Biologic and Clinical Significance of Red Cell Ferritin

By Mario Cazzola, Laura Dezza, Gaetano Bergamaschi, Giovanni Barosi, Vittorio Bellotti, Daniela Caldera, Maria Matilde Ciriello, Silvana Quaglini, Paolo Arosio, and Edoardo Ascari

Red cell ferritin was measured in normal subjects and patients with disorders of iron metabolism, inflammation, liver dysfunction, impaired hemoglobin synthesis, and increased red cell turnover by means of radioimmunoassays with antibodies to liver (basic) and heart (acidic) ferritins. The normal mean values for basic and acidic ferritin were 8.9 and 22.7 attogram (ag)/cell, respectively. The red cell ferritin content reflected changes occurring in tissues both in iron deficiency and iron overload. Basic ferritin was more closely related to the body iron status than acidic ferritin, and the acidic/basic ferritin ratio was increased in iron deficiency and decreased in iron overload. The major factor determining the red cell ferritin content appeared to be the transferrin saturation, that is, the distribution of iron between monoferric and diferric transferrin. This is in keeping with recent data indicating a competitive advantage of diferric transferrin in delivering iron to erythroid cells. In addition, the red cell ferritin content was increased in thalassemic patients with normal iron status, appearing to be inversely related to the rate of hemoglobin synthesis. The determination of red cell ferritin, based on a commercially available basic ferritin assay, may have clinical application. It can be used for evaluating the adequacy of the iron supply to the erythroid marrow, particularly in patients with increased red cell turnover. Moreover, it may be useful in evaluating the body iron status in patients with hemochromatosis and liver disease.

SINCE 1972, when the first sensitive immunoassay for ferritin was described, there has been an impressive number of studies on serum ferritin concentration in normal and disease states, and the clinical usefulness of the serum ferritin assay in evaluating body iron status is now well established.1,2

Attempts have also been made to measure ferritin in red cell extracts.3–7 Recently, Jacobs and coworkers8 and Cazzola et al.9 have shown that the red cells contain greater amounts of acidic (heart-type) ferritin than basic (liver- or spleen-type) ferritin, and that the red cell ferritin content reflects the abnormal body iron status both in iron deficiency and iron overload.

In the present work we have extended our preliminary studies10 by determining the amounts of basic and acidic ferritin in normal red cells and those from patients with disorders of iron metabolism, inflammation, liver dysfunction, impaired hemoglobin synthesis, and increased red cell turnover. This work has allowed us to study the relationship between red cell ferritin, body iron status, rate of hemoglobin synthesis, and red cell turnover in man.

MATERIALS AND METHODS

Subjects

Blood samples were obtained from normal subjects and hospitalized patients: clinical and hematologic data are shown in Table 1.

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0006-4971/83/6205-0018$02.00/0

than 0.85 g/dl; (D) fibrinogen concentration greater than 500 mg/dl. Of the 73 patients, 27 subjects had Hodgkin’s disease or non-Hodgkin’s lymphoma, 20 subjects had chronic infection, 16 subjects had collagen disease, and 10 subjects had solid tumor.

Liver disease. This group included 18 patients in whom liver dysfunction was diagnosed on the basis of clinical and laboratory data. In particular, all the patients had a serum bilirubin concentration greater than 15 mg/liter and variable elevations of serum transaminases and alkaline phosphatase.

Thalassemia. This group included: (A) 8 subjects with α-thalassemia trait and one subject with Hb H disease; (B) 34 subjects with heterozygous β-thalassemia; (C) 12 subjects with thalassemia intermedia.

Sickle cell trait. Four such subjects, having normal red cell counts, were studied.

Hemolytic disease. Thirteen subjects had hereditary spherocytosis and 5 subjects had autoimmune hemolytic anemia. Their reticulocyte counts ranged from 5% to 24%, or from 200 to 720 x 10^6/liter.

Sequential studies. Sequential studies were carried out in: (A) 5 patients with iron deficiency and 5 patients with iron overload undergoing appropriate treatment; (B) 4 patients with thalassemia complicated by iron deficiency; (C) 2 patients with thalassemia major, under transfusion therapy, at different levels of Hb.

Laboratory Methods

Red cell ferritin was assayed as previously described in detail. Pure red cell suspensions were prepared by means of the method of Beutler et al. Stroma were removed from red cell lysates by centrifugation, and ferritin concentration was then measured using radioimmunoassays with antibodies to human liver and heart ferritin.

In order to derive the red cell ferritin content, the Hb concentration of the red cell lysate was measured by the cyanmethemoglobin method, and red cell numbers calculated from this and the previous obtained mean corpuscular hemoglobin (MCH). The red cell ferritin content was expressed as ag/cell (ag = attogram, 1 ag = 10^-18 g).

Plasma iron and total iron binding capacity (TIBC) were determined by colorimetric analysis. Plasma ferritin was determined in duplicate using a radioimmunoassay based on antibodies against human liver ferritin (sensitivity 1 μg/liter). Red cell protoporphyrin was measured by direct spectrophotometry of oxygenated blood using the model 4000 Hematofluorometer (Environmental Sciences Associates Inc., Bedford, MA).

