Zinc Protoporphyrin-to-Heme Ratio and Ferritin as Measures of Iron Sufficiency in the Neonatal Intensive Care Unit

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Objectives To evaluate ferritin and zinc protoporphyrin-to-heme (ZnPP/H) ratios as biomarkers of iron status in neonates, determine how specific clinical events affected these measures, and assess how iron status changed during hospitalization.

Study design We performed a retrospective study of all infants with paired ferritin and ZnPP/H measurements between October 2014 and May 2016. Concordance of these measurements, effects of sepsis, red blood cell transfusion, erythropoietin treatment, and iron supplementation were assessed. Iron status was measured over time.

Results A total of 228 patients (mean birth weight 1.3 kg, median gestational age 29 weeks) were evaluated. Mean log ZnPP/H values in infants with and without sepsis were not significantly different (4.98 μmol/mol vs 4.97 μmol/mol, adjusted $P = .103$), whereas log-transformed ferritin values increased significantly during infection (5.23 ng/mL vs 4.04 ng/mL, adjusted $P < .001$). Ferritin also increased more significantly than ZnPP/H following red blood cell transfusion (ferritin: mean 5.03 ng/mL vs 4.0 ng/mL, $P < .001$; ZnPP/H: mean 4.85 μmol/mol vs 4.98 μmol/mol, $P < .001$). The mean iron supplementations at 30, 60, and 90 days were 5.4, 6.9, and 7.4 mg/kg/day, respectively. Ferritin values decreased with advancing postnatal age (adjusted $P < .001$), with 66% of ferritin values less than 76 ng/mL. Treatment with erythropoietin increased ZnPP/H, but not ferritin levels.

Conclusions Ferritin is more significantly affected by inflammatory events such as sepsis and transfusion than ZnPP/H, thus, ZnPP/H may be a more reliable marker of iron status in this population. Infants showed worsening iron sufficiency over time despite supplementation above American Academy of Pediatrics guidelines. *(J Pediatr 2017;■■:■■-■■).*

Iron sufficiency is critical for normal neonatal brain development.[1-4] Iron is a key cofactor for DNA and neurotransmitter synthesis. It is essential for myelination, with high concentrations seen in oligodendrocytes.[1] Animal studies of iron deficiency during critical periods of brain development have demonstrated truncated dendritic trees, reduced brain mass, and long-term memory deficits.[5-7] Human studies have shown outcomes consistent with these results with irreversible neurobehavioral impairments, such as memory deficits and changes in auditory recognition.[8]

The majority (~80%) of fetal iron accretion occurs in the third trimester of pregnancy, at a rate of 1.6-2.0 mg/kg/day.[9,10] Preterm infants are, thus, inherently iron deficient in the absence of third trimester maternal-fetal iron transport or postnatal supplementation. Perinatal risk factors, such as maternal diabetes mellitus and intrauterine growth retardation, can also result in full-term infants being iron deficient.[10]

Given their risk for iron deficiency, the American Academy of Pediatrics (AAP) recommends iron supplementation of 2-4 mg/kg/day for high-risk neonates.[9] Based on prior studies performed at the University of Washington (UW) neonatal intensive care unit (NICU),[11] we hypothesized that these guidelines may be inadequate to maintain iron sufficiency in NICU patients.

The ability to assess iron sufficiency is critical to supplementing infants appropriately. Two common measures of iron sufficiency used in neonates are ferritin and zinc protoporphyrin-to-heme ratio (ZnPP/H).[11-15] Ferritin binds and stores iron intracellularly, and also functions as an iron carrier in plasma, thus, plasma ferritin has been used as an indirect marker of iron sufficiency.[16] However, ferritin is an acute phase reactant, increasing with stress or inflammation, so may not be an ideal measure during periods of stress or inflammation. Iron is prioritized to erythropoiesis, and ZnPP/H measures zinc relative to iron incorporation into protoporphyrin IX in red blood cells. When insufficient iron is available, the proportion of zinc incorporated

AAP	American Academy of Pediatrics
Darbe	Darbepoetin
Epo	Erythropoietin
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
PENUT	Preterm Epo Neuroprotection Trial
UW	University of Washington
ZnPP/H	zinc protoporphyrin-to-heme ratio

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Supported by the Neonatal Biology Research Fund, University of Washington (K.G.). The authors declare no conflicts of interest.

https://doi.org10.1016/j.jpeds.2017.10.041

increases, thus, increasing ZnPP/H. Although ZnPP/H has been shown to be elevated in conditions of chronic inflammation in adult populations,[17,18] it is not currently known how ZnPP/H is affected by inflammation in neonates.

The objectives of this study were to determine if ZnPP/H and ferritin are correlated, evaluate how ZnPP/H and ferritin are affected by inflammatory states or erythropoietic stimulating agents, and evaluate iron sufficiency over time as measured by ZnPP/H and ferritin.

