Measurements of Serum Ferritin Used to Predict Concentrations of Iron in Bone Marrow in Anemia of Chronic Disease

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We determined serum ferritin, C-reactive protein (CRP), fibrinogen, and the erythrocyte sedimentation rate (ESR) in 73 patients with anemia of chronic disease. Nomograms of CRP, ESR, or fibrinogen vs ferritin concentrations were constructed and used to estimate the iron store in bone marrow. Iron stores estimated from the nomograms were compared with the results of staining cytological bone marrow smears for iron, the reference method for evaluating iron in bone marrow. In contrast to the results of Witte et al. (Clin Chem 1985;31:1011; Am J Clin Pathol 1986;85:202–6 and 1988;90:85–7), we observed that nomograms of CRP, fibrinogen, or ESR (i.e., acute-phase reactants not influenced by changes in iron metabolism) vs ferritin are not suitable to correct for the acute-phase component of changes in ferritin concentrations. For ferritin concentrations <70 μg/L, we found that iron deficiency, as judged from bone marrow iron stain, apparently was always present.

Additional Keyphrases: iron-deficiency anemia - acute-phase proteins - nomograms

Nearly 75% of the anemias in clinical practice are caused either by true iron deficiency or by poor utilization of iron. The latter is typical for anemia of chronic disease (1). Anemia of chronic disease is defined as anemia associated with chronic infectious, inflammatory, traumatic, or neoplastic illness and with characteristic disturbances of iron metabolism (1–3). For these patients the concentrations of iron measured in serum are low, despite iron stores that range from adequate to increased. Measurement of ferritin concentration in serum is often recommended as a useful estimate of iron stores or of iron deficiency. However, ferritin is also a so-called positive acute-phase protein. Therefore, especially in chronic disease, measurement of ferritin is not reliable for estimating bone marrow iron deficiency or overload. Apart from inflammation, liver cell damage can also increase the ferritin concentration, and in some malignancies very high ferritin concentrations have been measured, which are obviously not related to the body iron store.

Witte et al. (4–6) suggested that ferritin concentrations, if corrected for the acute-phase response, could still be used to estimate the iron stores in bone marrow, even in patients with anemia of chronic disease. They used nomograms describing the relation between ferritin concentrations and C-reactive protein (CRP) concentrations (4) or erythrocyte sedimentation rate (ESR) values (5, 6) to diagnose or to exclude iron-deficiency anemia in patients with chronic disease, in an effort to minimize the number of bone-marrow examinations (4–6).

To test the procedures suggested by Witte et al., we also examined the relation between ferritin concentrations and the ESR or between serum ferritin concentrations and CRP or fibrinogen concentrations, i.e., acute-phase markers not influenced by changes in iron metabolism. Staining of iron in cytologically obtained bone marrow was used as a reference method to determine the amount of iron in bone marrow.

Materials and Methods

Study population. We studied 73 patients with anemia and chronic disease (30 men and 43 women) from the Department of Internal Medicine and Rheumatology. Bone marrow aspirates and serum samples were taken during the same period. Informed consent was given by all patients. The criteria for patients to be included in the study were

- for men, hemoglobin <2.0 mmol/L and (or) hematocrit <0.40 L/L (reference values 2.13–2.75 mmol/L and 0.41–0.51 L/L, respectively),
- for women, hemoglobin <1.75 mmol/L and (or) hematocrit <0.35 L/L (reference values 1.88–2.50 mmol/L and 0.36–0.47 L/L, respectively),
- and, for both sexes, the presence of rheumatoid arthritis or other collagenous diseases (n = 16), chronic infectious disease (n = 40), chronic inflammatory bowel disease (n = 9), or chronic renal disease, due to inflammatory or infectious conditions (no patient was undergoing hemodialysis) (n = 8).

Patients with known recent hemorrhages, receiving iron-supplement treatment, or having received blood transfusions during the preceding three months, and patients with liver disease, as determined from above-normal transaminase concentrations in serum, were excluded from the study.