In order to evaluate any effect of cell age on the red cell ferritin content, studies on age-dependent red cell populations from 6 subjects were carried out. Blood samples enriched in reticulocytes were prepared as follows. Washed, leukocyte-depleted red cells packed cell volume (PCV) ~ 80% were pipetted into polyethylene tubes having internal dimensions of 0.5 x 10 cm. These tubes were centrifuged in a horizontal rotor at 10,000 g for 30 min at 5°C. After centrifugation, the upper and lower 10% of the red cell column were removed using insulin syringes and considered as top and bottom layer, respectively. The remaining was considered as middle layer. On each layer, reticulocyte count, pyruvate kinase activity, and red cell ferritin were estimated.

Statistical Analysis

Statistical analysis were performed using the SPSS package implemented on a Honeywell 6030 computer.

Frequency distribution curves of values for Hb concentration, plasma iron, TIBC, and transferrin saturation showed a near-normal distribution, while those of values for plasma ferritin, red cell protoporphyrin, and red cell ferritin showed a skew distribution. In this latter case, normalization was obtained by logarithmic transformation of the data.

Statistical analyses included: summary statistics, regression and correlation analysis, variance analysis, and discriminant analysis. The significance of the differences between means was tested by the F test or the Student’s t test.

RESULTS

The results obtained are summarized in Table 1, while the Pearson correlation coefficients between the examined parameters are reported in Table 2. The data shown in these 2 tables indicate that: (A) both the red cell ferritin types were related to the body iron status, but such a correlation was closer for basic ferritin than for acidic ferritin; (B) there was a significant correlation between the two ferritin types; (C) overall, the transferrin saturation was the major determinant of the red cell ferritin content, accounting for 54% variation in basic ferritin and 41% variation in acidic ferritin.

Normal red cells contained greater amounts of acidic ferritin than basic ferritin, the mean acidic/basic ferritin ratio being 2.53. Whereas the plasma ferritin concentration was markedly higher in males than in females, the sex differences were less consistent with respect to red cell ferritin, but still significant (Table 1).

Both in iron-deficiency anemia and iron-deficient erythropoiesis, the mean red cell ferritin content was lower than normal. Such decrease was higher for basic ferritin than for acidic ferritin, and therefore, the acidic/basic ferritin ratio was increased. There was a significant inverse correlation between red cell basic ferritin and red cell protoporphyrin in this group (Fig. 1). There was a negligible overlap between values for basic ferritin found in patients with iron-deficiency anemia and those found in normal subjects. In fact, 27 of 29 patients with iron-deficiency anemia had values for red cell basic ferritin lower than 3.0 ag/cell, while all normal subjects but one had values over this threshold.

Red cell ferritin was considerably increased in patients with iron overload. Such increase was comparatively higher for basic ferritin than for acidic ferritin, and therefore, the acidic/basic ferritin ratio was reduced in this group. As shown in Fig. 2, there were no significant differences between the various iron overload states with respect to the red cell ferritin content, the major determinant of this latter being the transferrin saturation.

The relationship between red cell ferritin and iron status was further confirmed by sequential studies carried out in a few patients. Two illustrative examples are reported in Fig. 3.
 vera showed, on the average, reduced values for red cell basic ferritin, which paralleled the reduction in transferrin saturation (Table 1). On the contrary, values for red cell acidic ferritin were normal, and the acidic/basic ferritin ratio was increased in these subjects.

As expected, patients with inflammation had low serum iron with low transferrin saturation and normal-to-low TIBC, increased plasma ferritin, and reticuloendothelial siderosis. In this group, the mean red cell ferritin content was low, with a normal acidic/basic ferritin ratio (Table 1). The red cell basic ferritin content was directly related to both the transferrin saturation and plasma ferritin concentration (Table 3).

Transferrin saturation was increased in 7 of 18 patients with liver disease, and plasma ferritin was elevated in 9 of 18 such subjects. However, there was no correlation between these two parameters ($r = 0.22$, $p = 0.19$). Both the red cell ferritin types were increased on the average (Table 1). The basic ferritin content was significantly correlated with the transferrin saturation ($r = 0.67$, $p < 0.001$), but not with the plasma ferritin concentration ($r = 0.32$, $p = 0.10$).

