Screening with Zinc Protoporphyrin for Iron Deficiency in Non-Anemic Female Blood Donors

Bent Møller Jensen, Steen Henrik Sands, Philippe Grandjean, Preben Wiggen, and Jørgen Dalhøj

Iron-depleted donors are at increased risk of developing anemia; if these donors could be identified by a screening test, iron supplementation or decreased donation frequency could be considered. Tests to determine serum ferritin, blood hemoglobin, and erythrocyte (Erc)-zinc protoporphyrin concentrations were examined in 679 consecutive female blood donors to identify donors with non-anemic iron deficiency. The test to determine serum ferritin is expensive and slow, whereas the two latter tests are rapid and less costly and could therefore be used for screening. Women in the fertile age groups had the lowest average serum ferritin values. In all, 93 women (13.7%) had depleted iron stores, as indicated by serum ferritin concentrations <14 μg/L. In these women, a much better correlation was found between Erc-zinc protoporphyrin and serum ferritin (r = -0.49, P <0.001) than between blood hemoglobin and serum ferritin (r = 0.31, P <0.01). These findings suggest that measurement of Erc-zinc protoporphyrin is superior to that of blood hemoglobin in identifying donors with non-anemic iron deficiency.

Additional Keyphrases: ferritin • hemoglobin

Repeated blood donation involves loss of iron, which may lead to a decrease of iron stores. Iron-depleted donors will therefore be at increased risk of developing anemia (1). Clinical anemia may result in reduced physical work capacity and impaired behavioral performance (2, 3).

Among female blood donors, the menstruating age groups are at particular risk of developing iron deficiency. To avoid iron depletion, potential donors may often have blood-hemoglobin (B-Hb) determined as a safeguard before phlebotomy. However, because some donors with hemoglobin concentrations within the normal range may nonetheless have depleted iron stores (4, 5), other tests are needed to detect latent iron depletion. Serum-ferritin (S-ferritin) is frequently assayed for this purpose because of the correlation to stainable iron in bone marrow (6, 7). S-ferritin is generally accepted as a better predictor of iron depletion than are S-iron and transferrin-iron-binding capacity, the latter two exhibiting a considerable diurnal variation. Unfortunately, S-ferritin determination must be carried out in a specialized laboratory and is considered rather expensive for screening blood donors.

Alternatively, iron status may be evaluated by quantifying erythrocyte-zinc protoporphyrin (Erc-ZPP) as shown in studies of donors with anemia or donors who became anemic after donation (1, 3). ZPP is produced when zinc instead of iron is incorporated into protoporphyrin IX in the last step of the heme synthesis; increased amounts are seen in cases of iron deficiency as well as lead intoxication (9). Erc-ZPP can be measured in a few seconds by front-face fluorometry with a hematofluorometer, and capillary blood or oxygenated venous blood samples can be used (9). The cost of the instrument is about US $5000, and no reagents are needed.

In one study, Erc-ZPP was as good as S-ferritin in predicting bone marrow iron content (10). However, another study found a relatively poor correlation between Erc-ZPP and S-ferritin in anemic donors (8). Also, an increase in Erc-ZPP concentration had a low predictive value in identifying donors who subsequently become anemic; this characteristic could lead to unnecessary exclusion of false-positive donors (1). However, the predictive value of a positive test result (increased Erc-ZPP) will vary with the prevalence of iron deficiency; differences in this regard are seen between remunerative and voluntary donor systems (11, 12).

We have assessed the usefulness of Erc-ZPP testing, as compared with B-Hb and S-ferritin, in screening for iron deficiency among female volunteer blood donors.

Materials and Methods

We examined 760 consecutive female volunteers who had been called for regular blood donation at the University Hospital blood bank. The sedimentation rate was determined for every donor (13). Eighty-one donors with a sedimentation rate >20 mm/h were excluded from the study because the possible presence of ongoing infection could cause temporary changes in S-ferritin concentrations.

For the remaining 679 donors, the median age was 37 years (range, 19–66). The number of previous blood donations was recorded from the donor register; the median was 12 (range, 1–68). Menstruation status was determined by interview; 87 of the women were postmenopausal.

