomitant inflammation. sTfR concentration is an indicator of functional ID that is not an acute-phase reactant, but challenges in its interpretation arise due to the lack of assay standardization, common reference ranges, and common cutpoints. It is unclear which indicators are best suited to assess excess iron status. The value of hepcidin, non-transferrin bound iron, and reticulocyte indices is being explored in research settings. Serum-based indicators are generally measured on fully-automated clinical analyzers available in most hospitals. Although international reference materials have been available for years, the standardization of immunoassays is complicated by the heterogeneity of antibodies used and the absence of physico-chemical reference methods to establish “true” concentrations. From 1988 to 2006, the assessment of iron status in the National Health and Nutrition Examination Survey (NHANES) was based on the multi-indicator ferritin model. However, the model did not indicate the severity of ID and produced categorical estimates. More recently, iron status assessment in NHANES has used the total body iron stores (TBI) model, in which the log ratio of sTfR to SF is assessed. Together, sTfR and SF concentrations cover the full range of iron status. The TBI model better predicts the absence of bone marrow iron than SF concentratio alone and TBI can be analyzed as a continuous variable. Additional consideration of methodologies, interpretation of indicators, and analytical standardization is important for further improvements in iron status assessment.

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Keywords

iron analysis; ferritin; soluble transferrin receptor; total body iron; NHANES; iron methodologies

Introduction

Biochemical assessment of iron status relies mainly on serum-based indicators. The focus of indicators has largely been deficiency states, whereas questions of measuring iron repletion and iron overload have received less attention. Importantly, even though a panel of iron status indicators is routinely used in clinical practice and as part of national nutrition surveys, numerous challenges remain with the laboratory measurement and interpretation of these data.

This article provides an overview of commonly available indicators of iron status and their analytical challenges as well as discussions related to a ratio of two measures to determine status and newer indicators of iron status. Iron status assessment in NHANES is also reviewed. Currently, the Biomarkers of Nutrition for Development (BOND)[5] project aims to provide evidence-based advice to researchers with an interest in the role of nutrition in health (1). Iron is one of six nutrients in the BOND project, and the iron expert panel has assembled comprehensive information on the current state of the art with regard to specific iron biology and available indicators for assessing iron status at the individual and population level. Upon completion, this work will be available in the peer-reviewed literature.

Commonly used iron status indicators and their analysis

Nature of commonly used indicators

Iron balance is a tightly controlled process that can be reflected in a number of iron status indicators. Its hallmark is rigorous regulation of absorption. There are three main body iron compartments that describe iron status inadequacy: iron stores, transport iron (iron to meet cellular requirements), and functional iron (iron available to tissues) (see Table 1). Depletion of each compartment leads to a different iron deficiency stage (2). Short-term variations in physiological iron needs are met by the release of iron stores, the majority of which are available as intracellular ferritin, predominantly in hepatocytes and specialized macrophages. The “gold standard” indicator that provides estimates of the size of the iron store is stainable bone marrow iron, but for obvious reasons it is not a practical measurement. Serum ferritin (SF) represents a small fraction of the body’s ferritin pool (Table 1), but the concentration of SF is reflective of the level of iron stores (3). Once iron stores are depleted, the first stage of iron deficiency (ID) is reached, namely iron depletion, but there are no erythropoietic consequences yet.

[5]Abbreviations used: ACD, anemia of chronic disease; BOND, Biomarkers of Nutrition for Development; EP, erythrocyte protoporphyrin; Hb, hemoglobin; ID, iron deficiency; IDA, iron deficiency anemia; NIST, National Institute of Standards and Technology; NTBI, non-transferrin bound iron; RBC, red blood cell; SF, serum ferritin; sTfR, soluble transferrin receptor; TBI, total body iron stores; TIBC, total iron-binding capacity; Tf, transferrin; TSAT, transferrin saturation; UIBC, unsaturated iron-binding capacity; ZPP, zinc protoporphyrin.

