Serum Ferritin Level Remains a Reliable Marker of Bone Marrow Iron Stores Evaluated by Histomorphometry in Hemodialysis Patients

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Background and objectives: As well as being a marker of body iron stores, serum ferritin (sFerritin) has also been shown to be a marker of inflammation in hemodialysis (HD) patients. The aim of this study was to analyze whether sFerritin is a reliable marker of the iron stores present in bone marrow of HD patients.

Design: Histomorphometric analysis of stored transiliac bone biopsies was used to assess iron stores by determining the number of iron-stained cells per square millimeter of bone marrow.

Results: In 96 patients, the laboratory parameters were hemoglobin = 11.3 ± 1.6 g/dl, hematocrit = 34.3 ± 5%, sFerritin = 609 ± 305 ng/ml, transferrin saturation = 32.7 ± 22.5%, and C-reactive protein (CRP) = 0.9 ± 1.4 mg/dl. sFerritin correlated significantly with CRP, bone marrow iron, and time on HD treatment (P = 0.006, 0.001, and 0.048, respectively). The independent determinants of sFerritin were CRP (β-coef = 0.26; 95% CI = 24.6 to 132.3) and bone marrow iron (β-coef = 0.32; 95% CI = 0.54 to 2.09). Bone marrow iron was higher in patients with sFerritin >500 ng/ml than in those with sFerritin ≤500 ng/ml. In the group of patients with sFerritin ≤500 ng/ml, the independent determinant of sFerritin was bone marrow iron (β-coef = 0.48, 95% CI = 0.48 to 1.78), but in the group of patients with sFerritin >500 ng/ml, no independent determinant of sFerritin was found.

Conclusions: sFerritin adequately reflects iron stores in bone marrow of HD patients.


A nemia is a common complication of chronic kidney disease (CKD) that mainly affects patients on hemodialysis (HD) therapy. It results from the reduced kidney production of erythropoietin (EPO), as well as from changes in iron homeostasis, which can lead to deficiency of this metal. Thus, the routine monitoring of iron stores is crucial for an adequate management of anemia in that population (1).

The most precise tool to evaluate iron status is the measurement of bone marrow iron content, which requires an invasive technique (2,3). Therefore, serum ferritin (sFerritin) and transferrin saturation are currently the main surrogate markers used in daily clinical practice for assessing iron status (4). However, sFerritin is also a positive acute-phase reactant (5). Because inflammation is a frequent finding in HD patients (6), it has been argued that sFerritin can lead to misinterpretation of iron status.

Thus, the aim of the present study was to analyze whether sFerritin is a reliable marker of the iron stores present in the bone marrow of HD patients. These stores were assessed by histomorphometric analyses of the bone marrow, a method capable of providing quantitative information about bone marrow iron content.

Materials and Methods

Subjects and Study Design

This cross-sectional study derived from the baseline data of a randomized controlled trial designed to compare the effects of two types of phosphate binders, calcium acetate (PhosLo) and sevelamer (Renagel), on mineral metabolism, bone remodeling, and progression of coronary calcification in patients undergoing hemodialysis over 1 yr (7). All patients were older than 18 yr and were receiving 4-h hemodialysis sessions, three times weekly, using hollow-fiber polysulfone or acetate membranes, at least for 3 mo. Dialysis water treatment included a reverse osmosis system. Exclusion criteria encompassed serious gastrointestinal disease, ethanol or drug abuse, active malignancy, HIV infection, chronic inflammatory disease, use of steroids, severe hyperparathyroidism [defined as intact parathyroid hormone (iPTH) >1000 pg/ml], body weight >100 kg, continuous use of antiarrhythmic or seizure drugs, pregnancy or breast-feeding, previous myocardial revascularization, and uncontrolled diabetes mellitus or hypertension (as deemed by the investigator).

All patients signed an informed consent. The study protocol was reviewed and approved by the local institutional ethics board (number 257/05).
Biochemical Parameters

Blood samples for the determination of biochemical parameters were obtained after an overnight fast and before the first weekly hemodialysis session. A minimal interval of 7 d was observed between intravenous (IV) iron administration and blood sampling.

