The Measurement and Interpretation of Serum Ferritin*

DONALD T. FORMAN, PH.D. and SHARON L. PARKER, PH.D.†

Departments of Pathology & Biochemistry, and
†Medical Allied Health Professions,
University of North Carolina, School of Medicine
Chapel Hill, NC 27514

ABSTRACT

Determination of serum ferritin is an important means of assessing body iron stores. Trace amounts of ferritin normally present in serum are detectable by sensitive radioimmunoassay techniques or an enzyme immunoassay procedure. Ferritin normally accounts for no more than a very small fraction of the total iron in serum, but generally maintains a stable concentration that is proportional to the much larger pool of storage iron in tissues. The serum ferritin assay, in contrast to other measurements of iron status such as hemoglobin, serum iron and iron-binding capacity, can distinguish differences in iron stores within the physiological range. In iron deficiency anemia, the concentration is below 10 ng per ml. Increased concentrations (above 200 ng per ml) are found in conditions with increased iron stores. The information it provides is similar to that obtained from bone-marrow aspirates stained for iron. In contrast to the percent transferrin-saturation and concentration of erythrocyte protoporphyrin, ferritin concentrations become abnormal before exhaustion of mobilizable iron stores and before the onset of anemia. Serum ferritin also provides a practical means of assessing new programs of iron supplementation, since it reflects various degrees of iron deficiency and overload.

Introduction

Ferritin is the major iron storage protein of mammalian tissues. Of the four to five grams of total body iron in the normal adult, approximately 65 percent is bound as hemoglobin, 15 to 20 percent as ferritin, three to five percent in bound myoglobin and the remainder as hemosiderin and iron containing enzymes. Ferritin is found predominantly in the liver, spleen, and bone marrow where it functions in recycling iron for hematopoiesis. It is ubiquitously distributed in many other tissues where its highly specialized protein structure enables it to sequester and store iron intracellularly. Increases in cellular iron result in the rapid synthesis of apoferritin, whereas iron depletion
syndromes result in the mobilization of iron from its storage proteins with a subsequent decrease in tissue ferritin.

Ferritin consists of a large spherical shell of 24 single protein subunits surrounding an inner core of insoluble ferric phosphate. The iron-free protein, apoferritin, has a reported molecular mass of about 450,000, which increases about twofold when the protein is fully saturated with iron. The iron appears to pass to and from the inner core through small channels in the protein shell. Recent data indicate that the ferritin can exist in multiple molecular forms (isoferritins) in different tissues. The various isoferritin molecules may represent varying percentage compositions of two types of subunits in the 24 subunit structure of the protein. The more basic isoferritins (L subunit) predominate in liver and spleen while the acidic forms (H subunit) are present in highest concentration in heart, kidney and certain malignant tissues. The physiological significance of these tissue isoferritins is not clear; however, the analytical significance may be very important because the isoferritins appear immunologically distinct, and these immunological differences in isoferritin populations may have important consequences.

Small, but clinically important concentrations of ferritin are detectable in serum in both normal and pathological conditions, with the concentration of serum ferritin being directly proportional to the level of body iron stores and the stainable iron in bone marrow. Consequently, the principal use of serum ferritin is in the diagnosis and management of iron deficiency and iron overload. This noninvasive technique, which is relatively simple and sensitive, can also provide the clinician and patient more information than serum iron levels, transferrin saturation, hemoglobin, hematocrit, red-cell indices, and erythrocyte protoporphyrin levels. The assay is particularly useful in differentiating true iron deficiency anemia from the various anemias of chronic diseases.

Methodological Aspects of Serum Ferritin Assays

Several procedures are available for the immunological quantitation of serum ferritin. These include a two-site immunoradiometric assay,* radioimmunoassay—competitive binding assay,† and an enzyme immunoassay procedure in development. The principle of the modified two-site immunoradiometric assay originally introduced by Addison is a two-stage reaction. Stage 1 involves the binding of human serum ferritin to a solid phase (plastic beads) and antihuman ferritin. Stage 2 involves the binding of radio-labelled (125I) antihuman ferritin with the insoluble antihuman ferritin complex generated in Stage 1. The solid phase is then washed, counted in a gamma counter, and the ferritin concentration is calculated by comparison to a standard curve.

