Zinc Protoporphyrin and Iron Deficiency Screening

Trends and Therapeutic Response in an Urban Pediatric Center

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Importance: Iron deficiency is the most common micronutrient deficiency among children worldwide, with iron-deficiency anemia associated with long-term adverse neurodevelopmental effects.

Objective: To understand the role of zinc protoporphyrin (ZPP) in iron deficiency screening in a low-income pediatric population, as well as to describe the prevalence and trends of abnormal ZPP and the response to iron therapy.


Setting: Boston Medical Center primary care center.

Participants: A total of 2612 children with baseline routine screening results for complete blood cell count, lead, and ZPP drawn between ages 8 and 18 months and at follow-up were included. Children with sickle cell disease or lead toxicity were excluded.

Intervention: Documented iron prescription.

Main Outcome Measure: Reduction of baseline abnormal ZPP at follow-up.

Results: Of 2612 children, 48% had an abnormal ZPP level at baseline. Among those with abnormal ZPP (n=1254), 18% were prescribed iron. Iron prescription was significantly associated with ZPP reduction (odds ratio, 1.5; 95% CI, 1.1 to 2.0) and greater mean change in ZPP (mean difference, −4.4; 95% CI, −7.2 to −1.5). In multivariate analysis, the effect of iron prescription on the reduction of abnormal ZPP was modified by hemoglobin level. Iron prescription was significantly associated with ZPP reduction among those with anemia (odds ratio, 2.4; 95% CI, 1.1 to 5.0). Iron was rarely prescribed in children without anemia; a substantial, but not statistically significant, trend to improvement in those prescribed iron with low-normal hemoglobin was found.

Conclusions and Relevance: Abnormal ZPP was common in this low-income population. Iron prescription was significantly associated with a larger reduction of ZPP. Our data suggest that ZPP may be appropriate for iron deficiency screening; further investigation is warranted to explore the role of ZPP among nonanemic children.

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Erythrocyte protoporphyrin (EP) is an intermediary metabolite that accumulates in red blood cells when there is insufficient intracellular iron available for incorporation into heme in the final step of its biosynthesis or when this step is inhibited by a competing substrate such as lead. It may be measured as free EP or indirectly through the measurement of zinc protoporphyrin (ZPP), which increases in red blood cells as EP levels increase. Zinc protoporphyrin has been found to be a sensitive and specific measure of ID in the absence of lead toxicity and may not be affected by inflammation. Zinc protoporphyrin is technically simpler and less expensive to measure than EP, making it attractive for screening purposes.

Until recently, low-income children in the United States have been shown to be at high risk for ID compared with their higher income peers. Data from the second National Health and Nutrition Examination Survey (1976-1980) showed a prevalence of ID of 20.6% among infants living in families with incomes below the federal poverty level compared with 6.7% among those living above the poverty level. Although poverty was associated with a higher risk for ID in multivariate analysis, data from the National Health and Nutrition Examination Survey IV (1999-2002) showed that this poverty differential has disappeared, with similar rates of ID among poor (9%) and nonpoor (7%) children aged 1 to 3 years. However, in a recent study of 198 low-income African American infants in Detroit, 32% of participants had abnormal ZPP, suggesting that ID may remain an important issue among low-income children.

This study uses clinical practice data to describe the prevalence of abnormal ZPP in a large population of low-income children screened for lead toxicity and ID, as well as the change of ZPP over time. We also describe iron prescription patterns and the clinical effectiveness of iron therapy, as well as explore the implications for ID screening in at-risk infants and toddlers.

**METHODS**

**STUDY SAMPLE**

This study was a retrospective, longitudinal analysis of data abstracted from the electronic medical records (EMRs) of pediatric patients seen for routine primary care at Boston Medical Center from January 1, 2002, through December 31, 2010. While not routinely used for ID screening, ZPP had been used in screening for lead toxicity. With the decline in prevalence of lead intoxication among US children, routine ZPP measurement was largely discontinued in 2010, but whole blood ZPP continued to be collected at Boston Medical Center for the purpose of ID screening.

Records for children who underwent their first routine primary care screening for anemia and lead intoxication with a complete blood cell count, ZPP, and blood lead assay between the ages of 8 and 18 months were reviewed. Children with sickle cell disease listed as an International Classification of Diseases, Ninth Revision diagnosis in the EMR or elevated lead value (>10 μg/dL) at baseline or follow-up were excluded from the study owing to their respective effects on Hb and ZPP. Children with laboratory values for ZPP listed in the EMR of greater than 100 or greater than 125 were set to missing since a precise value could not be attributed.

