A CLINICAL EVALUATION OF SERUM FERRITIN AS AN INDEX OF IRON STORES

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Abstract Measurements of serum ferritin were correlated with other hematologic laboratory indexes in 250 hospitalized subjects with anemia or disorders in iron metabolism. A geometric mean value of 4 ng per milliliter was found in 32 patients with uncomplicated iron-deficiency anemia, and one of 2930 ng per milliliter in 29 patients with iron overload. Among subjects with anemia from causes other than iron deficiency, the mean serum ferritin level was increased to 180 ng per milliliter (geometric mean in normal controls, 59 ng per milliliter, with a 95 per cent confidence range of 12 to 300 ng per milliliter), presumably reflecting the transport of red-cell iron to stores. The wide range of values found was shown to be related primarily to the magnitude of body iron stores in each case as evaluated by bone-marrow hemosiderin. In addition, however, inflammation, liver disease and increased red-cell turnover were shown to elevate the serum ferritin concentration to a degree disproportionate to that of iron stores. (N Engl J Med 290:1213-1216, 1974)

Ferritin is a high-molecular-weight iron containing protein that functions in the body as an iron-storage compound. Although predominantly intracellular, ferritin has been detected by relatively insensitive technics in the serum of patients with liver dysfunction and certain inflammatory neoplasms. Recently, immunoradiometric assays have been developed whereby ferritin can be quantitated in all human serum. Initial reports have indicated that in clinical disorders of iron balance, the level of serum ferritin reflects the level of body iron stores. In the present study, the usefulness of the serum ferritin assay for evaluating iron status has been examined with attention to diseases that influence the level independently of body iron stores.

Materials and Methods

Clinical and laboratory information on 250 patients, selected either because of anemia or because of suspicion of a disorder in iron metabolism, were reviewed. Serum iron, total iron-binding capacity and serum ferritin were measured in all subjects. Approximately a third of the patients were excluded from further analysis because the underlying disorder was poorly defined or complicated by additional disease. The patients were placed in a number of categories.

Uncomplicated iron deficiency was considered present when the transferrin saturation was less than 16 per cent, when total iron-binding capacity was greater than 400 µg per 100 ml and when there was no clinical or laboratory evidence of inflammation or liver disease. The 32 patients in this category had a mean hematocrit of 31 ± 5 per cent (± 1 S.D.), a serum iron of 37 ± 11 µg per 100 ml, a total iron-binding capacity of 479 ± 75 µg per 100 ml and a transferrin saturation of 8 ± 3 per cent.

Iron overload included 20 patients with transfusional siderosis as defined by a history of more than 20 transfusions without commensurate blood loss, and three patients with idiopathic hemochromatosis confirmed by liver biopsy who had not yet entered a phlebotomy program. The remaining clinical categories were selected without regard to iron status.

Inflammation was identified on the basis of clinical symptoms of several days' duration and one or more of the following factors: Westergren erythrocyte sedimentation rate greater than 40 mm per hour, white-cell count over 10,000 in the absence of a blood dyscrasia and a temperature higher than 37.5°C for at least 48 hours. The 39 patients in this category had a mean hematocrit of 30 ± 4 per cent, a white-cell count of 10.8 ± 5 x 10³, an erythrocyte sedimentation rate of 99 ± 36 mm per hour, a serum iron of 38 ± 11 µg per 100 ml, a total iron-binding capacity of 227 ± 81 µg per 100 ml and a transferrin saturation of 18 ± 5 per cent.