Methods

A retrospective chart review was completed on neonates admitted to the University of Washington NICU between October 2014 and May 2016. All infants who had at least 1 ZnPP/H and ferritin measurement on the same date were included, with no further inclusion or exclusion criteria. Demographic information including sex, race, gestational age, and perinatal risk factors was obtained. Additional information collected included delivery history, ferritin, ZnPP/H, complete blood count with reticulocyte counts, iron supplementation, and discharge comorbidities. A subset of infants included in this study were enrolled in the Preterm Erythropoietin (Epo) Neuroprotection Trial (PENUT; U01NS077953, NCT01378273). The PENUT trial is a phase III, double-blinded, randomized, controlled trial examining the use of Epo for neuroprotection in extremely low gestational age neonates. The randomization of patients to Epo vs placebo was not disclosed to the investigators, however, the PENUT Data Coordinating Center was involved in the analysis of this study, thus, allowing treatment with Epo to be appropriately adjusted for in our statistical results. This study was approved by the University of Washington Institutional Review Board.

All available ZnPP/H, ferritin, hematocrit, and reticulocyte percentage values were collected on every infant. Paired values of ZnPP/H and ferritin were collected preferentially, together with the infant's age and corrected gestational age at the time of laboratory collection. Number and volume of packed red blood cell transfusions were recorded. As per unit protocol, iron studies were collected every 2-4 weeks during NICU admission under most circumstances, with iron supplementation subsequently adjusted based on the laboratory results.

To assess the response of ZnPP/H and ferritin to states of inflammation, neonates with culture proven sepsis, and red blood cell transfusion were identified. Sepsis was defined as culture positive infection either 2 days before or 1 day after laboratory markers of iron status were obtained. Infants with necrotizing enterocolitis (NEC) were identified, however, no infants were diagnosed with NEC within 1 day after or 2 days before ZnPP/H or ferritin measurements, therefore, this marker of inflammation was not used in our analysis. NEC was defined as the presence of feeding intolerance with pneumatosis (ie, modified Bells stage II or above).[19] Recent red blood cell transfusion was defined as ZnPP/H and ferritin measurements within 7 days after transfusion.

Per the policy in the UW NICU, enteral iron supplementation was initiated beginning at 2 weeks of life once enteral feeds were at a minimum of 60-100 mL/kg/day. The starting dose of ferrous sulfate was 2-4 mg/kg/day. The iron dose was adjusted in increments of 2 mg/kg/day based on serum ferritin and ZnPP/H values or per PENUT trial protocol for infants enrolled in that study. Epo or darbepoetin (Darbe) were initiated per attending physician discretion or per PENUT protocol. Standard dosing for Epo and Darbe given for anemia is 400 units/kg 3 times per week and 10 mcg/kg/dose once weekly for a minimum of 2 weeks in the UW NICU. For the PENUT trial, subjects received Epo 1000 units/kg every other day for 6 doses followed by 400 units/kg 3 times per week through 32 postnatal weeks.

Per unit policy, delayed cord clamping is attempted in all infants born at less than 37 weeks gestational age. Delayed cord clamping for 30 or more seconds was achieved in 59% of this study cohort.

For subjects who remained hospitalized, total iron intake was calculated at 30, 60, and 90 days of life, and at the time of discharge from the NICU. Corrected gestational age, weight, method(s) of iron supplementation, and feeding volumes were recorded. Total iron intake was calculated as mg/kg/day and included enteral iron supplementation with ferrous sulfate, IV iron sucrose, and iron content of feeds as applicable.

Statistical Analyses

Prior to all analyses, ZnPP/H and ferritin values were log-transformed (base e) to alleviate the skewness observed in their distributions. To evaluate the relationship between ZnPP/H and ferritin in assessing iron stores, we calculated the Spearman correlation coefficient between all pairs of log ZnPP/H and ferritin measurements that were taken on the same date. The UW laboratory reports ZnPP/H values up to a maximum of 250 μmol/mol. ZnPP/H measurements recorded as >250 μmol/mol were conservatively assumed to be 251 μmol/mol. We further examined these relationships while adjusting for gestational age, day of life, and treatment with Darbe/Epo by fitting a linear mixed model[20] to account for correlations among multiple measurements per infant.

We compared the distributions of log-transformed ZnPP/H and ferritin in inflammatory states using the same linear mixed model framework where we adjusted for gestational age, day of life, and treatment with Darbe/Epo. The 2 inflammatory states of interest included sepsis and recent red blood cell transfusion.[21,22] We also examined a combination of these 2 inflammatory states as a representation of clinical inflammation.

The distributions of log ZnPP/H and ferritin in infants who were and were not treated with Epo or Darbe during their NICU course were also assessed. Adjustments were made for gestational age and day of life. Finally, we assessed the trends of log ZnPP/H and ferritin over time by examining the relationship between these 2 measurements and the infants' days of life at which the measurements were taken. We also adjusted for gestational age and treatment with Darbe/Epo while accounting for multiple measurements per infant. As more mature babies would likely be discharged earlier, we performed a subgroup analysis for infants 28 weeks of gestation or less, at birth.

FLA 5.5.0 DTD ■ YMPD9549_proof ■ December 4, 2017