High Prevalence of Iron Deficiency Despite Standardized High-Dose Iron Supplementation During Recombinant Erythropoietin Therapy in Extremely Low Gestational Age Newborns

Ashajyothi M. Siddappa, MD, FRCPCH[1,2], Rose M. Olson, MD[3,*], Miriam Spector, MPH[4], Elise Northrop, BA[5], Tara Zamora, MD[6,†], Ann M. Brearley, PhD[5], Michael K. Georgieff, MD[6], and Raghavendra Rao, MD[6]

Objective To assess the effects of protocolized recombinant human erythropoietin (r-HuEPO) therapy and standardized high dose iron supplementation on hematologic and iron status measures in a cohort of extremely low gestational age newborns (ELGANs).

Study design Charts of extremely low gestational age newborns admitted from 2006 to 2016 and who had received r-HuEPO per neonatal intensive care unit protocol were reviewed. The r-HuEPO was started at a dose of 900 IU/kg per week after 7 days of age and continued until 35 weeks postmenstrual age. Oral iron supplementation at 6-12 mg/kg per day was used to maintain a transferrin saturation of >20% during r-HuEPO treatment. Data on demographic features, hematologic and iron panel indices, red blood cell transfusions, and clinical outcomes were collected. Quartile groups were created based on serum ferritin levels at the conclusion of the r-HuEPO treatment and the quartiles were compared.

Results The cohort included 116 infants with mean gestational age 25.8 ± 1.5 weeks and birth weight 793 ± 174.1 g. The r-HuEPO promoted erythropoiesis as indicated by increasing hemoglobin, hematocrit, and reticulocyte count. Serum ferritin decreased over time and was ≤75 ng/mL in 60.2% of infants at the conclusion of r-HuEPO therapy; 87% received packed red blood cell transfusions. Transfusion volume, total iron intake, total iron binding capacity, and transferrin concentration differed among infants in the different serum ferritin quartiles ($P < .05$).

Conclusions In extremely low gestational age newborns, r-HuEPO therapy promoted erythropoiesis. Despite a biomarker-based standardized high-dose iron supplementation, the majority of infants had evidence of iron deficiency to a degree that is associated with reduced brain function. *(J Pediatr 2020;222:98-105)*.

Infants born preterm develop a decrease in hemoglobin (Hgb) that is most pronounced in those born at <28 weeks of gestation (extremely low gestational age newborns [ELGANs]).[1,2] This anemia of prematurity is the result of several factors, among which lower production of erythropoietin, frequent blood sampling, and decreased availability of nutrients that support erythropoiesis, including iron, folate, and protein, are the major ones.[1-3] Most ELGANs require multiple red blood cell (RBC) transfusions, iron supplementation, and in some situations, recombinant human erythropoietin (r-HuEPO) therapy to maintain Hgb levels.

Although RBC transfusions can improve anemia and hypoxia, there are notable risks including infection, electrolyte imbalance, circulatory overload, abnormally high serum ferritin concentrations, and mortality.[4-6] Associations between high serum ferritin concentrations and retinopathy of prematurity (ROP) and bronchopulmonary dysplasia (BPD) have been reported. r-HuEPO treatment mitigates the need for RBC transfusions and stabilizes or increases Hgb concentrations in preterm neonates.[6-14] r-HuEPO may also have a beneficial

BPD	Bronchopulmonary dysplasia
DOL	Days of life
ELGAN	Extremely low gestational age newborn
Hgb	Hemoglobin
HCT	Hematocrit
PMA	Postmenstrual age
RBC	Red blood cell
r-HuEPO	Recombinant human erythropoietin
ROP	Retinopathy of prematurity
TIBC	Total iron-binding capacity

From the [1]Department of Pediatrics, Hennepin Healthcare; [2]Department of Pediatrics, University of Minnesota; [3]University of Minnesota Medical School; [4]School of Public Health; [5]Division of Biostatistics, School of Public Health, and Biostatistical Design and Analysis Center, Clinical and Translational Science Institute; and [6]Division of Neonatology, Department of Pediatrics, University of Minnesota, Minneapolis, MN

*Current affiliation: Department of Medicine, Brigham and Women's Hospital, Boston, MA.
†Current affiliation: Division of Neonatology, University of New Mexico School of Medicine, Albuquerque, NM.