The iron supply provided by the transport iron compartment is mainly for red blood cell (RBC) production because the demand for iron for erythropoiesis is much larger than that for other tissues. If the supply can no longer be met, the second stage of ID, namely iron-deficient erythropoiesis, is reached without showing a notable decrease in hemoglobin (Hb) concentration. Indicators that provide information about the adequacy of iron supply are transferrin saturation (TSAT), and the concentrations of erythrocyte protoporphyrin (EP) and soluble transferrin receptors (sTfR) (4). TSAT represents the percentage of binding sites on all transferrin (Tf) molecules occupied with iron molecules (Figure 1) and is calculated as the ratio of serum iron to Tf or serum iron to total iron-binding capacity (TIBC). If unsaturated iron-binding capacity (UIBC) is measured, TIBC is calculated as the sum of serum iron and UIBC. The measured plasma or serum pool of iron is the fraction of iron that circulates bound primarily to Tf. The so called non-transferrin bound iron (NTBI), iron bound to low molecular weight proteins or other compounds, usually comprises less than 1% of the total plasma iron pool and is usually not detected in most routine assays (4). EP is a generic term for either the directly measured concentration of zinc protoporphyrin (ZPP), the form present in erythrocytes, or the free EP concentration measured after extraction. Although ZPP is often referred to as an indicator of iron status, for the purposes of this manuscript EP is used mainly because NHANES did not measure ZPP, but instead measured free EP. sTfR is a truncated fragment of the transferrin receptor 1 and it circulates in plasma bound to Tf.

Hb concentration is the key indicator for a functionally important iron deficit, specifically iron deficiency anemia (IDA) (Table 1) (5). The hematocrit or packed cell volume provides no additional information beyond Hb. IDA is one of several nutritional anemias; depleted folate or vitamin B12 stores can also cause anemia and low Hb concentrations. Non-iron related anemia can also be caused by blood loss, decreased or faulty RBC production, or destruction of RBCs.

Consideration of the common indicators requires that the biological confounding caused by inflammation be taken into account. Inflammation, a highly complex biological process (6), confounds interpretation of iron status indicators, especially of SF concentration because it increases in response to inflammation as well as to increased iron stores (Table 2). Discussions are ongoing regarding how to account for the amount of inflammation when interpreting iron status in a population (6).

In uncomplicated IDA (no inflammatory response), iron stores, transport iron, and functional iron (e.g., iron available to tissues) are all reduced (Table 3). As soon as iron supply to erythropoiesis becomes insufficient, transferrin production is upregulated to increase iron transport, transferrin receptor production is upregulated to facilitate iron delivery to cells resulting in an increase in serum sTfR, and ZPP is produced instead of heme resulting in an increase in EP. SF and Hb concentrations are important indicators in uncomplicated IDA.

In anemia of chronic disease (ACD), the inflammatory response is increased, which causes iron stores to be sequestered (7). SF, being an acute-phase protein, appears normal to increased even though functional iron is reduced. Transport iron shows a mixed response with reduced serum iron and Tf, increased EP, and normal sTfR concentration. When a

combination of IDA and ACD exists, the inflammatory response is increased, iron stores are reduced to normal, and functional iron is reduced. Transport iron indicators show reduced serum iron and Tf, normal to increased sTfR concentration, and increased EP. When inflammation complicates the interpretation of iron status, it is helpful to have data on several indicators.

The same iron status indicators mentioned above are used to determine iron adequacy or repletion, but concentrations of the indicators are neither reflective of ID nor of iron overload. In iron overload, SF and iron stores are increased and functional iron is normal. Transport iron indicators show increased serum iron, reduced Tf and EP, and normal sTfR. High SF concentration has a high sensitivity but low specificity to diagnose iron overload in hemochromatosis (8–10). The high within-person variability of TSAT limits its usefulness as an initial screening test for hemochromatosis (11), however elevated TSAT identifies patients at risk for organ iron overload that are eligible for further testing. EP has been shown to be of utility in distinguishing hemochromatosis from other conditions that lead to elevated SF as a result of ACD (12).