Transferrin saturation appeared to be an important factor in determining the red cell basic ferritin content within the thalassemia group too ($r = -0.48$, $p < 0.001$). Such a relationship, however, was significantly different from that observed in normal subjects and

### Table 1. Hemoglobin Level, Iron Status Data, and Red Cell Ferritin Contents in Normal and Disease States, Mean and Range

<table>
<thead>
<tr>
<th>Subjects (no.)</th>
<th>M/Hb (g/dL)</th>
<th>M/Hb (umol/L)</th>
<th>M/TIBC (umol/L)</th>
<th>M/Transferrin Saturation (%)</th>
<th>M/Protoporphyrin (g/dL Packed Red Cells)</th>
<th>M/Plasma Ferritin (g/Liter)</th>
<th>M/Basic Ferritin (g/Liter)</th>
<th>M/Blood Asayed</th>
<th>M/Acidic Ferritin (g/Liter)</th>
<th>M/Acidic Ferritin (g/Liter)</th>
<th>M/Acidic Ferritin/Acidic Ferritin Ratio</th>
</tr>
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<tbody>
<tr>
<td>Normal subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (68)</td>
<td>14.4</td>
<td>18</td>
<td>60</td>
<td>30</td>
<td>21</td>
<td>66</td>
<td>8.9</td>
<td>22.7</td>
<td>2.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(12.1-17.9)</td>
<td>(11-26)</td>
<td>(40-75)</td>
<td>(18-47)</td>
<td>(1-69)</td>
<td>(17-300)</td>
<td>(3.0-37.3)</td>
<td>(5.8-83.4)</td>
<td>(10.5-13.20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males (29)</td>
<td>15.5</td>
<td>19</td>
<td>58</td>
<td>33</td>
<td>20</td>
<td>115</td>
<td>11.1</td>
<td>27.8</td>
<td>2.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(13.3-17.9)</td>
<td>(12-26)</td>
<td>(48-71)</td>
<td>(20-45)</td>
<td>(1-60)</td>
<td>(19-300)</td>
<td>(4.0-37.3)</td>
<td>(7.5-83.4)</td>
<td>(0.81-8.59)</td>
<td></td>
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</tr>
<tr>
<td>Females (39)</td>
<td>13.7</td>
<td>16</td>
<td>60</td>
<td>27</td>
<td>21</td>
<td>44</td>
<td>7.5</td>
<td>19.5</td>
<td>2.57</td>
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<tr>
<td>(12.1-14.9)</td>
<td>(11-25)</td>
<td>(40-75)</td>
<td>(16-47)</td>
<td>(5-69)</td>
<td>(17-165)</td>
<td>(3.0-30.9)</td>
<td>(5.8-75.4)</td>
<td>(10.5-13.20)</td>
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</tr>
<tr>
<td>M/F comparison</td>
<td>t = 7.99</td>
<td>t = 2.92</td>
<td>t = 0.98</td>
<td>t = 3.34</td>
<td>t = 0.30</td>
<td>t = 7.39</td>
<td>t = 2.28</td>
<td>t = 0.16</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>p &lt; 0.001</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron deficiency anemia (29)</td>
<td>8.9</td>
<td>6</td>
<td>80</td>
<td>22</td>
<td>7</td>
<td>1.4</td>
<td>9.0</td>
<td>6.53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3.6-12.1)</td>
<td>(1-11)</td>
<td>(51-114)</td>
<td>(1-151)</td>
<td>(81-510)</td>
<td>(3-15)</td>
<td>(0.5-3.1)</td>
<td>(0.8-29.6)</td>
<td>(1.0-34.99)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron-deficient anemia (20)</td>
<td>12.7</td>
<td>8</td>
<td>75</td>
<td>11</td>
<td>99</td>
<td>11</td>
<td>3.0</td>
<td>11.6</td>
<td>3.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(11.7-14.1)</td>
<td>(4-12)</td>
<td>(59-101)</td>
<td>(5-18)</td>
<td>(46-221)</td>
<td>(5-22)</td>
<td>(0.9-13.6)</td>
<td>(3.2-58.0)</td>
<td>(0.93-20.90)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Iron overload

- Homozygous HLA-related hematological disorders (6)
  - Normal (11.5-15.5) (43-46) (45-54) (70-87) (7-45) (132-1.375) (141.0-527.0) (145.0-356.0) (0.63-2.98)
- Heterozygous HLA-related hematological disorders (11.5-18.7) (27-35) (45-55) (55-67) (1-33) (95-451) (29.6-65.0) (40.0-141.3) (0.68-3.76)
- Iron-loading anemia (21)
  - Normal (5.4-12.5) (18-48) (36-64) (47-95) (1-65) (199-3.150) (27.0-632.7) (35.0-502.7) (0.27-3.21)
- Pregnancy (14)
  - Normal (9.6-14.4) (5-24) (48-89) (5-45) (11-102) (5-160) (1-11-24) (10.0-57.2) (1-2.7-25.0)
- Polycythemia vera (6)
  - Normal (14.8-19.4) (6-19) (57-75) (9-32) (14-285) (13-153) (1-11-20) (4-6.3-35.5) (1-2.5-53.5)
- Inflammation (17)
  - Normal (5.9-14.7) (3-25) (26-84) (4-44) (3-295) (34-5.250) (0.5-38.0) (2-4.9-5.0) (0.4-20.51)
- Liver disease (18)
  - Normal (8.2-16.3) (8-36) (36-69) (16-80) (3-206) (5-6.825) (1-1.91-0) (3-3.4-298) (0.2-42.27)