Liver disease was diagnosed on the basis of clinical and laboratory evidence of primary liver dysfunction. All the 37 patients had a serum bilirubin higher than 1.2 mg per 100 ml and a serum alkaline phosphatase greater than 100 King-Armstrong U per milliliter. Alcoholism was considered to be the cause of the liver disease in 29 patients, all of whom were anemic. The mean hematologic values in this group were as follows: hematocrit, 30 ± 4 per cent; serum iron, 108 ± 57 µg per 100 ml; total iron-binding capacity, 209 ± 61 µg per 100 ml; and transferrin saturation, 55 ± 32 per cent. The remaining eight patients had acute viral hepatitis, and only one of these was anemic. The hematologic values in this group were as follows: hematocrit, 43 ± 3 per cent; serum iron, 186 ± 57 µg per 100 ml; total iron-binding capacity, 318 ± 45 µg per 100 ml; and transferrin saturation, 62 ± 26 per cent. An additional nine patients had combined evidence of liver dysfunction and an independent inflammatory process. These patients had a mean hematocrit of 29 ± 5 per cent, serum iron of 102 ± 76 µg per 100 ml, total iron-binding capacity of 211 ± 56 µg per 100 ml and a transferrin saturation of 46 ± 30 per cent.

Renal disease included patients with a history of chronic renal failure, a blood urea nitrogen greater than 40 mg per 100 ml and serum creatinine above 2 mg per 100 ml. Patients with an associated inflammatory process were excluded. The 15 in this category had a mean hematocrit of 21 ± 7 per cent, serum iron of 90 ± 51 µg per 100 ml, total iron-binding capacity of 270 ± 66 µg per 100 ml and a transferrin saturation of 32 ± 17 per cent.

Anemia with increased red-cell turnover included 15 patients, seven with ineffective erythropoiesis as evidenced by a hyperplastic bone

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marrow without an increase in the reticulocyte index (seven patients with megaloblastic anemia and one with sideroblastic anemia) and eight patients with hemolytic anemia identified by a reticulocyte index greater than 2.7

In a total of 87 patients a bone-marrow aspiration was performed, and iron stores were graded by two separate observers as absent, decreased, moderate and increased. “Decreased” corresponded to the average stores of adult females, whereas “moderate” corresponded to the average stores found in normal males.

Serum ferritin was estimated by means of a “two-site” immunoradiometric assay, the details of which have been described elsewhere.8 The serum ferritin concentration among normal subjects was normally distributed on a logarithmic scale.9 Accordingly, the statistical analysis of ferritin has been performed on logarithms. In normal subjects the geometric mean is 59, with a 95 per cent confidence range of 12 to 300 ng per milliliter.4 Student’s t-test was used to determine statistical significance.

Serum iron was measured by the method of Young and Hicks,10 and total iron-binding capacity by that of Lehmann and Kaplan.11 Hematologic measurements were made by means of standard methods on a Model 5 Coulter Counter (Coulter Electronics, Hialeah, Florida). Measurement of bilirubin, alkaline phosphatase, blood urea nitrogen and serum creatinine by standard technics using a multichannel Technicon AutoAnalyzer (Technicon Instrument Corporation, Tarrytown, New York).

Results

The effect of alteration in body iron balance on the serum ferritin was first examined (Fig. 1). In 32 patients with uncomplicated iron deficiency, the mean serum ferritin was 4 ng per milliliter, with a range of 1 to 14 ng per milliliter. Three patients with untreated idiopathic hemochromatosis had values of 3215, 6018 and 6100 ng per milliliter. In 20 patients with transfusional siderosis, the mean serum ferritin was 2713, with values ranging from 1350 in a subject given 20 to 14 ng per milliliter. Three patients with untreated idiopathic hemochromatosis had values of 3215, 6018 and 6100 ng per milliliter. In 20 patients with transfusional siderosis, the mean serum ferritin was 2713, with values ranging from 1350 to 1417. In nine additional patients who had both liver dysfunction and an inflammatory process, the mean serum ferritin was 801 ng per milliliter, with a range of 304 to 1417.