Finally, several other confounding factors should be noted for these status indicators (Table 2). Although sTfR and EP are more specific indicators of iron deficiency erythropoiesis, both are also elevated in increased erythropoietic activity, and EP is also elevated in lead poisoning. Hb is decreased in pregnancy due to hemodilution and increased in heavy smokers, people who live at high altitudes, and in a state of dehydration. The within-person biologic variability for serum iron (~30%) is much higher than for SF (~10–25%) or even sTfR (~10%) (13–16), necessitating the analysis of more than one sample for clinical evaluation. However, this is typically not done in population surveys. Also, because of the relatively high diurnal variability of serum iron (but without a consistent pattern) (14, 17), collection of a specimen from fasting persons was recommended in the clinical setting, but was recently shown not to have an advantage over random sampling (11).

Analytical considerations

Preanalytical conditions—For serum-based iron status indicators, the preanalytical conditions required to obtain a valid sample for laboratory analysis are fairly easy to achieve (Supplemental Table 1). Whole blood needs to be refrigerated and processed the same day (iron) or within a few days (SF and sTfR) after blood collection to obtain serum(18, 19); serum is stable for at least a week at 4°C and at least a year at ≤−20°C(20–22); and the sample can be subjected to up to 3 freeze-thawing cycles without negatively affecting the biomarker concentrations (20). For optimal long-term storage, serum should be stored at ≤ −40°C. Data from our laboratory show that serum iron, SF, and sTfR were stable for at least 10 y when stored at −70°C. While the preference for a particular anticoagulant in plasma and the influence of hemolysis and other preanalytical factors (e.g., bilirubin, lipemia) on the test result may be test specific, serum is usually the preferred matrix over plasma and iron measurement is generally more affected by hemolysis than SF and sTfR. For whole blood-based iron status indicators, fresh whole blood needs to be analyzed directly for Hb or refrigerated for 1–2 d prior to analysis (23) and refrigerated whole blood is stable for a week if protected from UV light for analysis of EP (4).

Laboratory methods—The measurement of hematologic indicators in general and Hb in particular is widely available in clinical and research laboratories by flow cytometry on fully-automated cell counters. These instruments can count different blood cells with good precision and high sample throughput. Battery-operated, hand-held hemoglobinometers can provide on-the-spot results from a single drop of blood collected in the field. However, these instruments require freshly collected blood and if capillary blood is used, proper sampling technique is critical to obtaining a valid specimen.

The measurement of serum-based biochemical indicators is generally carried out on fully-automated clinical analyzers available in many laboratories. Protein-based indicators such as SF, Tf, and sTfR, are measured by immunoassays, whereas serum iron, TIBC, and UIBC are measured on chemistry analyzers using a colorimetric reaction with ferrine or ferrozine as a chromogen to form a color complex with iron. Most clinical analyzers measure UIBC because it is more easily automated than TIBC. The measurement precision of UIBC is good at high concentrations as found in iron depletion, but worse at low concentrations in the presence of iron overload (24). Outside the United States, testing for UIBC and TIBC has mostly been replaced by Tf. The precision of other biochemical indicators is good, with between-run CVs generally ≤5%.

Using clinical analyzers offers advantages, such as high sample throughput and quick turnaround time with minimum operator involvement, the availability of commercial kits for various instrument platforms, relatively low reagent cost (with the exception of sTfR), and generally good precision. Possibly the biggest caution for using commercial kits on clinical analyzers is that the user has no control over reagent lot-to-lot variation and assay reformulations. This is especially critical when the assay is employed in longitudinal studies such as national nutrition surveys, because small assay shifts could be misinterpreted as changes in population status. Another disadvantage of clinical analyzers is that the required sample volume is typically ≥150 μL, which may be problematic for pediatric or capillary samples. Lastly, clinical analyzers are moderately expensive and require regular maintenance and periodic technical service. Other less common analytical techniques to measure iron status indicators are summarized in Supplemental Table 2.

Standardization of iron status indicators—International reference materials are available for most iron status indicators from the National Institute of Standards and Technology (NIST) (for iron in form of an iron wire [SRM 937] or an iron standard solution [SRM 3126A]), the WHO through the United Kingdom National Institute of Biological Standards and Control (for Hb [IS 98/708], SF [RM 94/572], and sTfR [RR 07/202]), or the European Institute for Reference Materials and Measurements (for Tf [ERM-DA470]). The NIST reference materials provide certified values assigned by the use of high-order reference methods. However, physico-chemical reference methods are not available for the protein-based iron status indicators to establish “true” concentrations, thus value assignment has been done through consensus after analysis by common clinical assays (SF, Tf) or after determining the protein content in the reference material (sTfR). As a result, moderate (SF, Tf) to large (sTfR) assay differences, as discussed above, can be observed in proficiency testing programs and have been documented in other studies (25, 26). The difficulties in standardizing immunoassays have been reviewed (27) and include the heterogeneity of the

antibodies used resulting in different epitope specificities, technical difficulties in producing a reference material that is identical to the circulating serum form, and difficulties in measuring intact proteins by mass spectrometry, including the absence of stable-isotopically labeled proteins that can be used as internal standards.