Methods. Bone marrow aspirates were obtained from the sternum; all smears were stained with May Grünwald–Giemsa and Perl iron stain. The grade of staining was classified by microscopic evaluation of macrophage staining (absent, trace, or positive) and sideroblast staining (>20% is positive).

Ferritin was measured with the immunoenzymometric assay Tandem-E Fer (cat. no. 14143; Hybritech, La Jolla, CA). Fibrinogen was measured according to the
method of Claus (7), and the ESR was determined according to Westergen (8). CRP was measured immuno-

noturbidimetrically (9) with a Cobas-Fara centrifugal analyzer, with antibodies and standard from Atlantic 
Antibodies (cat. no. 80285; obtained from Baxter, 
Maarseen, The Netherlands).

Results

As judged from bone marrow iron stain, 26 patients 
had "absent" iron stores, and eight patients had "trace" 
amounts. The remaining 39 patients were positive with 
respect to iron store in bone marrow. Analogous to the 
nomogram introduced by Witte et al. (6), Figure 1 shows 
data only for patients with serum ferritin concentra-
tions <160 μg/L (n = 30); the data for this subpopula-
tion are presented in Table 1. By this nomogram, six 
patients would be incorrectly diagnosed as having suf-
cient bone marrow iron stores, and two patients with a 
trace of iron (as judged from cytological examination) 
would also be classified as having adequate iron stores. 
No significant correlation was observed between CRP 
and ferritin concentrations <160 μg/L. The results ob-
served for fibrinogen vs ferritin are comparable with 
those for the ESR vs ferritin (see Table 1). All patients 
with ferritin concentrations <70 μg/L were iron-defi-
cient, as determined by iron stain of their bone marrow.

The mean, standard deviation, and median for the 
different analytes and for the different subpopulations 
are presented in Table 2.

Figure 2 presents the relation between ESR and fer-
ritin concentrations for all patients (n = 72). One patient 
with an extremely high ferritin concentration (3765 
μg/L), an ESR of 140 mm/h, and absent iron store was not 
depicted. Although a significant correlation is observed

![Graph](image)

Fig. 1. Nomogram (i.e., the relationship) between the serum ferritin concentration and the ESR for patients with ferritin concentrations <160 μg/L.

Figs. 1–3: According to Witte et al. (6), bone-marrow iron deficiency should be highly unlikely in the upper field (area above the regression line). Bone marrow iron stain absent (○), trace (△), or positive (▲).

between ferritin concentrations and the ESR, the nomo-
gram cannot be used to predict the iron store in bone 
marrow, because differentiation between groups of pa-
tients with absent, trace, or positive iron store, as judged 
from bone marrow iron stain, appears insufficient.

For CRP vs ferritin for the overall group (n = 72), the 
correlation is also highly significant. However, discrim-
ination between patients with absent, trace, or positive 
iron stores is still not possible. Data are presented in 
Table 1.

Figure 3 presents a nomogram for patients with 
rheumatoid arthritis, the same patient population con-
sidered by Witte et al. (6). For this subgroup, differen-
tiation between patients with absent, trace, or positive 
iron store in bone marrow also appears insufficient.

Discussion

As stated before, serum ferritin concentration can be 
used as a reliable marker to predict iron stores in bone 
marrow only when an acute-phase reaction is absent 
(10–12). In the present study, apart from a significant 
correlation, we observed no useful relation between 
ESR, fibrinogen, and ferritin concentrations. The rela-
tion between CRP and ferritin concentrations <160 
μg/L did not reach statistical significance, in contrast to 
the results of Witte et al. (4–6).

If we used the nomogram suggested by Witte et al. 
(ferritin concentrations <160 μg/L vs ESR, n = 30), six 
iron-deficient patients and two patients with trace iron 
stores (as judged from bone marrow iron stain) would be 
incorrectly diagnosed as having sufficient iron stores. 
Construction of nomograms that would include patients 
with ferritin concentrations >160 μg/L did not improve 
the value of the nomograms suggested by Witte et al. 
(4–6). Although highly significant correlations have 
been observed for both ESR and CRP vs ferritin (P <
0.001), the nomograms do not differentiate between 
patients with absent, trace, or positive iron stores in 
bone marrow. The overlap between the groups with 
absent, trace, or positive bone marrow iron stores is 
even greater when the nomograms with ferritin concen-
trations <160 μg/L are used (compare Figure 1 with 
Figure 2). In our study, patients with ferritin concen-
trations <70 μg/L appeared to be all iron-deficient, as 
judged from bone marrow iron stain, which is in ac-
cordance with other studies (13–15), although a cutoff 
value of 100 μg/L has also been reported (16). This difference 
might be caused by differences in the standardization of 
the ferritin test kits used (17).