- Thalassemia
  - α-Thalassemia (9)
    - Normal (12.6) (9-17) (57) (34) (48) (42) (14) (4.2-21) (10.0-159.9) (0.51-4.68)
  - Heterozygous β-thalassemia (34)
    - Normal (9.0-14.5) (9-34) (43-75) (14-69) (5-14) (15-285) (15.0-571.9) (20.0-488.9) (0.32-9.70)
  - β-thalassemia intermedia (12)
    - Normal (7.1-10.2) (25-39) (36-71) (45-77) (5-125) (105-1.870) (30.3-161.97) (18.5-452.1) (0.03-13.63)
  - Sickle cell trait (4)
    - Normal (11.5-16.4) (9-24) (49-107) (8-40) (14-103) (6-27) (1-0.6) (11.5-16.3) (0.02-11.50)
  - Hemolytic disease (11)
    - Normal (6.6-16.4) (9-29) (49-89) (14-50) (3-328) (11-1100) (3-7.3-33) (9.8-412.0) (1.04-13.74)

Arithmetic mean was calculated for Hb, plasma iron, TIBC, and transferrin saturation, while geometric mean was calculated for the other parameters.
patients with iron deficiency and iron overload. In fact, as shown in Fig. 4, at any value for transferrin saturation, the red cell ferritin content was greater in the thalassemic patients than in other subjects. In particular, patients with heterozygous β-thalassemia had normal body iron status and normal transferrin saturation but increased values for red cell ferritin with a reduced acidic/basic ferritin ratio (Table 1). Within the thalassemic patients having normal iron status, there was an inverse relationship between mean corpuscular hemoglobin (MCH) and red cell basic ferritin, as shown in Fig. 5. Thus, the lower the rate of hemoglobin synthesis, the higher the red cell ferritin content. There was no relationship between transferrin saturation and red cell basic ferritin \( r = 0.15, p > 0.05 \) within the group of subjects reported in Fig. 5. Nevertheless, the iron status was relevant to the red-cell ferritin content also in patients with thalassemia. In fact, as shown in Table 4, patients with α- or β-thalassemia trait complicated by iron deficiency showed normal-to-low values for red cell ferritin, and correction of iron deficiency resulted in an increase in such values.

In patients with sickle cell trait, the red cell ferritin content essentially reflected the body iron status. Although increased values for red cell ferritin were found in patients with hemolytic disease (Table 1), there was no relationship between red cell ferritin and reticulocyte count or degree of anemia in this group. The red cell basic ferritin content was also directly related to the transferrin saturation in this group (Fig. 6). However, such a relationship was significantly

![Fig. 1. Relationship between red cell basic ferritin and red cell protoporphyrin in patients with iron deficiency.](image1)