Laboratorial data comprised blood count as determined by an automated method. Serum iron was determined by a colorimetric method (Roche, Indianapolis, IN; normal reference value: 83 to 167 μg/dl for men, 49 to 151 μg/dl for women). Serum transferrin saturation, measured as a percentage, is the ratio of serum iron to total iron-binding capacity multiplied by 100 (normal reference value: 20% to 55%). sFerritin was determined by flow immunofluorimetry (normal reference value 36 to 262 ng/ml for men, 24 to 155 ng/ml for women; Abbott Laboratories, Abbott, IL). Serum iPTH was measured by a chemiluminescence assay (Immule; DPC, Los Angeles, CA); the range of normal values was 10 to 65 pg/ml. Serum albumin was measured by a colorimetric method (normal reference value: 3.4 to 4.8 g/dl). CRP was measured by a high-sensitivity immunometric assay (Immunoite DPC; lower limit for detection: 4 pg/ml). Single-pool Kt/V was calculated using the urea kinetic modeling formula (8).

Bone Biopsy

In this study, stored bone biopsies were used to evaluate bone marrow iron. The bone biopsies were carried out in either the right or left iliac crest, using a 7-mm inner diameter electrical trephine (Gautier Medical, Rochester, MN). Bone fragments were submitted to the usual processing for methyl methacrylate-embedded undecalciﬁed bone biopsy (9). To evaluate bone marrow iron, sections were stained with Perl’s Prussian blue. A histomorphometric analysis was performed using a semiautomatic system (Osteomeasure; Osteometrics, Atlanta, GA). The number of iron-stained cells per square millimeter of bone marrow was systematically counted in 10 ﬁelds, which is considered to be representative of the whole bone marrow section. The morphologic characteristics of the cells were of the reticuloendothelial lineage.

Statistical Analyses

Results are expressed as means ± SD or proportions. The relationship between sFerritin and other variables was assessed using either the Spearman or Pearson correlation coefﬁcient, when appropriate. One-way ANOVA followed by Tukey test was performed to evaluate the differences in sFerritin levels within the tertiles of bone marrow iron. Multiple linear regression analysis was conducted to identify the determinants of sFerritin, using the variables that were correlated in the univariate model. The comparison between the groups divided according to sFerritin levels (≤500 ng/ml or >500 ng/ml) was performed using either t test or Mann-Whitney test, where appropriate. For each group, multivariate regression analysis was performed using the following variables: time on HD, CRP and bone marrow iron. A p value <0.05 was considered signiﬁcant. All statistical analysis was carried out using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL).

Results

Demographic and clinical characteristics of the 96 patients are displayed in Table 1. The patients were relatively young and most of them were male (65%), with a mean time on HD of 36 ± 24 mo. The major cause of CKD was hypertension. The vascular access in 90% of the patients was native arteriovenous fistula. The majority (87%) of the patients were currently receiving erthropoiesis-stimulating agent (ESA), and half of them were receiving IV iron supplementation. The prior cumulative IV iron doses were not available. All of the laboratorial parameters were within the expected range for HD patients, except for CRP and iPTH, which were above the normal range. Of note, CRP higher than 0.5 mg/dl was found in 62% of the patients. The mean sFerritin was 609 ± 305 ng/ml. Only one patient had sFerritin <100 ng/ml, 42% of the patients had sFerritin ranging from 100 to 500 ng/ml, and 57% of them had sFerritin >500 ng/ml.

Iron-stained cells (bone marrow iron) were detected in 93 of 96 patients (97% ± 5 cells/mm²). Examples of bone marrow cells stained with Perl’s Prussian blue are shown in Figure 1. There was no difference in clinical characteristics between patients whose bone marrow cells stained (n = 93) and those whose cells did not stain (n = 3). The patient with the highest level of bone marrow iron content had normal levels of serum transferrin saturation (30%), high sFerritin (953 ng/ml), and normal CRP (0.25 mg/dl). Of note, this patient was in HD therapy for more than 8 yr.

Figure 2 shows the different sFerritin concentration of the patients divided according to the tertiles of bone marrow iron. The first, second, and third tertiles included patients with bone marrow iron levels from 0 to 58.1, 58.2 to 111.8, and 111.9 to 430.2 cells/mm², respectively. A significant difference in sFerritin was observed between the groups divided according to the tertiles of bone marrow iron content (Figure 2).

