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The nutritional assessment of iron status

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SUMMARY. In nutritional studies to assess the prevalence of iron deficiency, it has been common practice to define 3 stages of increasing severity: iron storage depletion as defined by a low serum ferritin, mild iron deficiency without anemia based on laboratory evidence of iron deficient erythropoiesis (IDE), and overt iron deficiency anemia (IDA). While this approach provides a broad perspective of impaired iron status, the main liabilities of iron lack are associated only with the more advanced stage of IDA. Consequently, the hemoglobin determination can be used to screen for nutritionally significant iron deficiency. Having identified anemia, more specific laboratory studies are needed to establish iron lack as the cause. The traditional measurements of iron deficient erythropoiesis (IDE) such as a low transferrin saturation, elevated erythrocyte protoporphyrin, or decreased mean corpuscular volume are commonly used. The major drawback in using these parameters is that they are affected similarly in individuals with the anemia of chronic disease (ACD), a common form of anemia in low socioeconomic populations. Because iron stores are invariably absent in individuals with uncomplicated IDA, a low serum ferritin concentration below 20 µg/L confirms the diagnosis of IDA when anemia is present. The main limitation of the serum ferritin is that it is falsely elevated to within the normal range when IDA develops in individuals with concurrent infection or chronic inflammation. When this occurs in a clinical setting, a bone marrow examination is commonly performed to identify IDA. Recent investigations indicate that this cumbersome procedure can be avoided by measuring an important new iron-related measurement, the serum transferrin receptor (TfR). Because the synthesis of TfR is upregulated with tissue iron deficiency, IDA can be identified readily by an elevated serum TfR. Importantly, the serum TfR is normal in individuals with the ACD but becomes elevated if these individuals develop IDA. The optimal combination of laboratory measurements for detecting IDA is the hemoglobin, serum ferritin and serum TfR.
Keywords: Iron deficiency anemia, ferritin, protoporphyrin, ànemia, iron deficiency, assesment, iron status.

RESUMEN. Evaluación del estado nutricional de hierro. En estudios nutricionales para evaluar la deficiencia de hierro, se han definido comunmente tres estadios de severidad creciente: agotamiento de las reservas de hierro, definido por bajos niveles de ferritina sérica, deficiencia de hierro moderada sin anemia basada en evidencia de laboratorio de eritropoyesis deficiente en hierro (EDH) y anemia por deficiencia de hierro (ADH). Aunque esta clasificación provee una amplia perspectiva de la alteración de estado de hierro, las principales consecuencias de la falta de hierro están asociadas con estadíos más avanzados de anemia por deficiencia de hierro. Consecuentemente, la determinación de hemoglobina puede usarse para tamizar la deficiencia de hierro nutricionalmente significativa. Una vez identificada la anemia, estudios de laboratorio más específicos son necesarios para establecer la falta de hierro como causa. Las mediciones tradicionales de eritropoyesis deficiente en hierro (EDH) tales como una baja saturación de transferrina, elevada protoporfirina eritrocitaria, o la disminución del volumen corpuscular medio se utilizan comúnmente. La principal desventaja de estos parámetros es que éstos están afectados de manera similar en individuos con la anemia de enfermedades crónicas, una forma común de anemia en poblaciones de bajo nivel socioeconómico. Debido a que las reservas de hierro se encuentran ausentes en la anemia por deficiencia de hierro sin complicaciones, una concentración de ferritina sérica por debajo de 20 µg/L confirma el diagnóstico de anemia por deficiencia de hierro una vez que la anemia está presente. La principal limitación de la ferritina sérica es que puede encontrarse falsamente elevada o en límites normales cuando la anemia por deficiencia de hierro se desarrolla en individuos con infección o inflamación crónica concurrente. Cuando esto ocurre en el medio clínico, un examen de médula ósea se realiza comúnmente para identificar la ADH. Las investigaciones recientes indican que este procedimiento puede evitarse midiendo un nuevo indicador relacionado al hierro llamado receptor sérico de transferrina (RTf). Debido a que la síntesis de RTf está regulada paralelamente con la deficiencia de hierro tisular, la ADH puede identificarse rápidamente por niveles elevados de RTf. Es importante destacar que el RTf es normal en individuos con anemia por enfermedades crónicas pero aumenta si estos individuos desarrollan ADH. La combinación óptima de las mediciones de laboratorio para detectar la ADH son la hemoglobina, la ferritina sérica y el RTf sérica.
Palabras clave: Deficiencia de hierro, evaluación, estado nutricional, ferritina, protoporfirina, anemia.