![Fig. 2. Relationship between transferrin saturation and red cell ferritin in patients with iron overload. HH, hom: homozygous HLA-related hemochromatosis; HH, het: heterozygous HLA-related hemochromatosis; RA: refractory hypoplastic anemia; CDA: congenital dyserythropoietic anemia; PASA: primary acquired sideroblastic anemia.](image2)
Table 3. Pearson Correlation Coefficients Between Various Parameters of Iron Status Within Patients With Inflammation (n = 73)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hb</th>
<th>Plasma Iron</th>
<th>TIBC</th>
<th>Transferrin Saturation</th>
<th>Hb/Plasma Iron</th>
<th>Hb/TIBC</th>
<th>Hb/Transferrin Saturation</th>
<th>Hb/In(Red Cell Protoporphyrin)</th>
<th>Hb/In(Plasma Ferritin)</th>
<th>In(Red Cell Protoporphyrin)/In(Plasma Ferritin)</th>
<th>In(Red Cell Protoporphyrin)/In(Red Cell Acidic Ferritin)</th>
<th>In(Red Cell Acidic Ferritin)/In(Red Cell Basic Ferritin)</th>
<th>In(Red Cell Acidic Ferritin)/In(Plasma Ferritin)</th>
<th>In(Red Cell Acidic Ferritin)/In(Red Cell Basic Ferritin)</th>
<th>In(Red Cell Acidic Ferritin)/In(Red Cell Basic Ferritin)</th>
<th>In(Red Cell Acidic Ferritin)/In(Red Cell Basic Ferritin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>1.0000</td>
<td>0.0881</td>
<td>-0.2648</td>
<td>-0.0389</td>
<td>-0.2480</td>
<td>-0.3019</td>
<td>-0.0712</td>
<td>-0.1392</td>
<td>-0.0615</td>
<td>-0.001</td>
<td>-0.001</td>
<td>-0.001</td>
<td>-0.001</td>
<td>-0.001</td>
<td>-0.001</td>
<td>-0.001</td>
</tr>
<tr>
<td>Plasma Iron</td>
<td>0.0881</td>
<td>1.0000</td>
<td>0.3381</td>
<td>0.8650</td>
<td>-0.3008</td>
<td>-0.0474</td>
<td>0.2975</td>
<td>0.1767</td>
<td>-0.1386</td>
<td>0.0229</td>
<td>-0.229</td>
<td>-0.001</td>
<td>-0.001</td>
<td>-0.001</td>
<td>-0.001</td>
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<tr>
<td>TIBC</td>
<td>0.2649</td>
<td>0.3381</td>
<td>1.0000</td>
<td>-0.0684</td>
<td>0.0234</td>
<td>-0.3648</td>
<td>-0.2891</td>
<td>-0.2476</td>
<td>0.0657</td>
<td>0.012</td>
<td>-0.002</td>
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<td>-0.002</td>
<td>-0.1367</td>
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<td>-0.1367</td>
</tr>
<tr>
<td>Transferrin Saturation</td>
<td>0.378</td>
<td>0.8650</td>
<td>-0.0684</td>
<td>1.0000</td>
<td>-0.2337</td>
<td>0.1235</td>
<td>0.4784</td>
<td>0.2836</td>
<td>-0.2185</td>
<td>0.017</td>
<td>-0.001</td>
<td>-0.192</td>
<td>0.017</td>
<td>-0.192</td>
<td>0.017</td>
<td>-0.192</td>
</tr>
<tr>
<td>In(Red Cell Protoporphyrin)</td>
<td>0.017</td>
<td>-0.3008</td>
<td>0.0234</td>
<td>-0.2337</td>
<td>1.0000</td>
<td>0.0775</td>
<td>-0.3005</td>
<td>-0.1992</td>
<td>0.1199</td>
<td>0.3109</td>
<td>-0.0474</td>
<td>0.1235</td>
<td>-0.0474</td>
<td>0.1235</td>
<td>-0.0474</td>
<td>0.1235</td>
</tr>
<tr>
<td>In(Plasma Ferritin)</td>
<td>0.004</td>
<td>0.345</td>
<td>0.0001</td>
<td>0.0149</td>
<td>0.005</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
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</tr>
<tr>
<td>In(Red Cell Basic Ferritin)</td>
<td>0.0712</td>
<td>0.2975</td>
<td>0.2891</td>
<td>0.4748</td>
<td>0.3005</td>
<td>0.5093</td>
<td>1.0000</td>
<td>0.4149</td>
<td>-0.6141</td>
<td>0.1392</td>
<td>0.1767</td>
<td>0.2476</td>
<td>0.4149</td>
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<tr>
<td>In(Red Cell Acidic Ferritin)</td>
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<td>0.006</td>
<td>0.0001</td>
<td>0.0014</td>
<td>0.005</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
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<td>0.001</td>
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<td>0.001</td>
<td>0.001</td>
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<tr>
<td>In(Acidic/Basic Ferritin)</td>
<td>0.0515</td>
<td>0.1386</td>
<td>0.0657</td>
<td>0.2185</td>
<td>0.1199</td>
<td>-0.2929</td>
<td>-0.6141</td>
<td>0.4652</td>
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<td>0.333</td>
<td>0.121</td>
<td>0.156</td>
<td>-0.006</td>
<td>-0.006</td>
<td>-0.006</td>
<td>-0.006</td>
</tr>
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</table>
RED CELL FERRITIN

Fig. 4. Relationship between transferrin saturation and red cell ferritin (liver assay) in normal subjects and patients with iron deficiency and iron overload (solid symbols and continuous line) and in thalassemic patients (open symbols and broken line). Although statistical analyses were performed on 150 and 55 subjects, respectively, only mean values are shown for clarity. The equations of the 2 regressions curves were: ln(RBC ferritin) = 2.02 + 0.0636 x transferrin saturation (continuous line), and ln(RBC ferritin) = 2.402 + 0.0456 x transferrin saturation (broken line). The two curves differed significantly with respect to both location ($F = 118.47, p < 0.001$) and slope ($F = 4.36, p < 0.05$). Discriminant analysis showed that 85.3% of normal subjects and patients with disorders of iron metabolism and 72.7% of thalassemic subjects could be completely separated.

shown in Table 5. In two patients with Cooley's disease, normal values for red cell ferritin were obtained only when red cell production was completely suppressed by transfusion therapy.

DISCUSSION

Iron-containing granules, visible on light microscopy after Perls staining, have been recognized in erythroblasts and erythrocytes for at least 40 yr. Using electron microscopy, Bessis and Breton-Gorius showed that cytoplasmic iron granules are composed of aggregated ferritin iron cores. Since then, attempts have been made to clarify the biologic significance of ferritin in erythroid cells, but that is still controversial.

The use of sensitive immunoassays has now allowed red cell ferritin to be quantitated. These investigations require, at least for research purposes, the simultaneous use of two immunoassays, one for basic ferritin and the other for acidic ferritin. It has been shown, in fact, that ferritin is present in tissues in multiple forms that differ structurally and immunologically, and that red cell ferritin is composed mainly by acidic isoferritins. The findings obtained by Jacobs and coworkers and by us in a preliminary study are compatible with the hypothesis that red cells contain two types of ferritin, one reacting with antibodies against liver or spleen ferritin, and the other reacting with antibodies against heart ferritin. The existence of two distinct ferritin types in red cells had been already suggested by Worwood et al. through studies of reticulocyte ferritin.