Table 1. Demographic and laboratory characteristics of the patients (n = 96)

<table>
<thead>
<tr>
<th></th>
<th>Age (years)</th>
<th>Sex (% male)</th>
<th>Diabetes mellitus (%)</th>
<th>Patients on ESA therapy (%)</th>
<th>Patients on IV iron supplementation (%)</th>
<th>Mean ESA dose (U/wk)</th>
<th>Mean IV iron dose (mg/wk)</th>
<th>hgb (g/dl)</th>
<th>Htc (%)</th>
<th>Serum iron (μg/dl)</th>
<th>TSAT (%)</th>
<th>sFerritin (ng/ml)</th>
<th>Albumin (g/dl)</th>
<th>CRP (mg/dl)</th>
<th>iPTH (pg/ml)</th>
<th>Kt/V</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48.6 ± 13</td>
<td>65</td>
<td>16</td>
<td>87</td>
<td>48</td>
<td>5831 ± 3122</td>
<td>72 ± 30</td>
<td>11.3 ± 1.6</td>
<td>34.3 ± 5</td>
<td>70.8 ± 28.9</td>
<td>32.7 ± 22.5</td>
<td>609 ± 305</td>
<td>3.7 ± 0.2</td>
<td>0.9 ± 1.4</td>
<td>343 ± 308</td>
<td>1.34 ± 0.2</td>
</tr>
</tbody>
</table>

*Values expressed as mean ± SD and proportions. CKD: chronic kidney disease, ESA: erthropoiesis-stimulating agent, IV: intravenous, hgb: hemoglobin, Htc: hematocrit, TSAT: transferrin saturation, CRP: C-reactive protein, iPTH: intact parathyroid hormone.
Serum ferritin and bone marrow iron stores

Ritin levels was observed only between the first and third tertiles \((P = 0.009)\).

In the univariate analysis, sFerritin levels correlated with bone marrow iron \((r = 0.34, P = 0.001)\), CRP \((r = 0.28, P = 0.006)\), and time on HD \((r = 0.20, P = 0.048)\). There was a positive correlation between bone marrow iron content and time on HD \((r = 0.26, P = 0.01)\). In the multivariate regression analysis, bone marrow iron content \((\beta = 0.32, P = 0.001, 95\% \text{ CI 0.54} \text{ to 2.09})\) and CRP \((\beta = 0.26, P = 0.005, 95\% \text{ CI 24.6} \text{ to 132.3})\) were independently associated with sFerritin levels \((r^2 = 0.194)\).

The comparison between patients with \(s\text{Ferritin} \leq 500\text{ng/ml}\) \((n = 41)\) and \(s\text{Ferritin} > 500\text{ng/mL}\) \((n = 55)\) is depicted in Table 2. The patients with \(s\text{Ferritin} \geq 500\text{ng/mL}\) had received HD therapy for a longer time and had higher CRP levels and a greater number of iron-stained cells per square millimeter of bone marrow.

A multivariate regression analysis was performed in both groups separately. sFerritin in the group of patients with \(s\text{Ferritin} \leq 500\text{ng/ml}\) was independently associated only with bone marrow iron content \((\beta = 0.48, P = 0.001, 95\% \text{ CI 0.48} \text{ to 1.78})\). However, in the group of patients with \(s\text{Ferritin} > 500\text{ng/ml}\), no independent determinant of sFerritin could be found.

**Discussion**

The present study showed that HD patients carry a high level of iron in bone marrow. There was a clear relationship between sFerritin and bone marrow iron content, measured by a quantitative method, suggesting that sFerritin does adequately reflect iron stores.

These results are in accordance with those of previous studies that have analyzed the correlation of sFerritin and iron stores. However, the majority of these studies determined these stores using qualitative methods with different scales \((3,10–15)\). The present study described for the first time a quantitative method for counting iron-stained cells in bone marrow of HD patients, showing that histomorphometry could be a useful tool for quantifying iron stores when a bone biopsy is available. To date, only qualitative methods have been used to correlate the number of iron-stained cells with the iron concentration in bone marrow.
Table 2. Comparison of the patients divided by sFerritin levels