INTRODUCTION

Reliable laboratory methods to evaluate iron status are of critical importance in efforts to improve the iron nutrition of a population. In countries where iron deficiency is less common, iron measurements are needed to assess prevalence rates in susceptible segments of the population and to monitor changes in iron status over time. In developing countries where iron deficiency is more prevalent, reliable measurements of iron status play an important role in the assessment of new intervention strategies. The iron measurements that are suitable for epidemiological studies have undergone a continual refinement during the past several decades and newer methods with greater specificity and sensitivity for identifying iron deficiency have been added. There are several considerations when selecting iron-related methods such as cost, suitability for capillary blood sampling, ease of laboratory performance and adaptability for field studies. There is no single method or combination of methods that is satisfactory for all purposes. The following discussion of available iron measurements will emphasize the measurement of serum transferrin receptor, an important new method for assessing iron status.

Definitions of iron status

The initial stage of iron lack is storage iron depletion in which the continuous supply of iron for hemoglobin production is adequate but no buffer of body iron reserves exists to cover short-term needs. The only practical method for identifying this early stage of iron deficiency in population studies is the serum ferritin concentration that varies directly with iron stores in otherwise normal individuals (1,2). Values below 10-20 μg/L indicate absent iron stores. The main limitation of the serum ferritin is that chronic infection or inflammation elevates the concentration 2-3 times higher than values representing iron stores (3). In developing countries where inflammatory diseases are more common, cut-off levels of 30 μg/L or higher have been used but these modified definitions should be established on the basis of bone marrow examinations or therapeutic iron trials (4).

A curtailment in the supply of transferrin-bound iron to developing red blood cells is referred to as iron deficient erythropoiesis (IDE). The serum iron, total iron-binding capacity (TIBC), and the transferrin saturation calculated as the ratio of serum iron/TIBC are the traditional indices of IDE. Unfortunately, these iron transport measurements are affected by numerous physiological and pathological processes and consequently have low specificity for identifying iron deficiency as the cause of IDE. Their major value is in excluding iron deficiency as the cause of anemia when the transferrin saturation is normal or increased. An elevated free erythrocyte protoporphyrin is more specific for iron deficiency but this measurement is increased with excess lead exposure as well as in the anemia of chronic disease (ACD). Microcytosis of circulating red blood cells as reflected by a low mean corpuscular volume is a useful index of IDE, but like most other laboratory measurements, it does not distinguish iron deficiency from inflammatory diseases.

The most advanced stage of iron lack is iron deficiency anemia (IDA), the severity of the anemia reflecting the degree of iron lack. Few, if any, of the nutritional consequences of iron deficiency such as decreased learning capacity in infants, impaired work performance in adults, and increased perinatal morbidity and mortality have been demonstrated in absence of anemia. Consequently, the hemoglobin concentration is an indispensible index of the impact of iron deficiency on health and well-being. The hemoglobin concentration should not be the only index of iron status because there are numerous diseases and deficiency states that result in anemia. One of the key laboratory measurements that is often used in tandem with hemoglobin to identify IDA is the serum ferritin assay. The combination of hemoglobin and serum ferritin measurements was used in a highly effective manner by Layrisse and coworkers to assess the efficacy of a large-scale program of food iron fortification in Venezuela (5). However, as discussed previously, falsely normal or elevated levels due to chronic inflammation diminish the utility of using only the serum ferritin and hemoglobin to identify IDA. The development of the serum transferrin receptor (sTfR) assay has circumvented many of the problems in identifying IDA in populations where infections and IDA are common.

Serum Transferrin Receptor

The movement of iron within the body is controlled by a specific membrane receptor for transferrin iron that varies in amount with the iron needs of the cell. The presence of a circulating form of this protein was first reported by a group of Japanese workers (6). Later investigators demonstrated that the serum form is a soluble fragment containing most of the large extracellular domain of the transferrin receptor (7). There have been several extensive reviews of this new iron-related measurement (8-10). The concentration of the serum transferrin receptor (sTfR) is directly proportional to the total body mass of transferrin receptor, 80% of which is derived from the red cell precursors in the bone marrow. There are only two conditions that elevate the concentration of the sTfR - an increase in red cell precursors in the bone marrow (erythropoiesis) and tissue iron deficiency. There are many forms of anemia that are associated with enhanced erythropoiesis but these are not encountered frequently enough in population studies to diminish the usefulness of the sTfR for identifying IDA.