There is a reasonable agreement between values for red cell basic ferritin obtained in different centers. In particular, Van Der Weyden et al., using a rapid and simple method, have obtained normal values very similar to those obtained in Cardiff and Pavia by

Table 4. Hb Level, Iron Status Data, and Red Cell Ferritin Contents in 4 Patients With Thalassemia Complicated by Iron Deficiency, Before and After Appropriate Treatment

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hb (g/dL)</th>
<th>MCH (pg)</th>
<th>MCV (fl)</th>
<th>Plasma iron (μmol/Liter)</th>
<th>TIBC (μmol/Liter)</th>
<th>Transferrin Saturation (%)</th>
<th>Red Cell Protoporphyrin (µg/dl Packed Red Cells)</th>
<th>Plasma Ferritin (µg/Liter)</th>
<th>Red Cell Ferritin Content (µg/Cell)</th>
<th>Basic Ferritin</th>
<th>Acidic Ferritin</th>
<th>Acidic/Basic Ferritin Ratio</th>
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</thead>
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<tr>
<td>(1) a-Thalassemia trait</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Associated with iron deficiency</td>
<td>10.1</td>
<td>17.1</td>
<td>55</td>
<td>7</td>
<td>86</td>
<td>8</td>
<td>168</td>
<td>12</td>
<td>28.0</td>
<td>10.9</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>After iron therapy</td>
<td>13.9</td>
<td>20.0</td>
<td>65</td>
<td>17</td>
<td>50</td>
<td>34</td>
<td>40</td>
<td>20</td>
<td>28.0</td>
<td>10.9</td>
<td>0.39</td>
<td></td>
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<tr>
<td>(2) Heterozygous ß-thalassemia</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Associated with iron deficiency</td>
<td>8.9</td>
<td>16.8</td>
<td>51</td>
<td>3</td>
<td>95</td>
<td>3</td>
<td>230</td>
<td>9</td>
<td>3.0</td>
<td>16.8</td>
<td>5.60</td>
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<tr>
<td>After iron therapy</td>
<td>11.4</td>
<td>21.0</td>
<td>69</td>
<td>22</td>
<td>64</td>
<td>34</td>
<td>40</td>
<td>45</td>
<td>52.6</td>
<td>76.3</td>
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<tr>
<td>(3) Heterozygous ß-thalassemia</td>
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<td></td>
</tr>
<tr>
<td>Associated with iron deficiency</td>
<td>9.0</td>
<td>17.6</td>
<td>56</td>
<td>9</td>
<td>88</td>
<td>10</td>
<td>277</td>
<td>6</td>
<td>4.0</td>
<td>14.6</td>
<td>3.55</td>
<td></td>
</tr>
<tr>
<td>After iron therapy</td>
<td>11.2</td>
<td>19.9</td>
<td>65</td>
<td>16</td>
<td>57</td>
<td>28</td>
<td>61</td>
<td>24</td>
<td>85.3</td>
<td>79.7</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>(4) Heterozygous ß-thalassemia</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Associated with polycythemia vera</td>
<td>16.7</td>
<td>19.5</td>
<td>61</td>
<td>10</td>
<td>59</td>
<td>17</td>
<td>74</td>
<td>13</td>
<td>15.8</td>
<td>24.6</td>
<td>2.19</td>
<td></td>
</tr>
<tr>
<td>After 131I treatment</td>
<td>12.1</td>
<td>21.2</td>
<td>70</td>
<td>20</td>
<td>54</td>
<td>37</td>
<td>35</td>
<td>39</td>
<td>30.9</td>
<td>48.7</td>
<td>1.58</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 5. Relationship between mean corpuscular hemoglobin (MCH) and red cell ferritin (liver assay) in patients with thalassemia having normal values for transferrin saturation and serum ferritin. The shaded area represents the normal reference range.
means of a more elaborate method, based on removal of leukocyte through blood filtration. However, it remains to be seen whether the above simple method also works in patients with increased WBC and/or inflammation, since contamination by leukocyte ferritin may become consistent in such conditions. Data on acidic ferritin have been obtained only in Cardiff and Pavia (at least to our knowledge). The absolute values for acidic ferritin were higher in Cardiff than in Pavia, but the behavior of acidic ferritin in disorders of iron metabolism was similar. Thus, as suggested in our preliminary work, the discrepancy could be removed by a standardization of acidic ferritin assays.