S-ferritin was measured by a sandwich radioimmunoassay (Bio-Rad, Richmond, CA) by using a Model NE 1600 gamma counter (Nuclear Enterprises, Edinburgh, Scotland); duplicate analyses were performed to secure an acceptable precision, and the total coefficient of variation was 13.4% at 6.08 μg/L, 5.9% at 29.09 μg/L, and 5.6% at 165.6 μg/L. B-Hb was determined by the cyanomethemoglobin method. Erc-ZPP was measured in mixed oxygenated venous blood in an Aviv hematofluorometer (Aviv, Lakewood, NJ) with results given as micromoles of zinc protoporphyrin per mole of hemoglobin (Fe) (9). Reference materials for ZPP are not available; an interlaboratory comparison study organized by the Danish National Institute of Occupational Health had shown satisfactory results. Nonparametric statistical methods included Mann–Whitney U-test and Spearman correlation coefficient.

Results

The 679 donors with a normal sedimentation rate had a median S-ferritin concentration of 27 μg/L (range, 5–177).
A total of 93 donors (13.7%) had an S-ferritin concentration <14 μg/L, suggesting potential iron depletion; 38 (5.6%) had <10 μg/L, thus indicating the presence of significant iron deficiency.

The median B-Hb concentration was 8.0 mmol/L (range, 6.8–10.6). Only two of the donors were anemic according to the routine criterion of B-Hb <7.00 mmol/L. The median Erc-ZPP value was 34 μmol/mol hemoglobin (range, 10–292).

In women in fertile age groups, the S-ferritin concentration was significantly lower than in postmenopausal women, but the two groups showed no difference in B-Hb and Erc-ZPP (Table 1). Also, donors with one to three previous phlebotomies (n = 104) had a slightly higher (P = 0.11) median S-ferritin concentration than did donors with more than three previous phlebotomies (n = 575). Again, these groups did not differ in hemoglobin or ZPP content. As expected, donors excluded from consideration because of increased sedimentation rate also tended to show increased S-ferritin concentrations (median, 31 μg/L; range, 5–108); hemoglobin and ZPP concentrations were similar to those seen in the other subjects.

In the women with normal sedimentation rate and an S-ferritin concentration <14 μg/L, hemoglobin was decreased and Erc-ZPP was increased (Table 2); the Spearman rank correlation coefficient for S-ferritin vs Erc-ZPP was −0.49 (P <0.001). For all donors, irrespective of S-ferritin concentration, the correlation coefficient was lower, −0.28 (P <0.001). Similarly, for women with S-ferritin <14 μg/L, the Spearman rank correlation coefficient for S-ferritin vs B-Hb was r = 0.31 (P <0.01), whereas in the total group, the r was only 0.15 (P <0.001).

Although we saw no close correlation between ferritin and Erc-ZPP, women with an Erc-ZPP above an upper reference limit of 45 μmol/mol hemoglobin had significantly decreased S-ferritin; in these women, hemoglobin was also decreased (Table 3).

If a low S-ferritin is accepted as a reliable diagnostic test for iron depletion, the specificity and sensitivity of the ZPP determination can then be determined at various cutoff values (Figure 1). For example, in screening for individuals with an S-ferritin concentration <14 μg/L, the specificity was found to be 0.87 and the sensitivity 0.34 for an Erc-ZPP cutoff value of 45 μmol/mol hemoglobin. At this value, the predictive value of a positive test (i.e., Erc-ZPP exceeding the cutoff) was 0.30 and that of a negative test 0.89 (Figure 2). Similarly, if a discrimination value of 10 μg/L was used for S-ferritin, the specificity remained 0.87 for the ZPP test, but the sensitivity increased to 0.63; in this case, the predictive value of a positive test was 0.22 and that of a negative test was 0.97.

### Table 1. B-Hemoglobin, S-Ferritin, and Erc-ZPP in 592 Premenopausal and 87 Postmenopausal Female Blood Donors

<table>
<thead>
<tr>
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<th>Premenopausal</th>
<th>Postmenopausal</th>
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<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Range</td>
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<tr>
<td>B-hemoglobin</td>
<td>8.0</td>
<td>6.8–10.6</td>
</tr>
<tr>
<td>S-ferritin</td>
<td>26*</td>
<td>5–177</td>
</tr>
<tr>
<td>Erc-ZPP</td>
<td>34</td>
<td>10–292</td>
</tr>
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*Significantly different from postmenopausal median (P <0.001, Mann-Whitney U-test). Women with increased blood sedimentation rates were excluded.