In an attempt to explain the wide range of serum ferritin levels observed in inflammation and liver disease, the influence of iron stores in these patients was examined. Iron deficiency was present in four patients with inflammation and in seven with liver disease. As shown in Figure 1, the serum ferritin levels in these patients were distinctly lower than in the group as a whole. A more detailed evaluation of iron stores was made in 75 patients who had a bone-marrow aspiration for evaluation of iron stores (Table 1). Patients with inflammation and liver disease were compared with a control group of patients with a variety of disorders exclusive of inflammation, liver disease or increased red-cell turnover. Serum ferritin values in all three groups of patients increased progressively with greater amounts of marrow hemosiderin. At each grade of marrow iron, however, values in patients with inflammation and liver disease were significantly higher than in the control group (p<0.05), except the group with liver disease, who had decreased stainable iron (only two pa-
hematologic and other laboratory measurements. The level is three times higher in males than in females, \(5^*\). A high correlation between that index and serum ferritin was observed in patients with anemia and inflammation. In patients with increased red-cell turnover, serum ferritin levels also appeared disproportionately increased in relation to bone-marrow hemosiderin. In a composite group of 15 patients, the mean serum ferritin level was 419 ng per milliliter. Seven patients with ineffective erythropoiesis had a mean serum ferritin of 434, and eight with hemolytic anemia a mean ferritin of 345 ng per milliliter. Of the 12 patients whose marrow hemosiderin was examined, four with diminished iron stores had values of 61, 142, 345 and 354 ng per milliliter; four patients with moderate iron stores had values of 240, 355, 620 and 1210 ng per milliliter, and four with increased iron stores had values of 550, 817, 1890 and 3067 ng per milliliter.

In chronic renal disease, the serum ferritin level appeared consistent with iron stores. In nine patients, the mean serum ferritin level was 32, with a range of 5 to 352 ng per milliliter. In an additional six patients, an elevation in serum ferritin to between 625 and 2900 ng per milliliter could be explained by the prior administration of iron; two of these patients had received nine and 13 transfusions, whereas four others had received iron dextran parenterally in doses ranging from 0.5 to 3.0 g of iron.

Correlations between serum ferritin and various hematologic and other laboratory measurements revealed a consistently high correlation only with the total iron-binding capacity. Within the various clinical disorders, the highest correlation between that index and serum ferritin was observed in patients with anemia and inflammation \( (r = -0.76, p < 0.001) \) (Fig. 2). When mean values of total iron-binding capacity and serum ferritin in the various clinical disorders were plotted, no disparities in this relation were observed (Fig. 2).

**Discussion**

Evidence has been obtained in previous studies that the serum ferritin level in normal subjects is proportional to the size of body iron stores. The serum ferritin level is three times higher in males than in females, \(6^*\). A finding consistent with the known sex difference in storage iron. Furthermore, a highly significant correlation has been demonstrated in normal subjects between serum ferritin level and radioiron absorption, which is a sensitive indirect measure of body iron stores. \(6^*\)

Pathologic disturbances in iron balance have a marked effect on the serum ferritin level. In iron deficiency, a mean of 5 ng per milliliter was reported in 21 subjects by Jacobs et al., \(3^*\), and we observed a mean of 4 ng per milliliter in 23 iron-deficient subjects. Similarly, in patients with iron overload due to either hemochromatosis or excess transfusion, the serum ferritin was invariably elevated. Any decrease in serum ferritin level may be interpreted as iron depletion, but an increased level does not necessarily mean iron overload. Thus, the serum ferritin may be increased by shifts of iron from the erythron to stores, as would occur with anemia not associated with blood loss, and by abnormalities in either hepatic or reticuloendothelial-cell function. It is this last effect that permits the distinction between iron-deficiency anemia and the anemia of inflammation. Although other measurements such as transferrin saturation and red-cell protoporphyrin reflect a decrease in iron supply to the erythroid marrow whether it is due to true iron deficiency or to an internal block in iron release from the reticuloendothelial cell, serum ferritin is decreased in the former but increased in the latter. From a practical standpoint, the finding of an elevated serum ferritin level in an anemic subject with inflammation would exclude inadequate iron stores and might obviate the need for bone-marrow examination.

