Ferritin in Serum: Diagnosis of Iron Deficiency and Iron Overload in Infants and Children

By Martti A. Siimes, Joseph E. Addiego, Jr., and Peter R. Dallman

Ferritin in serum was quantitated by radioimmunoassay to determine the usefulness of this assay in reflecting iron stores during normal development and in the diagnosis of iron deficiency and iron overload in infants and children. In the adult, serum ferritin has been reported to correspond to the magnitude of iron stores throughout a wide range. In 573 normal infants and children, we found the concentration of serum ferritin to parallel known changes in iron stores during development. The median serum ferritin concentration was 101 ng/ml at birth, rose to 356 ng/ml at 1 mo of age, and then fell rapidly to a median value near 30 ng/ml (95% confidence limits: 7-142 ng/ml) between 6 mo and 15 yr of age. In the adult, median concentrations diverged according to sex, 39 ng/ml in the female and 140 ng/ml in the male. In 13 children with iron-deficiency anemia, the serum ferritin concentration was 9 ng/ml or less. Overlap with the normal population was small, and no conditions were found to give "false" low values. In children with β-thalassemia major and sickle cell anemia, median values were elevated to 850 and 160 ng/ml, respectively. Possibly misleading, elevated values were obtained in some patients with acute infection and in acute lymphoblastic leukemia. The serum ferritin assay promises to be a useful tool in the evaluation of iron status, particularly in children.

The determination of ferritin in serum by radioimmunoassay is a promising new means to assess iron nutrition. Until recently, it was not possible to detect the small quantities of ferritin that are normally present in serum, 10-200 ng/ml. Since ferritin contains about 20% iron by weight, this represents only 0.2%-4% of the serum iron normally present in the adult. In the adult, serum ferritin concentration seems to reflect the magnitude of iron stores throughout a wide range, as shown by Addison et al. and Jacobs et al. Elevated serum ferritin values are reported in patients with excessive iron stores, and low concentrations are associated with iron deficiency. The procedure is far easier for the patient than other methods for estimating iron stores such as bone marrow biopsy, liver biopsy, or absorption of radioactive iron salts.

Further investigation is required to determine the diagnostic usefulness of the assay and its specificity and accuracy in reflecting iron stores.

The purpose of this study is to determine the usefulness of the serum ferritin...
assay in the diagnosis of iron deficiency and iron overload in infants and children. Features which make the test attractive for this age group are its potential for early diagnosis of commonly occurring iron lack and the small amount of blood required. In addition, the determination of developmental changes in normal infants and children is a further test of the hypothesis that serum ferritin reflects the status of body iron stores. Iron stores are relatively high at birth, rise during the first 2 mo of life, and then decrease rapidly during the first year of life.9,10 Serum ferritin values should vary correspondingly.

MATERIALS AND METHODS

Radioimmunoassay for Serum Ferritin

The assay consists of a series of incubations in which antibody-antigen reactions take place on the inner surface of polystyrene tubes. (Tube 2038, Falcon Plastics, Oxnard, Calif. 93030) The tubes are first coated with antihuman ferritin, which becomes adsorbed to the interior wall during the incubation. The remaining binding sites on the tube wall are then saturated with albumin, which has an affinity for polystyrene similar to that of ferritin. A diluted sample of serum, plasma, or ferritin standard is added to the tube, allowing ferritin to become bound to the antihuman ferritin coating the tube wall. The quantity of ferritin bound in this manner is then estimated by the capacity of its free binding sites to attach to 125I-labeled antihuman ferritin during an additional incubation. The radioactivity on the test tubes is counted after the nonbound, labeled antibody has been removed by washing.

The serum ferritin assay and the preparation of the reagents are based on the method of Addison et al.1 The use of the radioimmunoassay utilizing antibody-coated tubes incorporates the modification of Miles et al.,11 and is based on principles described by Catt and Tregear.12

Three electrophoretically distinct types of ferritin have been identified in man: spleen, liver, and reticulocyte ferritin.13 Spleen and liver ferritin also differ in amino acid composition.14 The three types are immunologically cross-reactive.15 Thus, it is likely that all types are measured in the serum ferritin radioimmunoassay, even though the assay is based on spleen ferritin.