Ratio of sTfR to SF as indicator of iron status

The ratio of sTfR to SF deserves separate discussion. This indicator is currently reported as part of the NHANES, but is less well known. There are several ways to express the ratio, and confusion exists as to how it is calculated, what it means, and what laboratory data can be used to calculate and interpret the ratio (Table 4). SF is a sensitive indicator until body iron stores (mainly in hepatocytes) are depleted, but concentrations below 12 μg/L are not indicative of the severity of the ID. On the other hand, serum sTfR is a sensitive indicator after body iron stores are depleted and concentrations keep increasing with increasing ID. Together, these 2 indicators assess the full range of iron status from severe deficiency to overload.

The logarithm of the ratio of sTfR to SF concentrations is linearly related to total body iron stores (TBI) expressed as mg/kg body weight, as shown in a unique phlebotomy study conducted in 14 healthy adult Caucasians (28). The sTfR assay used in that study was an in-house ELISA assay (29), shown to perform equivalently to the Ramco sTfR assay (30). The Ramco assay was shown to measure approximately 50% higher than the Roche sTfR assay (30).

TBI is one of several terms found in the literature for this indicator; body iron is the original term used by the investigators who developed this methodology (28), but some reports also used body iron stores or total body iron. Regardless of the term used, it is important to understand that TBI is not a measure of the quantity of iron in the individual's body. It merely provides a quantitative estimate of the size of the body iron store when iron is present in the store (values >0 mg/kg) or the size of the functional deficit that would need to be corrected before iron could again be accumulated in the store in an individual who is iron deficient (values ≤0 mg/kg). It is expressed as a continuous variable that is conceptually easy to interpret and indicates the severity of the iron deficit at the low end of the spectrum and the magnitude of the iron surplus at the high end of the spectrum. Some investigators have proposed that the term "body iron index" might be more appropriate. The formula for this relationship was validated using data from 3 published studies (31): a non-representative subset of healthy adult Caucasians participating in NHANES III (1988–1994) (31, 32), pregnant Jamaican women participating in an iron supplementation trial (33), and anemic Vietnamese women participating in an intervention trial with iron-fortified fish sauce (34). The same shortcoming of assay dependency as described above for the simple ratio also applies to the TBI indicator. TBI has been used in a study that employed an in-house multiplex ELISA sTfR assay believed to be equivalent to the Ramco assay (35) and in several NHANES data analyses that employed the Roche sTfR assay (36–38).

Alternatively, the utility of the simple ratio of sTfR to SF concentrations (both expressed in μg/L) was confirmed in an iron supplementation trial in pregnant women in Jamaica, where

the ratio of the supplemented group was significantly different from that in the nonsupplemented group (mean values of 470 vs. 1200, respectively) (33). However, because of large differences in sTfR assays, the cutpoint for the simple ratio (≤500 ample iron stores, >500 depleted iron stores) is assay dependent and can only be used with data generated by an assay that performs equivalently to the Ramco or Roche assay. The simple ratio has been used by several investigators who employed the Ramco sTfR assay (39–41) or an assay believed to be equivalent to the Ramco assay (42).

Finally, the sTfR index, calculated as the ratio of sTfR to the logarithm of SF, was introduced as an indicator to identify persons with depleted iron stores (43) and cutpoint values (mg/L) were published to distinguish between ACD (<1) and IDA (>2) or both conditions (>2) (7). The sTfR index was adopted on the Access Beckman Coulter analyzer for differential diagnosis of IDA and ACD (44). The interpretation of the sTfR index is also assay dependent. One study that employed the Roche sTfR assay (45) calculated the sTfR index, which is not appropriate because the Roche assay does not produce comparable results to the Beckman sTfR assay.