The radioimmunoassay technique is a competitive binding assay which utilizes a precipitating antiserum reagent to separate antibody-bound tracer from unbound tracer. The procedure is based on the competitive binding principles of radioimmunoassay as described by Yalow and Berson. Non-radioactive ferritin from patient samples, ferritin standards, and controls compete with a constant amount of (125I) ferritin tracer for binding sites on the ferritin antibody, which is held at a limiting concentration. The amount of labelled ferritin tracer which will bind to the antibody is inversely proportional to the amount of non-radioactive ferritin in the assay tube. A precipitating reagent solution containing a second antibody in a

* Fer-Iron, Ramco Laboratories, Inc., Houston, TX 77098.
† GammaDab-Ferritin RIA, Clinical Assays, Cambridge, MA 02139.
polymer solution is used to separate the antibody bound (\(^{125}\)I) ferritin from unbound labelled ferritin by immunoprecipitation. The antibody-bound and labelled ferritin is counted. A standard curve is prepared in a like manner and the concentrations of the patients samples are determined from the standard curve.

The enzyme immunoassay technique\(^2\)\(^-\)\(^9\) for human serum ferritin employs an antibody adsorbed on a solid phase. Adsorbed antibody against human ferritin is first allowed to react with ferritin. A second antiferritin antibody, which is labelled with an enzyme, is added. At least three enzymes, alkaline phosphatase, B-galactosidase, and horseradish peroxidase, have been used. The binding of the enzyme labelled antibody to the ferritin alters the activity of the enzyme. The resultant enzyme activity is correlated to ferritin concentration by means of a standard curve.

This technique has the advantage of using stable reagents that can be stored for many months at 4°C, and it eliminates the need for constant labelling of antigen or antibody with radioactive iodine. Intra-assay variation showed a reproducibility of less than 11 percent.\(^9\) The sensitivity of this technique appears sufficient for clinical purposes.

Each type of assay has its own attributes and problems with as large and complex an antigen as ferritin.\(^4\) In addition, there is a specificity problem owing to the immunological differences in the tissue ferritins used as immunizing agents or standards in the serum ferritin procedure. Since ferritin occurs in multiple molecular forms (isoferitins), and these can change markedly during development, iron status, and in malignancy, these differences can have important consequences for the standardization and interpretation of serum ferritin assays and for attempts to quantitate selectively distinct isoferitin populations (figure 1).

**Discussion**

Ferritin in serum was first reported\(^17\) in patients with liver damage in 1956. Its presence as a component of normal serum

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![Figure 1](https://example.com/figure1.png)
has been established after the development of sensitive radioimmunological techniques.

The positive correlation of serum ferritin levels with body iron stores, as assessed by marrow or liver biopsy Prussian Blue staining, has resulted in its increased use as a diagnostic aid in assessing the physiological status of iron stores. The mechanism of serum ferritin release is unknown. It may result from an active secretory process or from normal cell turnover or lysis. Several factors suggest that serum ferritin differs from tissue ferritin: (1) serum ferritin binds to Concanavalin A, whereas tissue ferritins have little binding affinity; (2) most of the serum ferritin contains little ferric iron even though tissue ferritins may be saturated with iron; (3) serum ferritin consists largely, if not entirely, of the relatively basic L subunit, but tissue ferritin contains both basic and acidic (H) subunits; and (4) approximately 25 percent of the liver ferritin is synthesized within membrane-bound polysomes. These differences in serum and tissue ferritins might suggest an active secretory process. However, it cannot be ruled out that these differences in physicochemical properties are simply a matrix effect.

In normal individuals, the levels of serum ferritin depend on age and sex. At birth, serum ferritin levels are approximately 100 ng per ml. This level falls during the first few months of life, but then returns to the 100 ng per ml range in young adults. Serum ferritin levels can also vary in females during the menstrual cycle and pregnancy. The reference range in normal adult males is 15 to 200 ng per ml with a mean of 90 ng per ml. Females have a lower range of 10 to 200 ng per ml and a mean of 50 ng per ml. Values in post-menopausal women and men tend to converge and continue to increase slightly with age. Serum ferritin levels under 10 ng per ml are found in iron deficiency and values in excess of 3,000 ng per ml are frequently found in iron overload.