All samples were diluted 1:20 and tested in triplicate. With this dilution, the best reproducibility was in the range of 3-1200 ng ferritin per ml serum or plasma. Values up to 2000 ng/mg and less than 3 ng/ml were distinguishable, but with less accuracy. Ten different concentrations of standards were analyzed with each assay (usually about 100 samples), and the results were used for drawing a standard curve. Radioactivity was counted in a Nuclear Chicago automatic gamma counter. The coefficient of variation of known normal samples in each assay was 90g.

Within any single assay there was closer agreement among triplicate determinations. Storage of serum or plasma at -15°C for as long as 4 mo resulted in no significant loss of ferritin.

Reagents

Human ferritin, for use as an antigen to stimulate the production of antiferritin in rabbits and as a standard in the assay, was prepared from human spleens according to the method of Linder and Munro.15 Purification of ferritin was based primarily on its heat stability and its high molecular weight. An aqueous homogenate was heated to 70°C for 10 min. After filtration and subsequent centrifugation at 15,000 g for 10 min, a brown, ferritin-rich fraction was precipitated by 50% saturated ammonium sulphate. This fraction was dissolved in water, purified on a Sephadex G-200 column, and further purified and concentrated to a smaller volume by centrifugation at 100,000 g for 4 hr. Acrylamide gel electrophoresis of this product revealed only one stainable band both for protein and for iron.16

Antihuman ferritin was prepared in rabbits. Human spleen ferritin in Freunds adjuvant, 1 mg/ml, was injected intramuscularly in two sites at a total initial dose of 1 ml, followed 3 wk later by a second dose of 0.25 ml. Serum used in this series of assays was collected 6 wk after the initial injection. Serum diluted to a titer of 1:64 with 0.8 M borate buffer, pH 8.4, in a volume of 0.5 ml, precipitated 100 μg of human ferritin in an equal volume.
Iodination of Anti-human Ferritin

The coupling of aminocellulose immunoabsorbent to horse ferritin was done according to the method of Addison et al.\textsuperscript{13} Iodination of the human antiferritin after its coupling to the immunoabsorbent and the subsequent elution of the \textsuperscript{125}I-labeled antibody by gradual acidification were done by the method of Miles et al.\textsuperscript{11} The elution was performed on Bio-Gel columns after neutralization and washing of the iodinated material. Solutions of acetic acid buffer, pH 4.5, and HCl, pH 3.0, 2.5, and 2.0, were used to elute the labeled antibody, which was collected in 1-ml fractions. Each fraction was buffered, counted, diluted, and tested with ferritin standards. The fractions containing antibody with the highest affinity were used for as many as several thousand assays.

Protein and Iron Content of Human Ferritin

Some technical difficulties have been reported in quantitating the protein content of ferritin precisely.\textsuperscript{15} We determined the protein content of human spleen ferritin by the method of Lowry et al.,\textsuperscript{17} using both bovine serum albumin and commercial horse ferritin as standards. Our results with the two standards were identical. The iron content of human spleen ferritin was 23.8\%, the same order of magnitude as values previously reported.\textsuperscript{18} Ferritin iron was estimated by the method of Weinfeld, which utilized orthophenanthroline as the color reagent.\textsuperscript{18}

Serum iron and iron-binding capacity were determined utilizing the Ferro-Chek kit (Hyland Laboratories, Costa Mesa, Calif. 92626), in which Ferrozine [3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4 triazine, disodium salt] is a sensitive iron color reagent for a spectrophotometric method.\textsuperscript{19} Microhematocrit was determined by standard techniques.

Subjects

Blood samples were collected in the Moffitt Hospital of the University of California, San Francisco (a heterogeneous city population), the San Francisco General Hospital (primarily an economically deprived group), the Letterman Army Hospital, San Francisco (dependents of Army personnel), and in a pediatric practice in San Rafael, a prosperous suburb of San Francisco (Table 1). The normal children were seen either in well-baby clinics or for routine examinations in an office practice. Because of the small amount of blood required for the serum ferritin assay (0.1 ml), samples could be obtained from the residuum of blood taken for other purposes.