Demographic and hematologic markers had similar distributions among subjects with complete blood cell count, ZPP, and/or lead values only at baseline and those with values at baseline and follow-up. Therefore, the study population was limited to those with complete data at both baseline and follow-up within 1 month to 2 years (N=3404 excluded), rendering a final study population of 2612. The study was approved by the Boston University Medical Center institutional review board.

**DEFINITIONS OF MARKERS OF IRON STATUS AND IRON PRESCRIPTION**

Thresholds for normal and abnormal values were based on those established by the US Centers for Disease Control and Prevention and by local laboratory normal values. Abnormal ZPP was defined as a ZPP value greater than or equal to 35 μg/dL. Zinc protoporphyrin was measured at the Massachusetts Department of Public Health State Laboratory Institute using hematoﬂuorometry.

Anemia was defined by Hb less than 11 g/dL (to convert to grams per liter, multiply by 10.0); low-normal Hb was defined by values equal to or greater than 11 g/dL and less than 12 g/dL; and high-normal Hb was defined by Hb greater than or equal to 12 g/dL. Other markers of iron status were defined based on local laboratory norms as follows: red cell distribution width greater than or equal to 14.5% and mean corpuscular volume less than 77 fl were abnormal.

If an iron prescription between the time of baseline and follow-up laboratory values was found in the EMR medication list under the search terms of iron or ferrous, a variable was created to reflect an intervening iron prescription. If no iron prescription was found, a negative value was attributed. Prescriptions for multivitamins containing elemental iron were not included, as these preparations do not contain iron at therapeutic doses.

**STATISTICAL ANALYSES**

All statistical analyses were conducted using SAS version 9.1 statistical software (SAS Institute Inc.). Frequencies were calculated for categorical variables, and means with standard deviations were calculated for continuous variables. Bivariate analyses comparing those with normal ZPP with those with abnormal ZPP at baseline were conducted to assess for differences in demographic characteristics. Longitudinal analyses were conducted on the group of children with abnormal ZPP at baseline to examine change over time of ZPP, Hb, and other associated markers of ID. Longitudinal analyses of hematologic markers were stratified by the presence of iron prescription as a potential intervening variable in clinical practice. The t test and χ2 test (or paired t test and McNemar test for paired data) were performed for all continuous and categorical variables, respectively.

Multivariate regression for categorical and mean reduction of abnormal ZPP was conducted to assess for an association of iron prescription with reduction of ZPP, controlling for a number of demographic characteristics associated with socioeconomic status and available through the EMR. Effect modification by Hb level was found based on descriptive results and hypothesis testing; therefore, stratified results are presented. A sensitivity analysis was performed for variation in ZPP reduction based on the length of time to follow-up. No substantial variation was found and combined results are presented.
DESCRIBED iron (improvement was greater in children who were pre-
dicators of iron status in both children who were pre-
were found at follow-up.

improvements in Hb, ZPP, and red cell distribution width
significantly to 41.3
line mean ZPP was 45.4
those who were not prescribed iron (n = 1024), the base-
also declined significantly at the time of follow-up. Among
the proportion of children with an abnormality in Hb
status as a primary intervening variable. Iron was pre-
scribed for 18.3% of the patients with abnormal ZPP at
baseline (N = 230).

Among those who were prescribed iron, the mean base-
line ZPP was 52.3 μg/dL, which decreased significantly
to 43.9 μg/dL at follow-up. Mean baseline Hb was greater
than the anemia threshold at 11.3 g/dL, which in-
creased significantly to 11.7 g/dL at follow-up. Other
markers of iron status—red cell distribution width and
mean corpuscular volume—improved significantly as well.
The proportion of children with an abnormality in Hb
also declined significantly at the time of follow-up. Among
those who were not prescribed iron (n = 1024), the base-
line mean ZPP was 45.4 μg/dL, which declined signifi-
cantly to 41.3 μg/dL at follow-up. Small but significant
improvements in Hb, ZPP, and red cell distribution width
were found at follow-up.

Significant improvement was noted in almost all in-
dicators of iron status in both children who were pre-
scribed and not prescribed iron. However, the degree of
improvement was greater in children who were pre-
scribed iron (Table 3). The odds ratio for the reduc-
tion of ZPP among those prescribed iron was 1.5 (95% CI, 1.1-2.0) and for improvement in Hb was 1.6 (95% CI, 1.2-2.2) compared with those not prescribed iron. Fur-
thermore, the absolute mean changes in ZPP and Hb were
significantly greater among those children who were pre-
scribed iron.

