Evidence That Stainable Bone Marrow Iron Following Parenteral Iron Therapy Does Not Correlate With Serum Iron Studies and May Not Represent Readily Available Storage Iron

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Key Words: Iron; Bone marrow; Iron deficiency; Parenteral iron

Abstract

We recently reported that parenteral iron therapy is associated with a characteristic pattern of iron staining on bone marrow aspirate smears. We now present clinical information from 6 patients who received parenteral iron and, at one or more points in follow-up, were found to have low or borderline low serum ferritin levels and/or serum iron levels, even though marrow aspirate smears revealed abundant stainable iron in the pattern characteristic of prior parenteral iron therapy. We conclude that stainable iron seen in this pattern does not correlate with serum iron studies and may not represent functionally available storage iron. This pattern of iron staining should not be used as evidence to withhold further iron therapy in patients who otherwise continue to have features of iron deficiency anemia.

Iron deficiency is a common cause of anemia. In this condition, erythrocytes have deficient hemoglobin content, and RBCs are usually microcytic and hypochromic. Determinations of serum iron level, total iron binding capacity (TIBC), the percentage of transferrin saturation, and/or the ferritin level are part of the basic chemical study of blood when evaluating for possible iron deficiency anemia. In iron deficiency, serum iron is typically decreased, while TIBC is elevated, and the percentage of transferrin saturation is decreased. A low serum ferritin level is virtually pathognomonic of iron deficiency.

Examination of bone marrow for iron is a widely accepted means of establishing the adequacy of body iron stores. Bone marrow evaluation to determine the presence or absence of storage iron may be undertaken when the results of other studies are equivocal, especially if the possibility of costly parenteral iron therapy is being considered. Potassium ferrocyanide (Prussian blue) stain may be applied to tissue samples to visualize microscopic nonheme iron within cells. Iron appears as variously sized intracellular blue granules or wispy amorphous intracellular deposits. Iron identified as such has generally been considered to represent the insoluble iron storage compound, hemosiderin. The examination of Prussian blue–stained bone marrow aspirate for the presence or absence of histiocytic iron granules has been considered the “gold standard” in evaluating iron-depleted states. The complete absence of staining is considered to represent iron deficiency.

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with serum iron studies and may not be a reliable indicator of functional storage iron.

**Materials and Methods:**

**Cases**

The cases represent bone marrow samples examined in our laboratory during an approximate 18-month period (January 2006–June 2007) that met the following criteria: (1) displayed the pattern of iron staining associated with parenteral iron therapy; (2) known date of last parenteral iron dose before the bone marrow procedure; and (3) known low serum ferritin and/or serum iron level before the bone marrow procedure, with no iron therapy between the date of iron studies and the bone marrow procedure. Six cases are reported.

<table>
<thead>
<tr>
<th>Case No./Sex/Age (y)</th>
<th>Clinical Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/59</td>
<td>Iron deficiency; gastrointestinal arteriovenous malformations; thrombocytosis</td>
</tr>
<tr>
<td>2/M/46</td>
<td>Celiac sprue with malabsorption</td>
</tr>
<tr>
<td>3/F/76</td>
<td>Iron deficiency due to gastrointestinal blood loss; negative endoscopy</td>
</tr>
<tr>
<td>4/F/67</td>
<td>Iron deficiency of unknown etiology</td>
</tr>
<tr>
<td>5/M/80</td>
<td>Iron deficiency; dialysis-dependent renal failure</td>
</tr>
<tr>
<td>6/M/44</td>
<td>Iron deficiency; Crohn disease with malabsorption</td>
</tr>
</tbody>
</table>

**Pathologic Evaluation**

For assessment of storage iron, aspirate smears were stained with Prussian blue. The selected cases demonstrated stainable iron in the pattern characteristic of prior parenteral iron therapy, as described in a previous report. For each case, medical records were obtained from the submitting physician and reviewed. The pertinent clinical history is described for each case.

**Results**

The 6 cases included 3 women and 3 men with ages ranging from 44 to 80 years. The clinical manifestations included iron deficiency anemia due to malabsorption, gastrointestinal blood loss, dialysis-related blood loss, and iron deficiency of unknown cause. Pertinent laboratory data, the timeline for the data, and timing of parenteral iron therapy in relationship to the date of bone marrow biopsy or aspiration are given in Table 1. Two patients received iron dextran (INFeD), 2 sodium iron gluconate (Ferrlecit), and 2 iron sucrose (Venofer) (Table 2). Photomicrographs illustrating the pattern of iron staining for each respective patient are provided Image 1, Image 2, Image 3, Image 4, Image 5, and Image 6.