Two groups of children were studied for the purpose of obtaining normal values. Group A included 430 children from birth to 15 yr of age who had no evidence of infection or other disease (Table 1). Subjects who were anemic, 57 of 430, were excluded. The criteria for anemia were a hematocrit of less than 33\% before 4 yr of age, below 36\% between 5 and 14 yr of age, and thereafter, 39\% for females and 42\% for males. These values are equal to and, in one case, exceeded the lower limits of normal hematocrit listed by the AMA Council on Food and Nutrition in 1968.\textsuperscript{20} Normal cord blood samples excluded patients with hemolytic disease, hyperbilirubinemia, infection, or prenatal history of maternal toxemia, or anemia, or family history of diabetes. Group B comprised 219 healthy children, aged 3–15 yr, from the Letterman Army Hospital, all of whom had serum iron and iron-binding capacity determinations. Of these 219 subjects, 19 were anemic by the same criteria applied in Group A and were excluded. The normal adults tested were healthy, nonanemic hospital and laboratory personnel.

<table>
<thead>
<tr>
<th>Table 1. Healthy Subjects</th>
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<tr>
<td>Group</td>
</tr>
<tr>
<td>A.</td>
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<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>B.</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

*Criteria for anemia listed in Materials and Methods.
†The group whose serum ferritin values are plotted on Fig. 1.
Statistical Analysis

The distribution of serum ferritin concentration in all age groups was markedly skewed. Logarithmic transformation of the data was found to result in a normal distribution. For this reason, geometric means or median values are calculated rather than arithmetic means. The "normal" limits of serum ferritin in children aged 6 mo–15 yr were computed as follows: The cumulative frequency of values at 2-ng/ml intervals was plotted on a log-normal system of coordinates and satisfactorily fitted a straight line, confirming the log distribution of the data. The geometric mean was the 50% point of the curve of distribution, and the 95% confidence limits were the values corresponding to ± 2 sigma. The geometric mean of 31 ng/ml was very close to the median value of 30 ng/ml. Since the above statistical method does not lend itself to small groups, the median values are given for all groups for purposes of uniformity. The significance of differences between groups was calculated by the Student's t test after a logarithmic transformation of the values.

RESULTS

Changes in Concentration of Serum Ferritin During Development

The serum ferritin concentrations of nonanemic, healthy patients from groups A and B are shown in Fig. 1. At birth, the median concentration of serum ferritin was 101 ng/ml, higher than during most of childhood. During the first month after birth, there was a steep rise to a median value of 356 ng/ml, followed by a decrease in ferritin concentration to 30 ng/ml at about 6 mo of age; thereafter, the median value remained near this level and was not significantly different in any age group between 6 mo and 15 yr. In this age group, 95% of the values were in the range of 7–142 ng/ml. In adults, the values diverged markedly according to sex. The median concentration in adult males was 140 ng/ml, in contrast to 39 ng/ml in adult females. In apparently healthy children of any age, the variation in ferritin values was large. Values below 20 ng/ml were common in children from 6 mo to 15 yr, in contrast to the newborn and the adult male.

Serum Ferritin Concentration in Relation to Serum Iron Saturation, Iron-Binding Capacity, and History of Iron Supplementation (Group B)

In the 197 children in group B who were not anemic and had a serum iron saturation of 16%, or more, the median serum ferritin was 25 ng/ml, and 95% of the group fell in the range of 7–85 ng/ml. These values are equivalent to, or somewhat lower than, those of Group A in which serum iron, in most cases, was not determined. The three nonanemic individuals in Group B who had a serum iron saturation of less than 16% also had a normal serum ferritin concentration. Three additional nonanemic patients not in Group B were found to have a low serum iron saturation, and two of these three had abnormally low serum ferritin concentrations. The range of values for the total group of six patients who fulfilled the laboratory criteria of latent iron deficiency was lower than normal, as shown in Table 2.

Of the 219 children (Group B), 41 received daily iron supplementation, and their median serum ferritin concentration was 31 ng/ml, compared with 22 ng/ml in the 178 children.