Serial phlebotomy studies in normal adults have shown that the sTfR concentration remains normal during the progressive depletion of iron stores (11). With the onset of tissue iron deficiency, the sTfR concentration increases in direct proportion to the severity of the deficiency. Because of the reciprocal changes in ferritin and sTfR at varying levels of body iron, the ratio of the logarithm of the receptor/ferritin

ratio bears a precise inverse relationship to body iron levels over a wide range from iron repletion to advanced IDA (11). This observation is hardly surprising in view of the tight reciprocal regulation of the synthesis of these two key iron proteins by means of the iron regulatory proteins (12). When the supply of iron to tissues is adequate, ferritin synthesis is upregulated to store any excess of intracellular iron. When the supply of iron to body tissues is inadequate, ferritin synthesis is reduced and the synthesis of transferrin receptor is enhanced to allow the cell to compete more effectively for the transferrin-bound iron in its environment.

The laboratory methods for measuring the sTfR are similar to the sensitive immunoassays that are used to assay the serum ferritin, differing only in regard to the immunological reagents. There are number of assays for the sTfR that are available commercially but, unfortunately, there is a wide range of reported normal values. The urgent need for standardization of sTfR assays has been repeatedly emphasized in the published literature (13). One important advantage of using the sensitive immunological assays of ferritin and sTfR is that only a few microliters of plasma or serum is required. In a recent investigation, ferritin and receptor measurements were performed on capillary blood spotted onto filter paper and allowed to dry (14). The sTfR results were identical to the spotted samples after correction was made for displacement of serum by red cells. However, the spotted ferritin values were significantly higher than serum ferritin values due to the release of ferritin from hemolyzed red blood cells. This problem was circumvented in part by using the receptor/ ferritin ratio of the spotted blood samples that proved to be as reliable as serum determinations for distinguishing normal subjects from those with IDA. The distinction between milder iron deficiency without anemia and either normal or IDA was less satisfactory with spotted samples than with serum. Recent studies have shown that the latter difficulty can be circumvented by using spotted capillary plasma samples rather than whole blood (15). However, in certain field conditions, the requirement for centrifugation of samples could be difficult. The use of capillary blood samples to prepare either whole blood or serum paper spots offers a significant advantage in field surveys by eliminating the need for venous sampling and thereby simplifying the storage and transport of specimens.

As mentioned previously, one problem in assessing iron status in lower socioeconomic segments of a population is the difficulty in distinguishing IDA from the ACD. The latter form of anemia, which is associated with a wide spectrum of chronic inflammatory or infectious disorders, is encountered frequently in clinical practice. The pathogenesis of this common anemia is still unclear (16,17). In one study in anemic patients above 65 years of age, 36% had IDA while 44% had ACD based on bone marrow examinations, the conventional method of making this distinction (18). Recent clinical studies indicate that this cumbersome procedure can be circumvented by measuring the sTfR.

An initial evaluation of the sTfR in anemic patients with inflammatory illnesses such as rheumatoid arthritis or chronic bacterial infections demonstrated that the sTfR remains normal in contrast to the distinct elevation in patients with IDA (19).

In a subsequent report, 129 anemic patient underwent bone marrow examinations to assess their iron status (20). The three diagnostic categories included 48 patients with IDA, 64 with the ACD, and 17 with both IDA and the ACD. The best separation between the groups was obtained with the ratio of receptor/ferritin that distinguished between patients with IDA and ACD and with a single exception, between those with ACD and both IDA and ACD. The key finding in this study was that the ratio of receptor/ferritin can be used to identify IDA even in the presence of inflammatory disease.

The utility of the sTfR is not limited to distinquishing IDA from other forms of anemia. Recent investigations have demonstrated its usefulness in the assessment of milder iron deficiency without anemia (21,22). These studies support the earlier work of Skikne et al. who demonstrated that the sTfR becomes elevated during the progression of iron deficiency well in advance of developing overt IDA (11).

CONCLUSIONS

Several recent clinical investigations indicate that tandem measurements of serum ferritin and sTfR offer major advantages in identifying IDA in population studies. These measurements have the practical advantage of requiring only a small sample of capillary blood and thereby permitting the transport and storage of samples on filter paper.

A significant limitation of the sTfR is poor standardization between laboratories and commercially available assay kits. In addition, more information is needed about the reliability of the receptor/ferritin index in the presence of other nutrient deficiencies such protein, vitamin A, folic acid, or vitamin B_{12}. Despite these limitations, the initial experience with coupled measurements of serum ferritin and sTfR suggest that this approach is a meaningful advance in our ability to assess the nutritional iron status of a population.

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