**Case Reports**

**Case 1**

A 59-year-old woman was referred for hematology consultation in November 2005 for evaluation of thrombocytosis. At that time, the platelet count was $1.389 \times 10^3/\mu$L ($1.389 \times$
Thomason and Almiski  /  STAINABLE BONE MARROW IRON AFTER PARENTERAL IRON THERAPY

The initial CBC revealed severe anemia (hemoglobin, 5.0 g/dL [50 g/L]; hematocrit, 15.5% [0.15]; and mean corpuscular volume [MCV], 81.2 µm³ [81.2 fL]). She was found to have decreased serum ferritin (3.0 ng/mL [7 pmol/L]) and serum iron (12.0 µg/dL [2.1 µmol/L]) levels. She was given transfusions of 3 U of packed RBCs and administered 250 mg intravenous (IV) Venofer each day for 3 consecutive days.

The patient was reevaluated in January 2006. The platelet count remained high (926 × 10⁹/µL [926 × 10⁹/L]), and she remained anemic (hemoglobin, 9.4 g/dL [94 g/L]; hematocrit, 29.9% [0.30]; and MCV, 77.4 µm³ [77.4 fL]). She continued to have laboratory findings of iron deficiency (ferritin, 9.0 ng/mL [2.1 µmol/L]; and iron, 18.0 µg/dL [3.2 µmol/L]). A bone marrow biopsy/aspiration revealed chronic myeloproliferative disease consistent with essential thrombocythemia. The evaluation also revealed abundant stainable iron in the pattern characteristic of parenteral iron therapy (Image 1), despite the clinical laboratory findings of iron deficiency. Subsequently, the patient received ten 250-mg doses of IV Venofer during a 3-month period, and the hemoglobin level normalized.

A gastrointestinal workup revealed gastritis and multiple arteriovenous malformations of the ileum without evidence of active bleeding.

**Case 2**

A 46-year-old man was monitored by a hematologist for refractory iron deficiency in the setting of malabsorption related to celiac sprue. He was given 2.5 g of IV INFeD on January 27, 2006. A CBC on March 20, 2006, revealed mild anemia (hemoglobin, 12.9 g/dL [129 g/L]; hematocrit, 37.3% [0.38]; and MCV, 78.1 µm³ [78.1 fL]); ferritin, males: 18-250 ng/mL; females: 13-160 ng/mL; Fe: males, 76-198 µg/dL; females, 26-170 µg/dL; TIBC: males, 262-275 µg/dL; females, 262-275 µg/dL; transferrin saturation: males, 20%-50%; females, 20%-50%.

### Table 2

Pertinent Laboratory Information and Timing in Relationship to Timing of Parenteral Iron Therapy and BMA

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Date of CBC</th>
<th>Hgb (g/dL)</th>
<th>Hct (%)</th>
<th>MCV (µm³)</th>
<th>Date of Ferritin Assay</th>
<th>Ferritin (ng/mL)</th>
<th>Date of Iron Assay</th>
<th>Fe (µg/dL)</th>
<th>TIBC (µg/dL)</th>
<th>Transferrin Saturation (%)</th>
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<tbody>
<tr>
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<td>9.4</td>
<td>29.9</td>
<td>77.4</td>
<td>1/11/06</td>
<td>9.0</td>
<td>1/11/06</td>
<td>18.0</td>
<td>457</td>
<td>3.9</td>
</tr>
<tr>
<td>2</td>
<td>5/19/06</td>
<td>12.4</td>
<td>38.5</td>
<td>81.9</td>
<td>5/19/06</td>
<td>24</td>
<td>5/1/06</td>
<td>28.0</td>
<td>266</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
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<td>8.6</td>
<td>28.1</td>
<td>74.9</td>
<td>6/21/06</td>
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<td>6/21/06</td>
<td>15</td>
<td>512</td>
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<td>2/14/07</td>
<td>8.9</td>
<td>28.2</td>
<td>91</td>
<td>2/13/07</td>
<td>18</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>9/29/06</td>
<td>5.0</td>
<td>16.5</td>
<td>74.3</td>
<td>9/7/06</td>
<td>8</td>
<td>9/7/06</td>
<td>17</td>
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<td>6/8/07</td>
<td>12</td>
<td>6/8/07</td>
<td>14.1</td>
<td>356</td>
<td>5</td>
</tr>
</tbody>
</table>

BMA, bone marrow aspiration; Hct, hematocrit; Hgb, hemoglobin; IV, intravenous; MCV, mean corpuscular volume; NA, not available; TIBC, total iron binding capacity.