Emerging iron status indicators

Hepcidin, NTBI, and reticulocyte indices are currently viewed as experimental indicators used mainly in the research setting. Hepcidin, the central regulator of iron homeostasis, increases with increasing iron status(46, 47). Hepcidin may be clinically useful in the diagnosis of some types of anemia, in the differentiation between IDA and ACD, and in patients with iron overload syndromes (48, 49). Because hepcidin is an important determinant of dietary iron absorption, it may guide safe iron supplementation in countries with high infection burden (46). Its role in determining whether iron status is replete is unclear. Hepcidin is an acute-phase reactant (50), is suppressed in the presence of increased erythropoietic activity unrelated to iron status (51), and has a large (49%) intra-individual variability (52). Hepcidin can be measured with good reproducibility in serum or urine by mass-spectrometry assays or immunoassays, but there are considerable assay differences and no standardization yet (53). A recent inter-laboratory comparison study identified a commutable secondary reference material, which–if used as a common calibrator–could harmonize assay results to an achievable equivalence of 7.7% from the current 28.6% (53).

NTBI has a postulated role in the pathogenesis of iron toxicity due to oxidative damage and the risk for increased susceptibility to infectious diseases such as malaria (54). NTBI appears in the circulation of patients with iron overload or for a short time after the administration of an iron dose and may thus be of interest to distinguish between iron repletion and iron excess. The fraction of plasma NTBI that is redox active and can be chelated is designated labile plasma iron (LPI) (55). The measurement of serum NTBI is fraught with technical difficulties related to the determination of heterogeneous chemical forms of circulating NTBI, as demonstrated in an international round robin by considerable method differences (40-fold variation) and large analytical variation (4.4–193%) (56). A second international round robin of current leading analytical assays for NTBI and LPI indicated good assay reproducibility, but still relatively poor correlation and agreement among assays (57). Recently, a new NTBI assay system utilizing a conventional automated

analyzer was reported to have good linearity, reproducibility, and comparability with HPLC (58).

Reticulocyte indices are sensitive indicators for iron-deficient erythropoiesis(59). For example, reticulocyte hemoglobin content is useful in assessing the functional iron available for erythropoiesis during the previous 3–4 days, while reticulocyte volume is a useful indicator when monitoring the therapeutic response of anemias. Automated flow-cytometric analysis provides acceptable precision and bias, yet method-specific reference intervals have to be currently used (59). Standardization of these measurements is encouraged. Preanalytical variation (related to specimen transportation and storage) represents the major source of inaccurate test results. The high sample related uncertainty requires that reticulocytes be analyzed without delay. Reticulocyte indices have been included in the American Academy of Pediatrics guidelines for the evaluation of childhood anemia (60) and have been used in patients with chronic kidney disease to manage iron status and predict the responsiveness to intravenous iron and erythropoiesis stimulating agents (61).

Iron status assessment in NHANES

Monitoring the iron status of the U.S. population has been an important component of the NHANES since its inception in 1971. All NHANES surveys have included a battery of hematologic and biochemical indicators in order to provide the best possible assessment (Supplemental Table 3) (62, 63). A matrix describing the laboratory methods by indicator and survey cycle is shown in Supplemental Table 4. Details for each method can be found on the NHANES website (64).

Three models that employed multiple iron status indicators were developed by an expert committee to assess the iron status of the U.S. population using data from NHANES II (1976–1980) (65). One of these models, called the ferritin model, has been used with data from several subsequent NHANES surveys conducted between 1988–2006 (32, 66, 67). The other 2 models (known as the MCV model and Hb shift model) have been discussed elsewhere (65). The ferritin model uses SF, TSAT and EP. To meet the definition of ID using the ferritin model, an individual had to have abnormal values for two or more indicators. Because the model included SF, it was believed more likely to capture the early stages of ID, including iron depletion (65).