Clinically, the principal use of serum ferritin is in the diagnosis and management of patients with iron deficiency and iron overload. Iron deficiency is present in more than 10 percent of women in the childbearing age. It is convenient to classify iron deficiency into three stages of increasing severity. The first is iron depletion, which is defined as the absence of free iron in the marrow. This early stage is reflected by the absence of Prussian Blue stainable iron in the bone marrow or a fall in serum ferritin below 10 ng per ml. With continuing iron loss, the transferrin saturation (serum iron/total iron-binding capacity) falls and the second stage of iron deficient erythropoiesis develops. While there is some impairment of hemoglobin synthesis at this stage, resulting in hypochromic microcytic red cells, the hemoglobin concentration is maintained in the normal range. As red cell production is further decreased, the final stage of iron deficiency anemia occurs as reflected in a significant fall in circulating hemoglobin. The laboratory features of the three stages of iron deficiency are summarized in table I.

There are several aspects regarding serum ferritin levels in iron deficiency
that should be emphasized. The first is that the level falls below 10 ng per ml in the earliest stage of iron depletion; however, as the free iron stores become exhausted, the level no longer reflects the severity of the iron deficiency state. The second point is that if iron deficiency anemia is not complicated by other clinical disorders, serum ferritin is highly specific for iron depletion. In one study of 32 patients with iron deficiency anemia, none had a value which exceeded 14 ng per ml.13

Because serum ferritin is essentially equivalent to a marrow examination for stainable iron, its diagnostic value is in clinical situations where the inconvenience, expense, and discomfort of a marrow examination is not desired. Another application for the measurement of serum ferritin is in the anemic patient with laboratory evidence of iron deficient erythropoiesis (microcytic hypochromic anemia and low transferrin saturation or serum iron/total iron-binding capacity). This hematologic picture occurs not only in true iron deficiency but also in clinical disorders such as chronic infection, inflammation, or malignancy where there is decreased iron release from the reticuloendothelial (RE) cell.11 This anemia of chronic disease is distinguished from true iron deficiency by an increase rather than a decrease in marrow iron, and the serum ferritin rises in parallel.11 Bone marrow sampling to exclude a defect in RE iron release in patients with suspected iron deficiency anemia may not be necessary when serum ferritin is less than 10 ng per ml.

There are certain disorders in which the serum ferritin concentration is disproportionately increased in relation to marrow iron stores (e.g., patients with infection or inflammation13 and patients with acute or chronic liver disease16). In these instances, serum ferritin at each level of marrow iron was increased about three-fold, as compared with hospitalized controls. However, even under these conditions the correlation between marrow iron and serum ferritin is still valid. When iron deficiency occurs in patients with infection or liver disease, a normal serum ferritin can be found despite absent iron stores. Serum ferritin assays have found an important role in monitoring iron balance in patients with renal failure treated by hemodialysis9 where iron deficiency commonly occurs as a result of blood loss.

The inherited form of iron overload, idiopathic hemochromatosis, is quite rare as compared to iron deficiency.11 Clinical detection of this condition is important because it can be effectively treated, and tissue damage from iron excess can often be reversed. Preclinical iron overload has been difficult to detect in the past because the available tests were unreliable or difficult to perform. Tests which have been utilized include transferrin saturation (serum iron/total iron-binding capacity), which is the least invasive, but it is a poor measure of body iron stores. Other tests that have been employed include urinary iron excretion following desferoxamine, which is a complex technique, and liver biopsy for stainable iron.

Serum ferritin measurements can play a major role in the early detection of idiopathic hemochromatosis.15 In patients with proven disease, the level is invariably greater than 2,000 ng per ml, and thus provides a distinct separation from normal individuals.

Once iron storage disease is diagnosed, serum ferritin has been found to be useful in monitoring iron stores during therapeutic phlebotomy. There is an orderly decline in the level during treatment which reaches the low range of normal when excess iron has been removed. Transferrin saturation is not usually helpful for this purpose because the levels often remain elevated in these patients despite normal body iron stores. Serum ferritin, on the other hand, can detect precisely the therapeutic end-point.
References