* Values are given in conventional units; conversions to Système International units are as follows: Hgb (g/L), multiply by 10.0; Hct (proportion of 1.0), multiply by 0.01; MCV (µL), multiply by 1.0; ferritin (pmol/L), multiply by 2.247; Fe and TIBC (µmol/L), multiply by 0.179. Reference ranges in conventional units are as follows: Hgb: males, 14-18 g/dL; females, 12-16 g/dL; Hct: males, 39%-54%; females, 37%-47%; MCV: males, 82-102 µm³; females, 78-101 µm³; ferritin: males, 18-250 ng/mL; females, 13-160 ng/mL; Fe: males, 76-198 µg/dL; females, 26-170 µg/dL; TIBC: males, 262-275 µg/dL; females, 262-275 µg/dL; transferrin saturation: males, 20%-50%; females, 20%-50%.
A gastrointestinal evaluation that included upper and lower endoscopy revealed no definite source of bleeding. She was given 2.0 g of IV Venofer on January 4, 2006. A CBC on January 9, 2006, revealed anemia (hemoglobin, 10.1 g/dL [101 g/L]; hematocrit, 32.3% [0.32]; and MCV, 91.3 µm³ [91.3 fL]). On June 21, 2006, serum iron studies revealed iron deficiency (serum iron, 15 µg/dL [2.7 µmol/L]; TIBC, 512 µg/dL [91.6 µmol/L]; and transferrin saturation, 3%). Bone marrow biopsy/aspiration performed on July 7, 2006, revealed low-level involvement by mature small B-cell lymphoproliferative disease, not otherwise specified. Storage iron was present in the pattern characteristic of prior parenteral iron therapy (Image 3). A CBC on the date of the bone marrow procedure revealed microcytic anemia (hemoglobin, 8.6 g/dL [86 g/L]; hematocrit, 28.1% [0.28]; and MCV, 74.9 µm³ [74.9 fL]). She was given three 2.0-g doses of IV Venofer in the period of July 20 to 28, 2006. A CBC on July 31, 2006, revealed persistent microcytic anemia (hemoglobin, 9.2 g/dL [92 g/L]; hematocrit, 30.1% [0.30]; and MCV, 78.9 µm³ [78.9 fL]).

**Case 4**

A 67-year-old woman was found to have iron deficiency anemia of unknown cause in July 2006. The CBC at that time revealed normocytic anemia (hemoglobin, 8.5 g/dL [85 g/L]; hematocrit, 15.5% [0.16]; and MCV, 84 µm³ [84 fL]), and the serum ferritin level was decreased (9 ng/dL [20 pmol/L]). She received 3 U of packed RBCs and seven 125-mg doses of Ferrlecit. Evaluation included upper and lower endoscopy, which revealed no definite source of gastrointestinal bleeding. She was reevaluated monthly...
from July 2006 to February 2007, and remained anemic (hemoglobin ranging from 7 to 11.6 g/dL [70-116 g/L] and MCV ranging from 83 to 97 µm³ [83-97 fL]). In the interval December 2006–January 2007, she received eight 125-mg doses of Ferlecit, with the last dose administered on January 12, 2007. A serum ferritin level obtained on February 12, 2007, was decreased (18 ng/mL [40 pmol/L]). A bone marrow biopsy/aspiration performed on February 14, 2007, revealed variably cellular marrow with adequate trilineage hematopoiesis and abundant stainable iron in the pattern associated with prior parenteral iron therapy (Image 4). A CBC on the date of the marrow examination revealed microcytic anemia (hemoglobin, 8.9 g/dL [89 g/L]; hematocrit, 28.2% [0.28]; and MCV, 74.3 µm³ [74.3 fL]). Following the bone marrow procedure, the patient received six 250-mg doses of IV Ferlecit during a 3-month period, and the hemoglobin level subsequently reached 11.2 g/dL (112 g/L).

Case 5

A 79-year-old man had a diagnosis of stage IV chronic kidney disease. On August 1, 2005, he was noted to be iron deficient and was given parenteral iron. He completed this course of therapy on August 20, 2005, after receiving 1,000 mg of Ferlecit in 8 divided doses. Additional Ferlecit therapy was begun on July 7, 2006, and he received eight 125-mg doses. Serum iron studies on September 7, 2006, revealed iron deficiency (ferritin, 8 ng/mL [18 pmol/L]; serum iron, 17 µg/dL [3.0 µmol/L]; TIBC, 356 µg/dL [63.7 µmol/L]; and transferrin saturation, 5%). A CBC on September 29, 2006, revealed severe microcytic anemia (hemoglobin, 5.0 g/dL [50 g/L]; hematocrit, 16.5% [0.17]; and MCV, 74.3 µm³ [74.3 fL]). A bone marrow biopsy/aspiration was performed on that day and revealed mild dyserythropoiesis. Storage iron was deposited in the pattern associated with parenteral iron therapy (Image 5). Subsequent evaluation revealed angiodysplasia-related gastrointestinal bleeding as the cause of the iron deficiency. Following cauterization of the abnormal gastrointestinal blood vessels, the hemoglobin level improved to 11.6 g/dL (116 g/L).