Cutpoints to define abnormal values for the indicators used in the ferritin model were validated in clinically-diagnosed patients whenever possible. This type of data was not available for children and adolescents, so cutpoints for these age groups were selected by identifying a value that kept the percentage of falsely identified individuals similar to that of women ages 20–44 y. Several types of studies provided data to validate the ferritin model for defining ID: a) comparison of anemia rates among individuals with abnormal values for <2 *vs.* ≥2 indicators used in the ferritin model(65, 68–70); b) assessment of whether the prevalence of ID (as defined by the ferritin model) followed expected patterns by selected demographic characteristics (65); c) examination of whether the prevalence of ID by the ferritin model changed in response to oral iron treatment (71); d) comparison of ferritin model prevalence with prevalence of iron depletion by estimated stores or bone marrow

staining (72, 73); and e) examination of whether including additional indicators in a regression model increased the predictive ability of the model above that observed for SF concentrations alone (74). One issue that complicates the interpretation of the preceding bone marrow studies is the use of different endpoints that may address slightly different stages in the development of ID. In specific, the ferritin model may assess a slightly later stage of ID than presence/absence of bone marrow iron stores (model includes TSAT and EP and thus detects iron-deficient erythropoiesis in addition to iron store depletion) and may therefore be expected to produce a lower prevalence. This is supported by findings from Cook et al. (72) that noted similar prevalences between the ferritin model and estimated body iron stores of −100 mg/kg.

In the early 2000s interest grew to consider a simpler approach to define iron status in NHANES. Assays for some of the indicators used in the ferritin model were labor intensive and no longer widely used, and it was becoming increasingly difficult to find laboratories that were willing to continue performing them. A simpler approach involving fewer iron status indicators may also reduce survey complexity and cost. Around that time, the validation of the TBI model was published (31), a WHO recommendation suggesting the use of SF and sTfR to assess the iron status of populations became available (75), and the fully-automated Roche sTfR assay that provided higher sample throughput and better precision than the Ramco sTfR assay was released (30). In 2005, CDC convened a workshop with experts in iron metabolism to discuss whether the ferritin model could be replaced with the TBI model starting with NHANES 2003–2004 and what the most appropriate approach to assess iron status in pregnant women would be. The advantages of using a simpler model had to be weighed against the possible effect on the ability to assess changes in iron status over time if the ferritin model was replaced. An important aspect of assessing iron status of populations is to ensure coverage of all high-risk groups. Prior to 2003, iron status indicators were measured in the entire population. From 2003 on, NHANES concentrated on assessing iron status in children ages 1–5 y and women of reproductive age (12–49 y). Pregnant women were oversampled during NHANES 1999–2006, which provided a sufficient sample size to calculate baseline estimates for an objective on ID in pregnant women for Healthy People 2010, the nation’s prevention agenda (76).

One of the strengths of the TBI model was the derivation from the direct calculation of body iron stores from serial phlebotomy (25), a “gold standard” method for assessment of TBI. Like the ferritin model, the TBI model has also been indirectly validated by: a) assessment of whether the prevalence of ID as estimated by the model followed expected patterns by selected demographic characteristics, such as age and sex (31, 72, 77); b) examination of the changes in body iron stores and the prevalence of low body iron stores in response to oral iron treatment (31); and c) comparison of SF, sTfR or the ratio of the two with absent stainable bone marrow iron in anemic patients (43, 78). Although the third group of studies provided some information, it cannot be seen as a direct validation because all of the patients were anemic and the indicators (SF and sTfR) were not combined in the same mathematical manner and may represent a different construct.

There was limited evidence that the TBI model may be valid for pregnant women: the pattern of body iron stores in pregnant Jamaican and Bolivian women followed expected

patterns (33, 77); unpublished data for US women in the third trimester of pregnancy presented at the workshop also supported its utility for this group.

The expert panel proposed that the ferritin model be replaced with the TBI model for assessing iron status in the population, including pregnant women. In order to permit secular trend analyses for Healthy People 2010, a gradual switch to the new model, with an overlap period during which both models can be applied (2003–2006), was recommended. The panel recommended that trimester-specific estimates be provided for pregnant women, regardless of which method was used to define their iron status. Table 5 provides advantages and disadvantages for these 2 models as well as a list of selected NHANES publications that used these 2 models.