In examining the possible determinants of the red cell ferritin content, one should first consider that red cell ferritin is a residue of erythroblast ferritin. Thus, a fundamental question is how much of the erythroblast ferritin remains in the daughter cells and how much is lost during maturation. Evidence has been obtained that ferritin may be lost during in vitro maturation of pig reticulocytes, and it has been suggested that such cells contain an intrinsic mechanism for metabolizing siderotic granules and ferritin. Such a mechanism does not appear to be active in man, but siderotic granules could be removed from reticulocytes by the spleen. In patients with increased erythropoiesis, Lipshitz et al. found appreciably higher concentrations of ferritin in the reticulocytes as compared with mature erythrocytes. The results of the present study show that, in patients with hemolytic disease and normal iron status,"

![Red cell ferritin (liver assay)](image)

**Fig. 6.** Relationship between transferrin saturation and red cell ferritin (liver assay) in patients with hemolytic disease. The shaded area represents the 97.5% confidence limits for normal values. Regression analysis of grouped data showed that the regression curve of normal subjects and that of hemolytic patients differed significantly with respect to location (\( F = 11.56, p < 0.001 \)), while they did not differ with respect to slope (\( F = 0.15, p > 0.05 \)).

![Reticulocytes](image)

**Fig. 7.** Values for reticulocyte count, pyruvate kinase (PK) activity, and red cell ferritin (RBC Ft) in different fractions of centrifuged blood samples from six subjects. T: top fraction; M: middle fraction; B: bottom fraction. (A and O) Normal subjects; (*) heterozygous \( \beta \)-thalassemia; (B) congenital dyserythropoietic anemia type II; (O) heterozygous \( \beta \)-thalassemia complicated by traumatic hemolysis; (\( \ast \)) anemia of chronic disease. Geometric mean values of each red cell fraction and \( F \) values for variance analysis between fractions were the following: reticulocyte count: 4.3% (T), 1.4% (M), 1.1% (B); \( F = 8.71, p < 0.01 \); PK activity: 18.3 IU/g Hb (T), 12.2 (M), 9.3 (B); \( F = 13.23, p < 0.001 \); red cell ferritin (liver assay): 27.2 ag/cell (T), 20.1 (M), 17.4 (B); \( F = 0.56, p > 0.05 \); red cell ferritin (heart assay): 60.0 ag/cell (T), 42.5 (M), 46.4 (B); \( F = 0.20, p > 0.05 \).

<table>
<thead>
<tr>
<th>Hb (g/dL)</th>
<th>Reticulocytes (%)</th>
<th>Plasma Iron (μmole/Liter)</th>
<th>TIBC (μmole/Liter)</th>
<th>Transferrin Saturation (%)</th>
<th>Plasma Ferritin (μg/Liter)</th>
<th>Red Cell Ferritin Content (ag/Cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.0</td>
<td>2.0</td>
<td>27</td>
<td>34</td>
<td>79</td>
<td>1.650</td>
<td>378.0 520.0 1.38</td>
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<tr>
<td>12.0</td>
<td>0.1</td>
<td>32</td>
<td>38</td>
<td>84</td>
<td>1.628</td>
<td>8.7 25.0 2.87</td>
</tr>
<tr>
<td>Patient 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.8</td>
<td>2.8</td>
<td>29</td>
<td>36</td>
<td>81</td>
<td>2.970</td>
<td>359.7 523.2 1.45</td>
</tr>
<tr>
<td>9.8</td>
<td>1.0</td>
<td>32</td>
<td>39</td>
<td>82</td>
<td>2.585</td>
<td>169.0 169.5 1.00</td>
</tr>
<tr>
<td>11.5</td>
<td>0.5</td>
<td>31</td>
<td>42</td>
<td>74</td>
<td>2.750</td>
<td>121.0 53.9 0.45</td>
</tr>
<tr>
<td>13.6</td>
<td>0</td>
<td>34</td>
<td>40</td>
<td>75</td>
<td>3.100</td>
<td>8.5 38.9 4.58</td>
</tr>
</tbody>
</table>
red cell ferritin is higher than normal (Fig. 6), thus indicating that reticulocytes contain more ferritin than mature red cells. On the other hand, our studies on the ferritin content of fractionated red cell populations show that variation due to the reticulocyte count is less significant than variation due to the iron status (Fig. 7).

In the present study, two factors appear to be important in determining the red cell ferritin content: (A) the transferrin saturation and (B) the rate of hemoglobin synthesis. Furthermore, such factors more closely affect the basic ferritin than the acidic ferritin. These findings may be explained as follows.