High values for iron-binding capacity were associated with low serum ferritin concentrations. The mean serum ferritin concentration was significantly lower.
Fig. 1. Serum Ferritin Concentrations During Development: Serum ferritin concentrations in healthy, nonanemic newborns, infants, and children of various age groups (Groups A and B) are shown with adult male and female values. The median value in each age group is indicated by a horizontal line. The dashed line encloses a square which includes the 95% confidence limits of the values between the ages of 6 mo and 15 yr.

Table 2. Serum Ferritin Concentration in Iron Deficiency and Iron Overload in Children Aged 6 mo–15 yr

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Median (ng/ml)</th>
<th>Range (ng/ml)</th>
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</thead>
<tbody>
<tr>
<td>Iron-deficiency anemia</td>
<td>13</td>
<td>3.4</td>
<td>1.5–9</td>
</tr>
<tr>
<td>Latent iron deficiency*</td>
<td>6</td>
<td>10.6</td>
<td>4.5–41</td>
</tr>
<tr>
<td>Thalassemia major</td>
<td>7</td>
<td>850</td>
<td>590–1830</td>
</tr>
<tr>
<td>Sickle cell anemia (SS)</td>
<td>14</td>
<td>163</td>
<td>49–180</td>
</tr>
<tr>
<td>Chronic hemolytic anemia†</td>
<td>19</td>
<td>242</td>
<td>96–920</td>
</tr>
<tr>
<td>Normal‡</td>
<td>486</td>
<td>30</td>
<td>7–1421</td>
</tr>
</tbody>
</table>

*No anemia, but serum iron saturation less than 16%.
†Hb SC, Hb H, G6PD deficiency and Coomb's positive hemolytic anemia.
‡95% confidence limits.
Iron-Deficiency Anemia

Serum ferritin concentrations were determined in 13 patients with iron-deficiency anemia. Hematocrit ranged from 18% to 29%, and serum iron saturation from 7% to 15%. Eleven serum ferritin determinations in this category were below the normal range, and the remaining two were borderline (Table 2), the total range being 1.5-9.3 ng/ml.

Patients with iron-deficiency anemia were treated with oral ferrous sulfate, about 6 mg of elemental iron per kg body weight per day for 2 mo. Compliance was satisfactory in the five patients studied with serial ferritin determinations and was checked by the amount of medication remaining at follow-up evaluations. During the first week of treatment, the serum ferritin concentration invariably rose to a peak, with a subsequent leveling off or a drop to a lower, but normal, level (Fig. 2). As shown, a marked rise in the serum ferritin may occur as early as a day or two after initiation of iron treatment.

Five individuals who were not iron deficient (normal iron saturation and no anemia) were also treated with oral iron at a lower dose of 2 mg/kg/day. There was no discernible change in the concentration of ferritin during iron treatment.

Chronic Hemolytic Anemias

Children with thalassemia major, sampled prior to transfusion, had marked elevations in serum ferritin, with a median value of 850 ng/ml, about 30 times greater than the median value for normal children (aged 6 mo-15 yr) and about 250 times greater than in iron-deficient patients. Those with homozygous sickle cell disease also had a high median concentration of serum ferritin, 163 ng/ml, about five times the normal childhood value. The median serum ferritin concentration in patients with a variety of other hemolytic anemias was similar to that of the sickle cell anemia group (Table 2). Except in the case of thalassemia major, however, there was overlap with values in the normal childhood range.

Other Conditions in Which the Serum Ferritin Concentrations Fell Outside the Normal Range

Table 3 includes some common conditions, such as fever and infection, in which we have found an elevation of serum ferritin. To date, we have found no conditions other than iron deficiency that are characterized by abnormally low values. Serum ferritin concentration was elevated only in some cases of fever or infection. For example, in children with acute otitis media, the median ferritin concentration was no higher than normal, but in children with acute respiratory infection, the values were significantly elevated.

High ferritin values were obtained in 14 children with acute lymphoblastic leukemia in complete remission, a median value of 202 ng/ml, with a range of 86–428 ng/ml. In three patients in relapse, the values were 361, 490, and 680 ng/ml.
Fig. 2. Serum Ferritin Concentration Prior to and During Iron Therapy in Iron-Deficient and Normal Patients: The marked early rise in serum ferritin concentration in six iron-deficient patients following iron therapy (broken lines) is in contrast to the lack of a response in a nondeficient patient treated with iron (solid line). For the sake of clarity, one representative response of the five nonanemic patients is shown.