Summary and research needs for iron status indicators

The laboratory assessment of iron status relies on a combination of biochemical indicators. Although it has been pointed out that there is a need to link these iron indicators better to meaningful clinical outcomes (79), such efforts must be underpinned by a sound understanding of the indicators available. Diagnosis of ID through SF concentration is complicated by concomitant inflammation, as is the case with ACD. Lack of assay standardization for sTfR, an indicator of functional ID less affected by inflammation, results in a lack of common reference ranges and cutpoints. It is unclear which indicators are best suited to assess adequate or excess iron status. Whereas laboratory methods for commonly used iron indicators are widely available and provide good precision, the comparability of results across assay platforms could be further improved for SF and require harmonization for sTfR to become a more widely used indicator. High-order reference methods and certified reference materials are needed for iron status indicators. At the same time, the availability of simple yet reliable assays that can multiplex the measurement of several indicators using minimal specimen volume would be beneficial for pediatric and capillary samples. The utility of newer iron status indicators, such as hepcidin and NTBI, is being explored mainly in the research setting, but laboratory methods for these indicators require further improvements in terms of comparability and for NTBI also in terms of the definition of the clinically most relevant forms of NTBI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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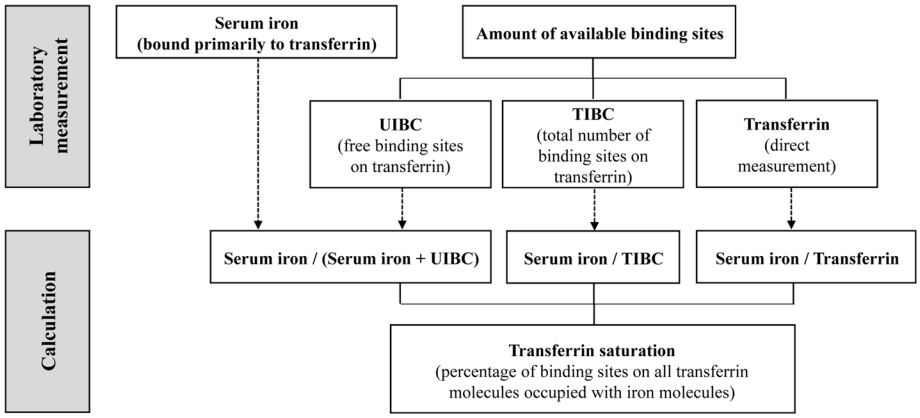

Figure 1.

Laboratory measurement of iron indicators needed to calculate transferrin saturation. TIBC, total iron-binding capacity; UIBC, unsaturated iron-binding capacity.

Table 1

Body iron compartments, their respective indicators, and the type of deficiency resulting from the depletion of each compartment

	Iron stores	**Transport iron**	**Functional iron**
Commonly used indicators	Stainable bone marrow iron	Transferrin saturation[1] (serum iron and either total iron-binding capacity, unsaturated iron-binding capacity, or transferrin)	Hemoglobin (whole blood)
	Ferritin (serum)	Protoporphyrin[2] (erythrocytes)	Hematocrit (whole blood)
		Soluble transferrin receptor (serum)	
Deficiency	*Iron depletion*	*Iron deficiency erythropoiesis*	*Iron deficient anemia*

[1]Transferrin saturation is calculated as the ratio of iron to transferrin or iron to total iron-binding capacity; if unsaturated iron-binding capacity is measured, the total iron-binding capacity is calculated as the sum of iron and unsaturated iron-binding capacity.

[2]Erythrocyte protoporphyrin can be measured directly as zinc protoporphyrin, the form present in erythrocytes, or as free erythrocyte protoporphyrin after extraction.

Table 2

Important confounders of iron status indicators[1]

Confounder	Indicator and direction of change	Comment
Inflammation	SF	Ferritin is a positive acute-phase protein
	Transferrin	Transferrin is a negative acute-phase protein
	Iron	
	EP	Release of cytokines leads to increased uptake and retention of iron in reticuloendothelial system cells, e.g., iron becomes sequestered and is not available for transport to the bone marrow for erythropoiesis
	Hb	
Increased erythropoietic activity	EP, sTfR	In thalassemia, sickle cell anemia, hemoglobinopathies
Lead poisoning	EP	Lead blocks the formation of heme and zinc protoporphyrin forms instead
Pregnancy	Hb	Plasma volume expansion results in hemodilution.
Dehydration	Hb	Volume of fluid in blood drops and hemoglobin artificially rises
Smoking	Hb	Compensation for decreased oxygen intake in heavy smokers
Altitude	Hb	Compensation for decreased oxygen intake due at high altitude

[1]EP, erythrocyte protoporphyrin; Hb, hemoglobin; SF, serum ferritin; sTfR, soluble transferrin receptor.