<table>
<thead>
<tr>
<th></th>
<th>sFerritin ≤500 ng/ml</th>
<th>sFerritin &gt;500 ng/ml</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 41)</td>
<td>(n = 55)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>48 ± 13.6</td>
<td>49 ± 12.6</td>
<td>0.71</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>66</td>
<td>64</td>
<td>0.82</td>
</tr>
<tr>
<td>Time on hemodialysis (months)</td>
<td>30 ± 24</td>
<td>41 ± 24</td>
<td>0.03</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>14</td>
<td>18</td>
<td>0.64</td>
</tr>
<tr>
<td>Patients on ESA therapy (%)</td>
<td>80</td>
<td>91</td>
<td>0.14</td>
</tr>
<tr>
<td>Mean ESA dose (U/wk)</td>
<td>6242 ± 3152</td>
<td>5560 ± 3104</td>
<td>0.96</td>
</tr>
<tr>
<td>Patients on IV iron supplementation (%)</td>
<td>54</td>
<td>44</td>
<td>0.35</td>
</tr>
<tr>
<td>Mean IV iron dose (per week)</td>
<td>77 ± 29</td>
<td>67 ± 32</td>
<td>0.17</td>
</tr>
<tr>
<td>Hb (mg/dl)</td>
<td>11.4 ± 1.6</td>
<td>11.3 ± 1.7</td>
<td>0.82</td>
</tr>
<tr>
<td>Htc (%)</td>
<td>34.7 ± 4.7</td>
<td>34 ± 5.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Serum iron (µg/dl)</td>
<td>71 ± 28.9</td>
<td>70.7 ± 29.2</td>
<td>0.95</td>
</tr>
<tr>
<td>TSAT (%)</td>
<td>36.2 ± 31</td>
<td>30 ± 12.4</td>
<td>0.19</td>
</tr>
<tr>
<td>sFerritin (ng/ml)</td>
<td>339 ± 116</td>
<td>810 ± 239</td>
<td>NA</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.8 ± 0.2</td>
<td>3.7 ± 0.2</td>
<td>0.33</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.7 ± 0.8</td>
<td>1.1 ± 1.8</td>
<td>0.02</td>
</tr>
<tr>
<td>iPTH (pg/ml)</td>
<td>359 ± 302</td>
<td>330 ± 315</td>
<td>0.65</td>
</tr>
<tr>
<td>Kt/V</td>
<td>1.32 ± 0.2</td>
<td>1.35 ± 0.25</td>
<td>0.58</td>
</tr>
<tr>
<td>Bone marrow iron (cells/mm²)</td>
<td>65 ± 49</td>
<td>120 ± 81</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Values expressed as mean ± SD and proportions. NA: not applicable, ESA: erithropoesis-stimulating agent, IV: intravenous, Hb: hemoglobin, Htc: hematocrit, TSAT: transferrin saturation, CRP: C-reactive protein, iPTH: intact parathyroid hormone.

The use of undecalcified plastic-embedded specimens is time-consuming and expensive. However, this technique could provide a well-preserved sample of bone marrow, in addition to preserving the real tissue structures. The thin section of the trephine biopsy permits an ideal evaluation of intracellular iron. In contrast, a bone marrow aspirate may be diluted with blood, causing skew in cell counts (17).

It is well known that the specificity and/or sensitivity of sFerritin may be reduced by conditions not associated with iron status. High sFerritin levels in HD patients may be the result of inflammation, infection, malnutrition, or malignancy. These conditions are associated with an increased production of proinflammatory cytokines that can induce liver synthesis of ferritin (1). Thus, despite being diagnostic of iron overload (3), high levels of sFerritin may also suggest that the patient is experiencing inflammation, a frequent condition in the HD population. In the present study, more than half of the patients had CRP levels higher than 0.5 mg/dl, and a positive correlation between sFerritin and CRP could be observed, as previously demonstrated by others (6). However, patients with sFerritin >500 ng/ml had higher levels of CRP and greater amount of bone marrow iron, suggesting that even in the presence of inflammation, sFerritin remains a reliable marker of iron stores.

Weiss and coworkers described how proinflammatory cytokines can affect iron homeostasis, increasing iron uptake within cells of the reticuloendothelial system by up-regulating the expression of the protein divalent metal transporter 1 (DMT1) and transferring receptor (18). In addition, the hepatic expression of the acute-phase protein hepcidin is also increased in inflammation. On one hand, this protein induces the retention of iron in macrophages by internalization and degradation of ferroportin, a transmembrane protein exporter of iron. This could at least partially explain the increased bone marrow iron content observed in the group of patients with sFerritin >500 ng/ml. On the other hand, hepcidin also inhibits duodenal absorption of iron, and in the long term, this could be associated to iron deficiency (18). Because hepcidin expression was not determined in the present study, this hypothesis could not be tested.

In summary, a new method for determining bone marrow iron content, based on counting iron-stained cells present in bone marrow, was demonstrated. According to this method, sFerritin remains a reliable marker of bone marrow iron stores in HD patients. However, HD patients with elevated sFerritin levels should be screened for inflammatory diseases to establish the necessity for iron supplementation. Although the number of iron-stained cells in bone marrow is the gold standard of iron stores, these patients may have decreased iron concentration circulation and thus limited availability of iron for erythropoiesis, even with increased intracellular iron. Additional care should be taken when prescribing small doses of iron for this population.

Acknowledgments

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Disclosures

None.

References