Case 6

The patient is a 44-year-old man with iron deficiency anemia secondary to gastrointestinal blood loss in the setting of Crohn disease. He received multiple cycles of IV INFED with a good response. Subsequently, the anemia recurred and was refractory to therapy. He received 500 mg of IV INFED on March 16, 2007. A CBC on June 8, 2007, revealed anemia (hemoglobin, 10.9 g/dL [109 g/L]; hematocrit, 34.7% [0.35]; and MCV, 80 µm³ [80 fL]), whereas iron studies revealed iron deficiency (ferritin, 12 ng/mL [27 pmol/L]; serum iron, 14.1 µg/dL [2.5 µmol/L]; TIBC, 356 µg/dL [63.7 µmol/L]; and transferrin saturation, 5%). Bone marrow biopsy/aspiration performed June 8, 2007, revealed normocellular marrow with trilineage hematopoiesis and stainable iron in the pattern characteristic of prior parenteral iron therapy (Image 6). Following the bone marrow procedure, the patient received three 500-mg doses of IV INFED during a 2-month period, and the hemoglobin level subsequently reached 11.7 g/dL (117 g/L).

Discussion

Common to all of the reported cases is a history of iron deficiency anemia treated with IV iron and clinical and/or laboratory features of persistent iron deficiency following therapy, at a time when a bone marrow sample revealed stainable iron in a pattern characteristic of prior parenteral iron therapy. The data indicate that for these cases, stainable iron in this pattern did not correlate with serum ferritin and/or iron levels.

The parenteral iron preparations used in these cases were Venofer, Ferlecit, and INFED. In each of these, elemental iron is bound to a carbohydrate moiety. These iron preparations are colloids composed of a central core of ferric oxide/oxyhydroxide surrounded by a shell of carbohydrate (sucrose in Venofer, dextran in INFED, and gluconate in Ferlecit). Following administration, the iron-carbohydrate complex is taken up by reticuloendothelial cells, where the iron is separated from the carbohydrate. Once free of the carbohydrate, iron is stored as soluble ferritin or insoluble hemosiderin or released into plasma and bound to transferrin for transport to other tissues.

Storage iron is identified microscopically by the use of special histochemical staining of bone marrow specimens. The most reliable specimens are aspirate smears and aspirate particle specimens. Core biopsy specimens may reveal iron, but absent staining may represent a false-negative result owing to leaching of iron from the specimen during decalcification. Iron identified by microscopy is generally considered to represent the insoluble iron storage compound, hemosiderin. It appears as variously sized and shaped granules and amorphous wispy material that is randomly distributed with reticuloendothelial cells.

The peculiar appearance of stainable iron following parenteral iron therapy (Images 1-6) is dissimilar to that observed in patients who have not received this therapy. Following parenteral iron therapy, stainable iron is visualized as relatively uniform intracellular granules that tend to form curvilinear arrays within marrow stromal or reticuloendothelial cells, with amorphous wispy iron deposits being unapparent.

The pattern of iron staining following parenteral iron therapy, in combination with knowledge that patients with this pattern may continue to have clinical and laboratory
evidence of functional iron deficiency, raises some interesting questions. Does the iron observed in this pattern represent hemosiderin or possibly some other form of stainable iron? In particular, might it represent iron that remains bound to the carbohydrate moiety in the pharmaceutical preparation and thus be unavailable for physiologic use? If so, this would explain why this pattern of iron staining does not correlate with serum ferritin or iron levels, assays that may better represent functionally available iron.

In 5 of the 6 cases, additional parenteral iron therapy following the bone marrow procedure resulted in resolution of anemia and/or a significant rise in the hemoglobin level. This suggests that the additional therapy corrected the functional iron deficiency and that a portion of the therapeutic iron may readily become part of the functional iron pool while the remaining portion may not.

Conclusion

Our observations show that following parenteral iron therapy, stainable bone marrow iron in the pattern characteristic of parenteral iron therapy does not always correlate with serum iron and/or ferritin levels. In this situation, it seems that this stainable iron does not represent readily available storage iron. This pattern of iron staining should not be used as evidence to withhold further parenteral iron therapy in patients who otherwise continue to have features of iron deficiency anemia.

References