Discrepancies between our results and those of Witte 
et al. (4–6) cannot be ascribed to the use of different test 
kits for the determination of ferritin. The correlations 
between several ferritin test kits were good (17). More-
ever, if the discrepancies were caused by differences in 
standardization, construction of nomograms for ferritin 
concentrations extending over different ferritin concen-
tration ranges (Table 2) should give results comparable 
with those of Witte et al. (6), but this is not the case.

To exclude discrepancies that might be introduced by 
differences in patient population, we presented results
Table 1. Relation between Acute-Phase Markers (x) and Serum Ferritin (y) in Patients with Ferritin Concentrations <160 µg/L and for the Composite Group

<table>
<thead>
<tr>
<th></th>
<th>Patients with ferritin &lt;160 µg/L (n = 30)</th>
<th>All patients (n = 72)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>Slope</td>
</tr>
<tr>
<td>ESR, mm/h</td>
<td>0.45</td>
<td>0.67</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>-0.04</td>
<td>-0.06</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>0.52</td>
<td>21.17</td>
</tr>
</tbody>
</table>

*One patient with a ferritin concentration of 3765 µg/L is not included in the regression analysis.

Table 2. Mean, Standard Deviation, Median, Minimum, and Maximum Values for the Overall Group and Subpopulations

<table>
<thead>
<tr>
<th>Ferritin &lt;70 µg/L (n = 14)</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR, mm/h</td>
<td>61</td>
<td>26</td>
<td>53</td>
<td>33</td>
<td>117</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>34</td>
<td>31</td>
<td>31</td>
<td>9</td>
<td>123</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>4.5</td>
<td>0.5</td>
<td>4.6</td>
<td>4.0</td>
<td>5.4</td>
</tr>
<tr>
<td>Ferritin, µg/L</td>
<td>27</td>
<td>16</td>
<td>19</td>
<td>7</td>
<td>51</td>
</tr>
<tr>
<td>Ferritin &lt;160 µg/L (n = 30)</td>
<td>ESR</td>
<td>71</td>
<td>31</td>
<td>68</td>
<td>16</td>
</tr>
<tr>
<td>CRP</td>
<td>30</td>
<td>25</td>
<td>29</td>
<td>9</td>
<td>123</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>4.9</td>
<td>1.1</td>
<td>4.6</td>
<td>3.8</td>
<td>8.2</td>
</tr>
<tr>
<td>Ferritin</td>
<td>51</td>
<td>46</td>
<td>76</td>
<td>7</td>
<td>160</td>
</tr>
<tr>
<td>Overall group (n = 73)</td>
<td>ESR</td>
<td>84</td>
<td>34</td>
<td>82</td>
<td>15</td>
</tr>
<tr>
<td>CRP</td>
<td>50</td>
<td>50</td>
<td>33</td>
<td>8</td>
<td>230</td>
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<tr>
<td>Fibrinogen</td>
<td>5.4</td>
<td>1.7</td>
<td>5.2</td>
<td>1.8</td>
<td>9.5</td>
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<tr>
<td>Ferritin</td>
<td>364</td>
<td>517</td>
<td>208</td>
<td>7</td>
<td>3765</td>
</tr>
</tbody>
</table>

Fig. 2. Relationship between ferritin concentrations and the ESR for the overall group (n = 72)

Fig. 3. Relation between ferritin concentrations and the ESR for patients with rheumatoid arthritis and other collagen diseases (n = 16)

for patients with rheumatoid arthritis (Figure 3), the subpopulation comparable with the patient population of Witte et al. (6). Again, no differentiation appeared possible between patients with absent, trace, or positive iron stores in bone marrow.