PRESCRIPTION PATTERNS AND CLINICAL EFFECTIVENESS OF IRON PRESCRIPTION

Iron prescription patterns are shown in the Figure. In
this primary care clinical practice, iron was prescribed
for anemia only 35.5% of the time and in 18.3% of all

children with elevated ZPP. Among those with abnor-
mal ZPP at baseline, iron was prescribed for 40% of those
with anemia, 17.6% of children with low-normal Hb, and
8.3% of children with high-normal Hb.

Multivariate regression for categorical reduction of ab-
normal ZPP and mean change in ZPP at follow-up is shown in Table 4. Initial logistic and linear regression
models included Hb, a constructed Hb level interaction
term, and the following demographic covariates: sex, in-
surance type, and race/ethnicity. The Hb level interac-
tion term was found to be significant in both models, so
Hb value was removed and the models were run sepa-
rately for each Hb level.

Among those with anemia and abnormal ZPP at base-
line (n = 189), 81.5% of those prescribed iron and 69.7%
of those not prescribed iron showed a reduction of ZPP
at the time of follow-up. Children who were prescribed
iron had 2.4 times the odds of a reduction of ZPP (odds
ratio, 2.4; 95% CI, 1.1-5.0). However, at low-normal Hb
levels, iron was prescribed only 17.6% of the time; chil-
dren prescribed iron showed a substantial, although not
statistically significant, association with reduction of ZPP.
At high-normal values of Hb, iron was prescribed only
8.3% of the time, and no significant association was seen.
Iron prescription was also associated with a larger, but not
statistically significant, mean reduction of ZPP at the time
of follow-up in children who were anemic and those
with low-normal Hb.

Until recently, screening for anemia with Hb testing was
recommended by the AAP for infants aged 9 to 12 months
who were at high risk for ID anemia.15 Because more re-

Table 1. Sample Demographics for 2612 Participants

<table>
<thead>
<tr>
<th></th>
<th>Normal ZPP</th>
<th>Abnormal ZPP</th>
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</thead>
<tbody>
<tr>
<td>No. of children</td>
<td>1358 (52)</td>
<td>1254 (48)</td>
</tr>
<tr>
<td>Male</td>
<td>653 (48)</td>
<td>611 (49)</td>
</tr>
<tr>
<td>Insurance typea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Public</td>
<td>1007 (79)</td>
<td>937 (79)</td>
</tr>
<tr>
<td>Private</td>
<td>206 (16)</td>
<td>183 (15)</td>
</tr>
<tr>
<td>Uninsured</td>
<td>65 (5)</td>
<td>64 (6)</td>
</tr>
<tr>
<td>Race/ethnicityb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>29 (2)</td>
<td>35 (3)</td>
</tr>
<tr>
<td>African American</td>
<td>904 (67)</td>
<td>790 (63)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>166 (12)</td>
<td>187 (15)</td>
</tr>
<tr>
<td>White</td>
<td>77 (6)</td>
<td>67 (5)</td>
</tr>
<tr>
<td>Other</td>
<td>130 (10)</td>
<td>126 (10)</td>
</tr>
<tr>
<td>Unknown</td>
<td>52 (4)</td>
<td>49 (4)</td>
</tr>
<tr>
<td>Age, mean (SD), mo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>10.6 (1.9)</td>
<td>10.6 (2.0)</td>
</tr>
<tr>
<td>Follow-up</td>
<td>20.8 (5.0)</td>
<td>20.4 (5.1)</td>
</tr>
<tr>
<td>Time to follow-up, mean (SD), mo</td>
<td>10.1 (1.6)</td>
<td>9.8 (4.7)</td>
</tr>
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</table>

Table 4

<table>
<thead>
<tr>
<th></th>
<th>No. (%).</th>
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<tr>
<td>Time to follow-up</td>
<td></td>
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<tr>
<td>Baseline</td>
<td></td>
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<tr>
<td>Follow-up</td>
<td></td>
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<tr>
<td>Mean (SD), mo</td>
<td></td>
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<tr>
<td>10.0 (2.0)</td>
<td></td>
</tr>
<tr>
<td>20.4 (5.1)</td>
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<tr>
<td>10.1 (1.6)</td>
<td>9.8 (4.7)</td>
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</table>
cent data suggest that ID without anemia may also be associated with adverse neurodevelopmental outcomes, new recommendations from the AAP in 2010 called for screening for ID in high-risk populations and routine anemia screening with Hb measurement in all children at 1 year of age.9 High-risk populations include those with conditions that disproportionately affect low-income children such as prematurity, low birthweight, and poor nutritional status.37-41