Iron is distributed to body tissues by the plasma transferrin. It has been recently shown that the transferrin iron pool is not homogeneous but is composed of monoferric and diferric transferrin, the latter having a far greater capacity to dispense iron to tissue. In subjects with iron overload, transferrin saturation is high and most of the plasma iron is bound to diferric transferrin; erythroblasts of such subjects likely take up more iron than that required for heme synthesis. In studies of iron uptake by normal human erythroblasts in vitro, May et al. have found that increasing transferrin saturation from 18% to 81% doubled iron uptake, with about two-thirds of such excess iron being incorporated in compounds other than heme. Thus, the increased iron uptake by the erythroblasts would explain the high values for red cell ferritin content found in patients with iron overload. On the other hand, patients with iron deficiency have low plasma iron, almost completely bound to monoferric transferrin. Their erythroblasts take up less iron than that required for heme synthesis, and therefore need less ferritin as an intracellular iron carrier or depot. This would explain the low ferritin contents of iron-deficient red cells. Due to a block in the release of iron from the reticuloendothelial cell to transferrin, patients with inflammation have low plasma iron, mainly bound to monoferric transferrin. As expected, their red-cell ferritin content was normal to low, which is similar to that of subjects with iron-deficient erythropoiesis. However, the direct correlation between red cell and plasma ferritin found in these patients is not clear. A possible explanation is that ferritin synthesis in inflammation may be also a nonspecific systemic response of tissues. Indeed, high levels of ferritin have been found both in granulocytes and monocytes in such conditions. Thus, in inflammation, the red cell ferritin content may be a result of the balance between the negative effect of reduced iron supply to erythroid marrow and the nonspecific stimulus to ferritin synthesis.

The second determinant of red cell ferritin appeared to be the rate of hemoglobin synthesis. In thalassemia, globin chain synthesis is variably impaired and hemoglobin synthesis is consequently reduced, the end-result being poorly hemoglobinized erythrocytes. We found high values for red cell ferritin in thalassemic patients, even in those with normal iron status and normal transferrin saturation. This can be explained by assuming that thalassemic erythroblasts take up normal amounts of iron, but part of the metal cannot be utilized for hemoglobin synthesis. Such excess iron would be stored in ferritin molecules, thus producing increasing amounts of red cell ferritin. The inverse relationship between red cell ferritin and mean corpuscular hemoglobin (Fig. 5) is in keeping with this hypothesis. However, it should be noted that the data reported in Table 4 indicate that the amount of iron entering erythroblasts is also relevant to the red cell ferritin content in thalassemic patients.

A natural question is whether the two ferritin types measured by the liver (or spleen) and heart assays have different metabolic functions within erythroid cells. Previous studies by Gabuzda and Gardner showed the existence of two distinct pools of ferritin in human bone marrow, and more recent studies have provided evidence that, within erythroid cells, one pool could be used in the intracellular transport of iron for heme synthesis and the other for iron storage. Basic ferritin is usually found in iron storage organs (liver and spleen), and in red cells, this ferritin type seems to be closely related to the iron status; therefore, it may function as an intracellular depot of iron that is in excess of that required for heme synthesis. Acidic ferritins are usually found in organs having high iron requirements for metabolic activities (e.g., heart) and are capable of taking up and donating the metal more rapidly than basic ferritins. In red cells, acidic ferritin appears to be less influenced by the iron status than basic ferritin. Moreover, the acidic/basic ferritin ratio has been found to decrease with erythroblast maturation. Thus, it is possible that acidic ferritin has an active role within erythroid cells, behaving as an intermediate for the transfer of iron from the plasma membrane to the mitochondria for incorporation into heme. A model of possible pathways of iron within heme-synthesizing erythroid cells is shown in Fig. 8.

From a clinical standpoint, the red cell ferritin assay is more cumbersome than the plasma ferritin assay and obviously cannot be employed in subjects who have been recently transfused. Nevertheless, in spite of this and the low number of studies performed, a few clinical applications of the red cell ferritin determination, based on a commercially available basic ferritin assay, can be singled out.

First, the red cell ferritin content reflects the bal-
ance between iron supply to the erythroid marrow and its utilization for the hemoglobin synthesis. Thus, it can be used to evaluate the adequacy of iron supply and to diagnose relative iron deficiency in patients with increased red cell turnover.43

Second, it is well known that liver disease may elevate the plasma ferritin concentration to a degree disproportionate to that of iron stores.43,44 Since the red cell ferritin content does not appear to be influenced by abnormalities in liver function, it can be useful in the evaluation of iron status of such patients. It has been recently shown that the ratio of red cell to serum ferritin discriminates patients with alcoholic liver disease and iron overload from those with idiopathic hemochromatosis.45 In addition, the red cell ferritin content identified patients with idiopathic hemochromatosis after phlebotomy, who, despite normal serum ferritin concentrations, had persistently raised iron stores.46 In the present study, the red cell ferritin content was the best marker of iron overload in heterozygotes for hemochromatosis with partial expression of the disease.

There may be other clinical applications of the red cell ferritin assay (an example is reported in Table 5), and the number will certainly increase in the next years.

NOTE ADDED IN PROOF

In the interval between initial acceptance of this manuscript and its publication, we have used a two-site immunoradiometric assay (IRMA) based on a mouse monoclonal antibody against human heart ferritin for measuring red cell ferritin. This IRMA did not recognize human liver ferritin up to a concentration of 10,000 µg/liter. On the average, the values obtained with this assay in normal subjects and patients with iron deficiency and iron overload were lower than those found with the radioimmunoassay based on polyclonal antibodies against human heart ferritin. The monoclonal antibody will be available on request in the future for interlaboratory standardization and further studies on red cell ferritin.

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Biologic and clinical significance of red cell ferritin

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