Table 3

Response of iron status indicators to a depletion of body iron compartments with and without concomitant inflammation and to an overload of body iron compartments[1]

Compartment	Indicator	IDA	ACD	IDA + ACD	Overload
Stored iron	SF	Reduced	Normal to increased	Reduced to normal	Increased
Transport iron	Iron	Reduced	Reduced	Reduced	Increased
	Transferrin	Increased	Reduced	Reduced	Reduced
	TSAT	Reduced	Reduced	Reduced	Increased
	EP	Increased	Increased	Increased	Reduced
	sTfR	Increased	Normal	Normal to increased	Normal
Functional iron	Hb	Reduced	Reduced	Reduced	Normal
Inflammatory response	n/a	Normal	Increased	Increased	n/a

[1]ACD, anemia of chronic disease; EP, erythrocyte protoporphyrin; Hb, hemoglobin; IDA, iron deficiency anemia; IDA + ACD, combined iron deficiency anemia and anemia of chronic disease; n/a, not applicable; SF, serum ferritin; sTfR, soluble transferrin receptor; TSAT, transferrin saturation

Table 4

Approaches to express and interpret the ratio of soluble transferrin receptor to serum ferritin[1]

Ratio	Calculation	sTfR assay used to establish ratio	Cutpoint value and definition
Total Body Iron[2] (mg/kg)	− [log(sTfR/SF) – 2.28229]/0.1207	In-house ELISA that is equivalent to Ramco assay and has known relationship to Roche assay	≤0: iron deficit >0: iron surplus
Simple ratio[2]	sTfR/SF	In-house ELISA that is equivalent to Ramco assay and has known relationship to Roche assay	≤500: ample iron stores >500: depleted iron stores
sTfR index (mg/L)	sTfR/log SF	First ELISA from R&D Systems; then adopted on Access Beckman Coulter analyzer	<1: ACD >2: IDA or IDA + ACD

[1]ACD, anemia of chronic disease; ELISA, enzyme linked immnosorbent assay; IDA, iron deficiency anemia; IDA + ACD, combined iron deficiency anemia and anemia of chronic disease; SF, serum ferritin; sTfR, soluble transferrin receptor.

[2]Units of both sTfR and SF in the ratio are μg/L

Table 5

Characteristics and selected NHANES publications for the serum ferritin and total body iron model

Ferritin model	Total body iron model
Model characteristics	
• Model does not indicate degree of severity of iron deficiency	• Model assesses entire range of iron status
• Model may assess slightly later stage of iron deficiency than presence or absence of bone marrow iron stores (i.e., lower prevalence than serum ferritin alone)	• Model derived from direct calculation of body iron from serial phlebotomy, a “gold” standard method to assess body iron
• Model may detect iron deficient erythropoiesis in addition to iron store depletion	• Model better predicts absence of bone marrow iron than serum ferritin alone
• Model produces categorical yes/no estimate only	• Model may provide better estimate of impact of iron intervention (amount of iron absorbed)
	• Total body iron can be analyzed as continuous variable
Selected NHANES publications	
• NHANES III (1988–1994) prevalence of iron deficiency in persons 1 y and older (32)	• NHANES 2003–2006 iron deficiency in children 12–24 mo and nonpregnant women 12–49 y of age (36)
• NHANES 1999–2000 iron deficiency in persons 1 y and older (66)	• NHANES 1999–2006 iron status in pregnant women 15–39 y of age (37)
• NHANES III (1988–1994) *vs.* 1999–2002 anemia prevalence among children 12–59 mo and women 20–49 y of age (67)	• NHANES 2007–2010 iron, anemia, and iron deficiency anemia among children 1–5 y of age (38)