However, how best to screen for ID without anemia in routine clinical practice is unclear. Serum ferritin has been shown to be an insensitive measure of ID in infants.32,42,43 False-negative results are common in the setting of inflammation or recent illness, including mild viral infections, further limiting its use for widespread screening.18,20,21,44-47 Other measures suggested in the AAP guidelines were serum transferrin receptor-1 saturation and reticulocyte hemoglobin level, neither of which is routinely available in clinical practice. Zinc protoporphyrin has been used both to screen for lead toxicity and for ID. It has been found to have greater sensitivity than Hb and ferritin in the detection of ID, and evidence suggests that it may be affected by inflammation less than ferritin.21,22,47 Additionally, ZPP is inexpensive ($11.03 privately; A. Mengistab-Daniel, MT [ASCP], Boston Medical Center, written communication, August 2012) and could be an attractive and feasible screening measure for ID.22,24

Table 2. Longitudinal Trends in Markers of Iron Status Among Those With Abnormal ZPP at Baseline

<table>
<thead>
<tr>
<th>Iron Deficiency Marker</th>
<th>Iron Not Prescribed (n = 1024)</th>
<th>Iron Prescribed (n = 230)</th>
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<tbody>
<tr>
<td></td>
<td>Baseline Follow-up Mean Difference (95% CI)</td>
<td>Baseline Follow-up Mean Difference (95% CI)</td>
</tr>
<tr>
<td>ZPP, mean (SD), µg/dL</td>
<td>45.4 (12.1) 41.3 (17.6) 4.09 (2.93 to 5.25)</td>
<td>52.3 (24.5) 43.9 (14.0) 8.45 (5.23 to 11.61)</td>
</tr>
<tr>
<td>Hb, mean (SD), g/dL</td>
<td>11.8 (0.8) 12.0 (0.8) 0.09 (0.04 to 0.14)</td>
<td>11.3 (0.9) 11.7 (0.8) 0.37 (0.25 to 0.48)</td>
</tr>
<tr>
<td>RDW, mean (SD), %</td>
<td>14.6 (1.6) 14.4 (1.5) 0.19 (0.09 to 0.29)</td>
<td>15.6 (2.0) 15.2 (2.0) 0.41 (0.14 to 0.68)</td>
</tr>
<tr>
<td>MCV, mean (SD), fL</td>
<td>77.1 (4.8) 77.1 (4.9) 0.02 (0.00 to 0.04)</td>
<td>74.2 (6.7) 75.1 (6.3) 0.90 (0.40 to 1.39)</td>
</tr>
</tbody>
</table>

Abbreviations: Hb, hemoglobin; MCV, mean corpuscular volume; NA, not applicable; OR, odd ratio; RDW, red blood cell distribution width; ZPP, zinc protoporphyrin.

SI conversion factor: To convert Hb to grams per liter, multiply by 10.0.

Table 3. Change in Markers of Iron Status Among Those With Abnormal ZPP at Baseline

<table>
<thead>
<tr>
<th>Iron Deficiency Marker</th>
<th>Total</th>
<th>Iron Prescribed (n = 230)</th>
<th>Iron Not Prescribed (n = 1024)</th>
<th>OR (95% CI)</th>
</tr>
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<tbody>
<tr>
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<tr>
<td>ZPP reduction, No. (%)</td>
<td>834 (66.5) 168 (73.0)</td>
<td>866 (65.0)</td>
<td>1.5 (1.1 to 2.0)</td>
<td></td>
</tr>
<tr>
<td>Hb improvement, No. (%)</td>
<td>693 (55.3) 148 (64.4)</td>
<td>545 (33.2)</td>
<td>1.6 (1.2 to 2.2)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Hb, hemoglobin; OR, odds ratio; ZPP, zinc protoporphyrin.

SI conversion factor: To convert Hb to grams per liter, multiply by 10.0.

Figure. Rates of iron prescription. ZPP indicates zinc protoporphyrin.

Several findings of potential importance emerged from this analysis of routinely collected EMR data from a low-income urban pediatric population. Nearly half of the children studied had an abnormal baseline ZPP, most of whom did not have anemia. This finding is not statistically different from the 52% prevalence of abnormal ZPP found...
in a smaller study by Lozoff et al of African American infants in Detroit. Furthermore, in our more ethnically diverse study population, abnormal ZPP was not significantly associated with race/ethnicity, suggesting that this condition affects low-income children regardless of race/ethnicity. However, these data cannot be compared directly with the national prevalence estimate of ID of 9.2% in this age group as this figure is based on the National Health and Nutrition Examination Survey definition of ID, which requires that at least 2 of 3 measures of iron status be abnormal. This finding suggests that practitioners may need to be more vigilant about ID screening and treatment to avoid potentially permanent adverse neurodevelopmental outcomes disproportionately affecting children from low-income families.

