Refining the assessment of body iron status\(^1,2\)

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Body iron is found in three compartments. Metabolically active iron in iron-requiring proteins and enzymes is termed the functional compartment. The largest component of this compartment is hemoglobin in erythroid elements. Iron absorbed from the gut in excess of that required for the functional compartment is incorporated into storage proteins constituting the storage compartment. Ferritin is the major storage protein. The reticuloendothelial system and the liver are the major storage tissues. Storage and functional compartments are linked by a small transport compartment consisting of transferrin iron. Transferrin iron is delivered to tissues via transferrin receptors expressed on individual cells. Liabilities of iron deficiency correlate only with functional compartment depletion. To accurately assess body iron it is imperative to understand which compartment any given test is able to evaluate.

Soluble ferritin is released from cells in direct proportion to cellular ferritin content. The molecular identity and genetic origin of this serum ferritin are matters of ongoing study. Serum ferritin is readily measured by assays for L-subunit-rich tissue ferritin and is directly proportional to the size of the storage compartment. Serum ferritin is the most applicable measurement for establishing the size of the storage iron compartment.

A soluble transferrin receptor is also released in direct proportion to the number of cellular receptors expressed. It is an 85-kDa truncated extracellular domain of the receptor produced by proteolytic cleavage by a serine protease predominantly at the surface of the exosome within the multivesicular body (1). This serum receptor is the single best measure of the functional compartment.

The cellular content of both ferritin and transferrin receptor is precisely and reciprocally regulated at a posttranscriptional level through interaction of an iron-response-element binding protein (IREBP) with iron-responsive elements (IREs) of ferritin and transferrin receptor mRNAs (2). Non-iron-saturated IREBP has mRNA binding activity whereas when saturated, IREBP is a cytoplasmic aconitase lacking mRNA binding capacity. IREBP interaction with the IRE at the 5' end of ferritin mRNA reduces ferritin synthesis by limiting polysome formation, binding to IREs at the 3' end of transferrin receptor mRNA increases receptor synthesis by stabilizing the mRNA. Given this reciprocal regulation, it is predictable that a close inverse relation will exist between serum transferrin receptor and serum ferritin.

The first serum receptor assay, a two-site immunoradiometric assay (IRMA) that made use of the commercially available monoclonal antibodies OKT9 and B 3-25, gave mean values of 251 and 256 µg/L in healthy males and females, respectively (3). An enzyme-linked immunoassay (EIA) with monoclonal antibodies produced against purified placental transferrin receptor gave mean values that were 20-fold higher, 5.6 mg/L, without a sex difference (4). A polyclonal EIA also measured values in the same concentration range as the monoclonal EIA (5). In this issue, Cooper and Zlotkin (6) report on an indirect enzyme-linked immunosorbent assay that makes use of an industry-supplied monoclonal antibody and with which they obtained a mean value of 3.87 mg/L. The disparity among the IRMA and the monoclonal and polyclonal EIA is due to a systematic difference in the IRMA between free and bound receptor. Whereas the EIA are based on antibodies raised to receptor-ligand complexes and adsorbed with ligand, the IRMA is based on antibodies raised to membrane-bound receptor, which is largely free of ligand. Serum transferrin receptor always circulates bound to ligand. Despite these differences, the first three assays show remarkably similar relative changes in response to disordered erythropoiesis. Erythropoietic disorders are not reported in the study by Cooper and Zlotkin (6).

The serum transferrin receptor assays evaluated thus far indicate that iron deficiency is associated with a three- to fivefold increase in transferrin receptor concentration. In a careful phlebotomy study to sequentially reduce body iron, serum ferritin concentration fell progressively until the storage compartment was depleted (7). This equates with a serum ferritin concentration of 12 µg/L. During this period of storage depletion the serum transferrin receptor concentration changed little. As functional compartment depletion progressed serum receptor showed a highly predictable increase proportional to the magnitude of the iron deficit (7). By contrast, serum ferritin showed little change. Between initial storage iron depletion and the development of anemia, serum receptor provides the only accurate assessment of functional compartment depletion. Other measurements, including mean cell volume, red cell...
distribution width, and free erythrocyte protoporphyrin are less sensitive, less predictable, and later indicators than is the serum receptor.

Because serum ferritin is sensitive to storage compartment change whereas serum receptor reflects the functional compartment, it is logical to combine the two in a ratio. Given their reciprocal regulation, the ratio appears to have a solid theoretic basis. When data from the phlebotomy study (7) are used, the ratio of receptor to ferritin describes a perfect log-linear relation to body iron, ranging from < 100 in the presence of stores to > 2000 once significant functional depletion occurs. A median ratio of 500 corresponds with the point of depleted stores. The utility of the ratio was further confirmed in a recent iron-supplementation study in pregnant women in Jamaica in which the ratio in the supplemented group (mean value: 470) was strikingly different from that in the nonsupplemented group (mean value: 1200) (8).

Serum transferrin receptor shows great promise for evaluating iron status in persons in whom this has traditionally been difficult, including growing children, pregnant women (9), and persons with anemia of inflammation (10). In the former two situations, iron stores are usually depleted, making serum ferritin a relatively unhelpful index. An independent indicator of the functional compartment greatly facilitates the definition of iron status. In inflammation, serum ferritin is spuriously elevated because it is an acute-phase reactant. Consequently, iron evaluation of aspirated bone marrow was previously required to resolve the anemia of inflammation from that of iron deficiency. Serum receptor remains normal in inflammation; thus, measurement of serum receptor is the only noninvasive test for resolving these two anemias. When the two conditions coexist, serum receptor tracks the functional iron deficit.

In the accompanying article (6), serum receptor is shown to be a robust measurement with little interassay and intrasubject variability. It will be important to evaluate perturbations of erythropoiesis and iron status with the current assay. If observations of stability are found consistently, the utility of the serum receptor measurement will be further enhanced. This is particularly important in relation to a recently reported application of the receptor measurement in which it and serum ferritin were able to identify erythropoietin abuse in athletes (11). If a single sample is sufficient for a reliable and accurate measurement, then the value of the assay as a screening mechanism is greatly enhanced.

REFERENCES