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THE ZINC PROTOPORPHYRIN/HEME RATIO IN PREMATURE INFANTS: HAS IT FOUND ITS PLACE?

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There is need for a sensitive clinical index of early iron depletion in growing premature infants. In prematurity, serum ferritin level (the common clinical index of tissue iron status) may be artifactually high, and, at best, it indicates late stages of tissue iron depletion. Finding a sensitive index is critical because tissue iron depletion in fetal life (eg, intrauterine growth restriction and maternal diabetes)^{1,2} or early life³ after premature birth may impair cognitive development. Recent changes in clinical practice may place premature infants at greater risk for tissue iron depletion. Neonatologists are avoiding packed erythrocyte transfusions, a rich iron source, due to the implementation of lower hemoglobin/hematocrit triggers for transfusions and lower phlebotomy losses accompanying improved respiratory stability. Although it is unclear which premature infants are candidates for recombinant erythropoietin (rhEpo), the use of rhEpo adds additional risk for tissue iron depletion.

In this issue of *The Journal*, Miller et al⁴ present a pilot study examining the utility of measuring serial zinc protoporphyrin/heme (ZnPP/H) ratios in evaluating impaired erythrocyte iron delivery and guiding iron therapy in premature infants. ZnPP/H ratio is an underused, sensitive measure of iron deficient erythropoiesis in older patients.⁵ Zinc protoporphyrin level reflects incomplete iron incorporation into protoporphyrin, as zinc substitutes for iron when supply is limited. ZnPP/H ratio is more sensitive than hemoglobin or plasma ferritin in diagnosing iron-deficient erythropoiesis in adults⁶ and older term infants.⁷ ZnPP/H ratio also is used to monitor iron therapy.⁷ Lott et al⁸ and Juul et al⁹ have reported that cord and early postnatal ZnPP/H ratios are inversely correlated with gestation and reflect differing fetal iron status. The current work by Miller et al complements these earlier studies, taking the field one step further by evaluating whether serial ZnPP/H ratio can help guide iron supplementation in hospitalized premature infants.

ZnPP/H ratio could be practical for screening and/or monitoring therapy in premature infants. It is potentially sensitive, can be performed on small sample volumes, is stable on storage for 10 days, and is inexpensive. Sample volumes of 50 μ L may be taken from the same EDTA microtube used for a complete blood count. Plasma bilirubin interferes with the assay, but rinsing with saline removes this pigment, improving the assay's specificity. The test can be done in less than 1 minute, and rinsing with saline adds only 10 minutes to the process. Although not universally accessible, the machine is portable, economical to purchase (\$5500), and inexpensive to use. (Obtaining 15 samples per week would cost around \$20 per week in supplies.) Although other tests that measure reticulocyte hemoglobin content through sophisticated flow cytometry may also monitor iron status in this population,¹⁰ ZnPP/H ratio is a simpler test.

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The study by Miller et al extends the current knowledge regarding ZnPP/H ratio in premature infants. Although the study is not randomized and the study population is heterogeneous, the study does evaluate the response to oral iron supplementation and the lack of measured iron toxicity. In combination with the earlier reports by Juul et al⁸ and our group¹¹ demonstrating that ZnPP/H ratios in growing premature infants reflect iron status, evidence is mounting that ZnPP/H ratio may be clinically useful in this difficult-to-monitor population. Normative or reference data aids in interpreting ZnPP/H ratios. The Figure shows mean (± 2 standard deviations [SD]) ZnPP/H ratios in young children, based on current reports in the literature. 7-9,12-18 In the figure, cord or first postnatal week ZnPP/H ratios after premature delivery are shown as -3, -2 and -1 months preterm. Because cord ZnPP/H ratios are higher after episodes of fetal hypoxemia,^{8,9} ratios with potential hypoxic exposures are excluded. Term cord blood and term postnatal ratios are also shown. The figure demonstrates that mean postconceptional ZnPP/H ratios fall with increasing duration of gestation.

Miller et al used their laboratory's reference ratios based on corresponding newborn-corrected gestational age to guide iron supplementation.⁴ The highest ZnPP/H ratios are found in the most premature newborns, possibly due to their greater fetal growth velocity, greater relative increase in blood volume, and greater iron accretion needs.⁹ ZnPP/H ratios fall with increasing postconceptional age. Because nutritional goals for premature infants generally match fetal nutritional needs at equal postconceptional age, it is logical to use corrected gestational age-matched reference ratios; however, it is unclear whether the more stringent postnatal age-matched reference ratios for ZnPP/H should be used. Work by Griffin et al¹⁹ and our group¹¹ has shown that ZnPP/H ratios in premature infants at or after discharge from the neonatal intensive care unit were higher than the more stringent normal limits for postnatal age, but normal for corresponding postconceptional age.

Further study of the role of the ZnPP/H ratio in prematurity is warranted. The field will benefit from the reporting of additional cord blood ratios from premature deliveries without evidence of fetal hypoxia to help further refine the postconceptional reference ranges. It will also be clinically important (albeit difficult) to study whether maintaining growing premature infants in the more stringent postnatal reference range or in the postconceptional reference range is associated with a better neurologic outcome. Prospective, randomized trials of high versus low iron supplementation monitored by ZnPP/H ratios in growing premature infants not yet transfused could be also accomplished. Without the compounding effect from transfusions, one could examine whether the ZnPP/H ratio discriminates between iron-deficient erythropoiesis and slow recovery from the anemia of prematurity. Future studies of the ZnPP/H ratio could also address the question of whether oral iron supplementation is adequate to meet the needs of rhEpo-treated premature infants. If clinical response to rhEpo is poor, then future studies could help address whether the ratios discriminate between iron-deficient erythropoiesis and inadequate rhEpo dosage. Studies on the role of the ZnPP/H ratio in older preterm infants may investigate using the ratio to adjust iron supplements and prevent the transition from iron-deficient erythropoiesis to the more extreme condition of tissue iron depletion. If ongoing studies in premature infants continue to support its utility, the underutilized ZnPP/H ratio may one day become part of routine clinical practice.

Glossary

RhEpo, Recombinant erythropoietin; SD, Standard deviation; ZnPP/H, Zinc protoporphyrin/heme.

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Figure.

ZnPP/H ratio ($\mu\text{mol/mol}$) on the vertical axis (in log scale) versus age in months (MO) or years (YR) in premature and term infants on the horizontal scale. Cord or first postnatal week ZnPP/H ratios after premature delivery are demarcated as -3 , -2 , or -1 months (based on number of months preterm). Cord blood ratios from term infants are included. Positive months indicate postnatal age of term infants; ≤ 12 MO includes values between 6 and 12 months, ≤ 24 MO includes values between 13 and 24 months, and ≤ 5 YR includes values between 25 months and 5 years. Mean is indicated by the bold line connecting the boxes, $+2$ SD is shown by the line connecting the triangles, and -2 SD is represented by the line connecting the circles. Note that $80 \mu\text{mol/mol}$ is a commonly reported $+2$ SD for older children and adults.⁸ The figure is constructed from the reports in literature.^{7-9,12-18}