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effect on neurodevelopment.[15,16] However, adequate iron supplementation is essential for sustaining erythropoiesis during r-HuEPO treatment.[17] The amount of iron necessary to support erythropoiesis, while not depleting iron stores and risking tissue iron deficiency, is not known. Inadequate iron supplementation may predispose to tissue iron deficiency, including brain iron deficiency owing to prioritization of iron to RBCs over other organs during development.[18] The aim of our study was to examine the effects of r-HuEPO therapy combined with a biomarker-based standardized iron dosing strategy on hematologic measures and the iron status of ELGANs.

Methods

This is a retrospective chart review of ELGANs born at Hennepin County Medical Center between 2006 and 2016 after the institution of a standardized r-HuEPO and iron supplementation protocol. All infants who were <28 weeks of gestation at birth and received r-HuEPO per protocol were included. Exclusion criteria were transfer to an outside facility, death, and failure to complete r-HuEPO treatment per protocol. The study was approved by the Institutional Review Board at Hennepin County Medical Center.

Per protocol, r-HuEPO therapy was initiated after 7 days of life (DOL) at a dose of 300 U/kg, 3 times a week (Monday, Wednesday and Friday) and continued until a postmenstrual age (PMA) of 34 weeks and 6 days. Baseline iron studies were obtained before starting r-HuEPO therapy. The dose of r-HuEPO was adjusted weekly for infant's weight. Iron supplementation was started 1 week after the initiation of r-HuEPO by adding iron dextran at a dose of 0.5-1.0 mg/kg per day to total parenteral nutrition after a test dose.[19] Oral iron supplementation was started using ferrous sulfate when full volume feeding was achieved. The starting oral iron supplementation dose was 6 mg/kg per day, and the maximum dose was 12 mg/kg per day. The iron dose was adjusted to maintain iron saturation (transferrin saturation) of >20%. The iron dose was decreased to maintenance dose (2-4 mg/kg per day) 1 week after r-HuEPO was discontinued. In addition to r-HuEPO and iron supplementation, infants received 50 μg/d of folate and 25 IU/d vitamin E supplementation.[20] r-HuEPO was terminated before 35 weeks PMA in some infants by the attending neonatologist owing to clinical concerns of infection, hypertension, or severe ROP. These infants were excluded from the analysis.

Hematologic indices, including Hgb (g/dL), hematocrit (Hct [%]), mean corpuscular volume (fL), red cell distribution width (%), reticulocyte count (%), and platelet count (10^9/L), and iron status panel, including serum iron (μg/dL), serum ferritin (ng/mL), total iron-binding capacity (TIBC, μg/dL), transferrin saturation/iron saturation (%), and serum transferrin (mg/dL) were measured weekly for the first 4 weeks and then every other week until r-HuEPO was completed. Transferrin saturation (%) was calculated using the formula: serum iron/TIBC × 100.

Demographic, clinical, hematologic, and iron indices data from each infant were extracted from the electronic charts. Growth rate (grams per day) was calculated using the birth and discharge weights. RBC transfusion data extracted included number of transfusions and the volume of packed RBC received by the infant. The total amount of iron (parenteral + oral) received during the r-HuEPO therapy was calculated. Clinical outcomes during hospitalization were extracted, including the occurrence of ROP, BPD, intraventricular hemorrhage, patent ductus arteriosus, necrotizing enterocolitis, and days of mechanical ventilation.

Statistical Analyses

Weight, length, and head circumference Z-scores were calculated using the Fenton 2013 growth charts for preterm infants.[21] Two-sided paired *t* tests were used to assess changes in the hematologic and iron indices. Quantile groups were created based on the SF values at 35 weeks PMA as follows: <40 ng/mL (lowest quartile), 40-89 ng/mL (middle 2 quartiles), and ≥90 ng/mL (highest quartile). These cutoffs were determined by finding the 25th and 75th quantiles and rounding to the nearest multiple of 5. ANOVA and χ^2 tests were used to assess differences among serum ferritin quartile groups. Significant covariates underwent a post hoc 2-sample *t* test with unequal variance to further compare the highest and lowest quartiles. A multivariate logistic regression model was fit and summarized to assess the overall effect of serum ferritin quartile on BPD risk. To examine the effect of serum ferritin quartile on ROP, a multivariate ordered logistic regression model was used. Cubic splines with 2 knots for iron and hematologic factors were fitted to graphically display the trend of each index. Cubic splines were fitted with all data, but truncated at 38 and 42 weeks PMA for iron and hematologic indices, respectively, owing to insufficient data to observe meaningful trends beyond those periods. All analyses and graphing were performed with R version 3.4.0 (The R Foundation, Vienna, Austria). A *P* value of <.05 was considered statistically significant.