Table 3. Serum Ferritin Concentration in Common Acute Infections in Children Aged 6 mo–15 yr

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number</th>
<th>Median (ng/ml)</th>
<th>Range (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Otitis media</td>
<td>8</td>
<td>39</td>
<td>6.0–185</td>
</tr>
<tr>
<td>Upper respiratory infection</td>
<td>12</td>
<td>167</td>
<td>18–510</td>
</tr>
<tr>
<td>Lower respiratory infection</td>
<td>7</td>
<td>115</td>
<td>28–320</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>6</td>
<td>79</td>
<td>28–195</td>
</tr>
<tr>
<td>FUO</td>
<td>12</td>
<td>138</td>
<td>17–262</td>
</tr>
<tr>
<td>Normal</td>
<td>486</td>
<td>30</td>
<td>7–142*</td>
</tr>
</tbody>
</table>

*95% confidence limits.
Stability of Serum Ferritin in One Individual

Sequential samples in one individual tended to fall within a relatively narrow range. Among seven normal adult subjects, the range of values in 3–11 blood samples over the course of 2 wk fell within a range of ±4% to ±12% of the mean. Furthermore, there was essentially no difference between ferritin concentration in samples drawn at 9 a.m., at noon, and at 4 p.m. The stability of serum ferritin concentration in normal individuals is in contrast to marked diurnal variation of serum iron.21

DISCUSSION

The clinical role of the serum ferritin assay lies in its tendency to parallel the size of iron stores during normal development as well as in iron deficiency and iron overload. The concentration of iron storage compounds9,10 and of serum ferritin are both high at birth, increase further for 1–2 mo, and then decrease rapidly, maintaining low levels after 6 mo of age. Diminution of iron stores during this period is a normal developmental consequence of rapid growth during a period when the major physiologic source of food, i.e., milk, is a poor source of iron.

In iron deficiency, the serum ferritin assay seems to have an important advantage over serum iron and iron-binding capacity in that low values are almost invariably diagnostic. Iron-deficiency anemia has sometimes been difficult to distinguish from the anemia of infection, since the serum iron and per cent iron saturation can be low in both conditions.22 The fact that infection is associated with a normal or elevated serum ferritin concentration should add to its diagnostic value. It is uncertain whether a low serum ferritin concentration anticipates a depression in the saturation of serum iron in nutritional iron deficiency as it seems to in blood loss.2

Although serum ferritin concentration seems to reflect the size of iron stores under most conditions, there may be certain exceptions. Infections and acute leukemia are two examples. Increased serum ferritin concentrations have also been reported in adults with acute myeloblastic leukemia, chronic leukemia, and Hodgkin’s disease.26 In myeloid leukemia, the white blood cell count was the only hematologic parameter that correlated with serum ferritin.26 We found increased serum ferritin concentrations in children with acute lymphoblastic leukemia, whether in complete remission or in relapse. Although the values appeared to be higher during relapse, there was no correlation with the white cell count. Another example of possible failure of serum ferritin to reflect iron stores is found during the course of iron therapy in iron-deficient patients. The serum ferritin concentration rises abruptly during the first week and then stabilizes or falls, a pattern that is unlikely to correspond to iron stores. It will be difficult to explain such exceptions until the physiologic basis for variations in serum ferritin is understood.

From the evidence at hand, the serum ferritin assay promises to be a useful tool in the evaluation of iron stores. The sample size required is sufficiently small to be obtainable by finger or heel stick, and thus avoids the need for a venipuncture. This is a particularly important consideration in the age group between 6 mo and 3 yr, when the incidence of iron deficiency is at its peak,
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and when it is often technically difficult to obtain several milliliters of peripheral blood. The alternative of bone marrow aspiration for iron staining has the disadvantage of being more painful than blood sampling and, moreover, is difficult to interpret in children. Decreased stainable iron (compared with the adult male) is so common in children that it is of little use in distinguishing an iron-deficient patient from the normal population.

ACKNOWLEDGMENT

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