We conclude that the nomograms suggested by Witte et al. (4–6) cannot be used to estimate iron deficiency in patients with anemia of chronic disease. The idea of correcting the serum ferritin for increases attributable to the acute-phase response, by using an acute-phase protein that is not influenced by changes in iron metabolism, is attractive. However, as our investigations have shown, one cannot eliminate the acute-phase component of serum ferritin changes by using the ESR, CRP, or fibrinogen as independent acute-phase markers. Hence, we conclude that the use of a "corrected value" for serum ferritin to evaluate iron deficiency in patients with anemia of chronic disease appears not possible.

References
8. Westergren A. Studies of the suspension stability of the blood in


Determination of Creatinine in Serum and Urine by Cation-Exchange High-Pressure Liquid Chromatography

Aimo Harmoinen, Pekka Sillanaukee, and Hannu Jokela

We describe an HPLC method for quantifying creatinine, separating the analyte from other compounds in serum and urine by cation-exchange chromatography and measuring its absorbance at 234 nm. The precision of the method (CV) varied from 2.9% (mean creatinine concentration, 31 μmol/L) to 1.7% (361 μmol/L) within a series of assays and from 3.9% (34 μmol/L) to 2.4% (391 μmol/L) between series. A comparison with the Jaffé method, as performed with a Technicon SMA analyzer, gave the regression line $Y_{\text{HPLC}} = 1.00X_{\text{Jaffé}} - 12.0$ ($n = 141$, $r = 0.998$, and $S_p = 19$). Results also are comparable with those of an enzymatic method, if the enzymatic method is standardized with a serum-based standard when serum samples are measured. An aqueous standard has to be used for enzymatic determination of creatinine in urine.

Additional Keyphrases: bilirubin interference · Jaffé, enzymic methods compared · glomerulus function

The concentration of creatinine in serum and creatinine clearance are generally accepted as reliable indices of the glomerular filtration rate. The methods most commonly used for quantitative determinations of creatinine are based on the Jaffé reaction, involving alkaline sodium picrate. This reaction, however, is not specific, being affected by numerous metabolites and drugs (1–5). Many attempts to improve the specificity of the method have not been altogether successful. Some recently developed enzymatic methods (6–8) are promising, but a high concentration of bilirubin in the sample is still a problem (9).

Some scientists have used cation-exchange chromatography to separate creatinine from interferents (10–13); HPLC methods for creatinine determination have also been described (14–19). Unfortunately, most of these methods are very laborious (14–16), and some require a complicated assay system (14, 17). The HPLC method we describe has some advantages over the methods published earlier. The simple sample handling, the isocratic buffer system, and the short retention time of creatinine in the proposed method result in a rapid assay that is therefore also suitable in routine work.

Materials and Methods

Reagents. All chemicals were reagent grade. Creatinine standard was purchased from NIST (U.S. Department of Commerce National Institute of Standards and Technology, Gaithersburg, MD 20899). The stock solution of creatinine (10 mmol/L) was prepared by adding 113.1 mg of creatinine to 100 mL of 0.1 mol/L HCl reagent. The working standard was prepared by diluting the stock solution 10-fold with distilled water. The mobile phase was 75 mmol/L lithium acetate (Fluka Chemie AG, Buchs, Switzerland) buffer, pH 7.1. The proteins of the serum samples were precipitated with trichloroacetic acid (TCA), 100 g/L.

Instruments. A Model 5000 liquid chromatograph with a variable-wavelength UV-100 detector (Varian Instruments, Walnut Creek, CA) was used. Sample (20 μL) injection was carried out with an MSI 660 autosampler (Kontron AG, Zürich, Switzerland). A Model C-1B Chromatopac (Shimadzu Corp., Kyoto, Japan) was used as recorder. A cartridge column system (Chrompack, Middelburg, The Netherlands) was used for separation: The glass column (100 mm × 3 mm) was packed with Ionospher C, a silica-based cation-exchanger with sulfate as functional group.

Sample preparation. Vortex-mix 300 μL of serum

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Received May 10, 1990; accepted January 10, 1991.