An interesting correlation was observed in these studies between total iron-binding capacity and serum ferritin level. It is well known that iron-binding capacity increases with depletion of iron stores \(10^*\) and is decreased in a variety of anemias in which iron stores

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**Table 1. Relation between Serum Ferritin and Bone-Marrow Hemosiderin.**

<table>
<thead>
<tr>
<th>MARROW IRON</th>
<th>CONTROL</th>
<th>PATIENTS WITH INFLAMMATION</th>
<th>PATIENTS WITH LIVER DISEASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO. SERUM FERRITIN*</td>
<td>NO. SERUM FERRITIN*</td>
<td>NO. SERUM FERRITIN*</td>
<td></td>
</tr>
<tr>
<td>ng/ml</td>
<td>ng/ml</td>
<td>ng/ml</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>12</td>
<td>6 (1-37)</td>
<td>2</td>
</tr>
<tr>
<td>Diminished</td>
<td>8</td>
<td>51 (21-163)</td>
<td>6</td>
</tr>
<tr>
<td>Moderate</td>
<td>5</td>
<td>159 (60-253)</td>
<td>9</td>
</tr>
<tr>
<td>Increased</td>
<td>2</td>
<td>589 (442-669)</td>
<td>8</td>
</tr>
</tbody>
</table>

*Geometric mean (range given in parentheses).
would be expected to increase. Past experience has led us to believe that the total iron-binding capacity was of limited usefulness in the assessment of iron stores. For example, Bainton and Finch observed a value below 300 µg per 100 ml in 25 per cent of a group of 115 patients with iron-deficiency anemia who responded to iron. In view of the close reciprocal relation observed in this study to total iron-binding capacity and serum ferritin, however, the clinical usefulness of the former needs to be re-evaluated.

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REFERENCES

BACKGROUND READING
References 7 and 11

ESCHERICHIA COLI K1 CAPSULAR POLYSACCHARIDE ASSOCIATED WITH NEONATAL MENINGITIS

JOHN B. ROBBINS, M.D., GEORGE H. MCCracken, JR., M.D., EMIL C. Gotschlich, M.D., FRITS ØRSKOV, M.D., IDA ØRSKOV, M.D., AND LARS A. HANSON, M.D.

Abstract Examination of 77 strains of Escherichia coli from the cerebrospinal fluid of neonates with meningitis revealed 65 (84 per cent) with the capsular (K1) polysaccharide. The Esch. coli K1 capsular antigen has been shown to be immunochemically identical to the meningococcal Group B polysaccharide. These cerebrospinal-fluid Esch. coli K1 strains were associated with at least seven different somatic (O) and three flagella (H) antigens. In contrast, Esch. coli K1 strains were found in 14 of 36 (39 per cent) blood cultures of neonates without meningitis and in approximately 15 per cent of blood, urine and stool cultures from adults and rectal cultures from infants. The mean median lethal dose in a mucin-enhanced mouse model for 31 neonatal cerebrospinal-fluid K1 isolates was 168 organisms. In contrast, the mean lethal dose for 10 neonatal non-K1 isolates was 58,000 and was greater than 10,000 organisms for six non-K1 enteropathogenic strains and five K1 strains isolated from normal infant stools. The high prevalence of the K1 antigen in neonatal meningitis suggests that this capsular polysaccharide is related to Esch. coli invasiveness in the newborn. A protective effect of anti-capsular antibody in a mouse model suggests that immunity to neonatal Esch. coli K1 meningitis may be mediated by serum antibodies. (N Engl J Med 290:1216-1220, 1974)

NEONATAL meningitis due to Escherichia coli remains an intriguing and baffling problem to the clinician and scientist. Despite advances in antimicrobial chemotherapy, the combined mortality and morbidity of Esch. coli neonatal meningitis remains high; there are few normal survivors. Data from a recent cooperative neonatal meningitis study illustrates this problem.* Among 133 infants with purulent meningitis studied from July, 1971, to April, 1973, 50 (38 per cent) cases were caused by Esch. coli, 41 (31 per cent) by Group B beta-hemolytic streptococci, seven (5 per cent) by Listeria monocytogenes, and 35 by one or more of 16 different pathogens. The mortality rate for Esch. coli meningitis was 35 per cent, and the morbidity of the

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