### Table 2. B-Hb and Erc-ZPP in Female Volunteer Blood Donors with Low or Normal S-Ferritin Concentrations

<table>
<thead>
<tr>
<th></th>
<th>&lt;14 (n = 93)</th>
<th>≥14 (n = 586)</th>
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<tr>
<td></td>
<td>Median</td>
<td>Range</td>
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<tr>
<td>B-Hb, mmol/L</td>
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<tr>
<td>Erc-ZPP, μmol/mol Hb</td>
<td>42*</td>
<td>19–123</td>
</tr>
</tbody>
</table>

*Significantly different (P <0.001, Mann-Whitney U-test) from group with normal S-ferritin concentrations.

### Table 3. S-Ferritin and B-Hb in Female Volunteer Blood Donors with Normal or Increased Erc-ZPP Concentrations

<table>
<thead>
<tr>
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<th>&lt;45 (n = 558)</th>
<th>≥45 (n = 121)</th>
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<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>S-ferritin, μg/L</td>
<td>29</td>
<td>5–177</td>
</tr>
<tr>
<td>B-Hb, mmol/L</td>
<td>8.0</td>
<td>7.0–10.6</td>
</tr>
</tbody>
</table>

*Significantly different between groups: *P <0.001, **P <0.01.

**Fig. 1.** Sensitivity and specificity of Erc-ZPP for detecting iron depletion at various cutoff values, compared with an S-ferritin discrimination value of 14 μg/L.

### Discussion

In a group of consecutive female volunteer blood donors, a high number (13.7%) exhibited an S-ferritin concentration <14 μg/L, suggestive of depleted iron stores. In agreement with a previous investigation (14), we found that premenopausal donors were at increased risk of having a low S-ferritin. Also, women with more than three blood donations had a somewhat lower S-ferritin; similar observations have been published previously (12).

The best blood test available to detect depleted iron stores in the bone marrow is probably S-ferritin analysis (6). However, practical constraints prevent this analysis from being carried out just before blood donation, and its expense would rule out its use for screening large numbers of blood donors. Because of the speed and low cost of Erc-ZPP determination, we assessed the usefulness of this test as a simple way to identify iron-depleted donors who should be offered iron supplements or who should perhaps be advised not to donate blood this time.
In our consecutive cohort of volunteers, Erc-ZPP correlated better than did B-Hb with S-ferritin, in particular in the low-ferritin area. Thus, although women with depleted iron stores were unlikely to be identified by a hemoglobin test, Erc-ZPP is potentially a good screening method for this purpose. ZPP has a high specificity (above 0.80) for cutoff values between 45 and 55 μmol/mmol hemoglobin, but the sensitivity varies considerably with the cutoff values chosen, both for Erc-ZPP (Figure 1) and for S-ferritin.

Although the predictive value of a negative test result (i.e., a low Erc-ZPP) is high, the predictive value of a positive test (i.e., a high Erc-ZPP) is quite low in the group studied. However, in a higher prevalence of iron deficiency, this situation would tend to be considerably improved (11). With the very low prevalence of environmental lead pollution in Denmark, lead exposure is unlikely to influence the ZPP concentrations in the women examined. Other explanations must be sought for the high number of women with an increased Erc-ZPP despite S-ferritin concentrations >14 μg/L. Possibly some donors in an early stage of iron deficiency have an ineffective erythropoiesis, which results in an increased Erc-ZPP, although S-ferritin may remain >14 μg/L. Data to support this hypothesis are lacking at present. Also, because ZPP forms a stable bond with hemoglobin, the Erc-ZPP concentration would reflect the average iron availability during the formation of circulating erythrocytes, i.e., the last four months (9, 16); short-term variations in iron stores could tend to decrease the correlation between ZPP and ferritin.

The data suggest that Erc-ZPP could be a useful test to identify donors with non-anemic iron deficiency, especially when the prevalence is relatively high, i.e., with remunerated donors used or high donation frequencies. The cost of a single test is minimal, because no reagents are needed. A hematofluorometer could be available for testing all donors, female and otherwise, considered at risk. To avoid excluding donors on the basis of a false-positive result, one might determine S-ferritin in subjects with an increased Erc-ZPP value, before subsequent phlebotomy is considered. Using the ZPP test in this way will generate fewer S-ferritin analyses in donors with normal iron stores. Thus, the use of Erc-ZPP as a screening test should reduce, probably by about 80–90%, the number of S-ferritin analyses needed.

Another strategy could be to consider every donor with an increased Erc-ZPP at risk of developing anemia. Iron supplements could be recommended in these cases. However, blood donations might be unnecessarily reduced if donors were excluded from phlebotomy despite normal S-ferritin concentrations. To assess the diagnostic validity of ZPP determination, further studies are needed to compare Erc-ZPP concentrations with iron stores in the bone marrow.

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References