Our second important finding was that although ZPP and other markers of iron status improved in our study population regardless of iron prescription, those prescribed iron had significantly greater improvements in ZPP and Hb at the time of follow-up. Our results suggest that ZPP could be useful in monitoring the response to therapy after iron is initiated.

Additionally, after controlling for demographic variables, we found that the relationship between iron therapy and the reduction of ZPP was modified by Hb level. In our population, children with abnormal ZPP and anemia showed a significant reduction of ZPP and a trend toward greater linear change in ZPP. However, a significant association between iron therapy and ZPP reduction at nonanemic levels of Hb was not found. However, this finding should be interpreted with caution. During the time of study, routine screening for ID without anemia was not yet recommended and rates of iron prescription were low in these patients, leaving little power to detect a reduction of ZPP with iron therapy. Despite this limitation, we found a nonsignificant association with reduction of ZPP and greater linear change among children with low-normal Hb who were prescribed iron. Further investigation is warranted to explore the effect of iron on ZPP when monitoring the response to therapy at nonanemic levels, and whether iron therapy should be considered for children with low-normal Hb and elevated ZPP.

An interesting secondary finding reflected the rates of iron prescription in routine clinical practice; even when anemia was present, an iron prescription was documented for only 35% of children. This finding indicates room for improvement in adherence to routine clinical practice guidelines, and it may relate to provider knowledge gaps or challenges of practicing with high-risk populations, such as loss to follow-up and general barriers to health services.

Our study has several limitations. The subjects comprised a convenience sample of children attending a hospital-based primary care clinic; thus, the findings cannot be generalized to the general population of low-income children. Furthermore, as a retrospective analysis of EMR data, we had limited ability to control data quality. It is possible that this analysis did not capture all iron prescriptions; written prescriptions or those called in directly to a pharmacy may not have been documented in the EMR. However, this would lead to a type 2 error or underestimation of the treatment effect.

In addition, we could study only those variables obtained during routine anemia screening. Therefore, we were unable to compare or combine ZPP with other measures of iron status such as ferritin. We had limited socioeconomic data beyond insurance status and race/ethnicity; therefore, we were unable to assess for potential confounders such as parental education, immigration status, or health literacy that could be useful when considering targeted interventions. Furthermore, while we excluded those with evidence of lead toxicity or sickle cell anemia—2 conditions that disproportionately affect this low-income urban population—we were not able to exclude other rarer causes of anemia apart from ID. However, other causes of anemia, such as thalassemias, are not known to affect ZPP.

Finally, in our data set, we were only able to assess for documented iron prescription and not measures of patient adherence such as prescription fill rates, patient surveys, medication cap devices, or inspection of returned medicine containers. Prior studies in this population found a low rate of adherence to a multivitamin-iron supplement prescribed to prevent ID. Therefore, iron prescription is not necessarily
indicative of receipt of therapy, particularly in a high-risk, low-income population that may encounter financial, social, and cultural barriers to health care. However, given this limitation, our finding of a significant relationship between iron prescription and reduction of ZPP is particularly relevant.

While several limitations exist with the use of routinely collected EMR data, there are also unique strengths to this study. This data set allowed for the analysis of a large sample size of ZPP data at multiple points. Furthermore, our results represent experience from clinical practice with low-income families, integrating the social, economic, and cultural challenges that affect health services delivery in this at-risk population of young children.

Our findings indicate that ID among low-income children, as measured by elevated ZPP, may be higher than national estimates suggest. Iron prescription was significantly associated with a greater degree of reduction of ZPP, particularly among anemic patients, and with a trend toward improvement among those with low-normal Hb values. Our findings suggest that ZPP may be a useful and practical measure for ID screening and monitoring in clinical practice. Further investigation and prospective studies are indicated to assess the use of ZPP as a screening measure for ID and monitoring the response to therapy, particularly in preanemic children.

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Author Contributions: Dr Magge had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Magge, Sprinz, Adams, and Meyers. Acquisition of data: Sprinz, Adams, and Meyers. Analysis and interpretation of data: Magge, Sprinz, Adams, Drainoni, and Meyers. Drafting of the manuscript: Magge, Sprinz, and Adams. Critical revision of the manuscript for important intellectual content: Magge, Drainoni, and Meyers. Statistical analysis: Magge. Administrative, technical, and material support: Magge, Sprinz, Adams, and Drainoni. Study supervision: Adams, Drainoni, and Meyers.

Conflict of Interest Disclosures: None reported.

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