Results

The medical charts of a total of 209 infants were reviewed, and 93 infants were excluded from final analysis. Ten infants were transferred to another facility before completion of r-HuEPO treatment, 33 infants died, 31 infants did not receive r-HuEPO, and r-HuEPO was stopped early in 15 infants (3 owing to positive blood culture, 8 owing to concern for infection, 1 owing to high serum ferritin, 1 owing to stage 3 ROP, 1 owing to hypertension, and in 1 infant for an unclear cause). In addition, data from 4 infants were not included (in 1 infant r-HuEPO and iron were started on DOL 14, in 1 infant r-HuEPO was started at 31 weeks, and in 2 infants transfusion data were not available). Thus, the final analysis included 116 infants (**Figure 1**; available at www.jpeds.com). Demographic features of the infants are shown in **Table I**. The mean duration of total parenteral

Table I. Demographics of all study participants (n = 116)

Characteristics	Birth	Discharge
Male	55 (47.4)	–
Race/ethnicity		
African American	48 (41.4)	–
African	8 (6.9)	–
Caucasian	15 (12.9)	–
Hispanic	32 (27.6)	–
Other	13 (11.2)	–
Cesarean delivery	78 (67.2)	–
Antenatal steroids (%)	100 (86.2)	–
5-Minute Apgar score	7.1 ± 1.6	–
Weight (g)	793 ± 174.1	3242 ± 621.1
Weight Z-score	0.02 ± 0.95	−0.66 ± 0.87
Length (cm)	33.0 ± 3.01	48.2 ± 3.07
Length Z-score	0.08 ± 1.05	−1.2 ± 1.23
Head circumference (cm)	23.0 ± 1.85	34.3 ± 1.95
Head circumference Z-score	−0.12 ± 0.94	−0.44 ± 0.96
PMA (wk)	25.8 ± 1.45	40.4 ± 3.38

Values are mean ± SD for continuous variables, or number (%) for categorical variables.

nutrition was 27.6 ± 13.2 days. Enteral feedings were started on DOL 5.96 ± 4.7; 73.3% were started on maternal breastmilk, 4.3% on donor breastmilk, 9.5% received a combination of maternal and donor breast milk, and 13.0% received formula. Enteral feedings were fortified on DOL 26.5 ± 14.6. At discharge, 15.5% of infants were exclusively on maternal breast milk, 14.6% received a combination of maternal breast milk and formula, and 69.9% received only formula; 96% of infants (n = 111) needed mechanical ventilation for a mean duration of 32.0 ± 32.9 days. The incidence of intraventricular hemorrhage was 33.0% (n = 38), severe intraventricular hemorrhage (grades 3 and 4) was 8.6 % (n = 10), necrotizing enterocolitis was present in 6.0% (n = 7), and patent ductus arteriosus was present in 66.4% (n = 77). The incidence of infants needing surgery, including patent ductus arteriosus ligation and gastrostomy tube placement, was 40.5% (n = 47). The incidence of ROP (all stages) was 77.6% (n = 90), and stage 3 ROP was present in 7.8% of infants (n = 9). BPD was present in 70.7% of infants (n = 82).

r-HuEPO therapy was initiated on DOL 9.7 ± 2.8. Parenteral iron supplementation was started on DOL 13.6 ± 9.0, and oral iron supplementation was started on DOL 28.0 ± 13.1; 87% of the infants (n = 101) received RBC transfusions, with 78 infants receiving a transfusion during the first week after birth. The first transfusion was on DOL 5.4 ± 5.2, and the last transfusion was on DOL 26.2 ± 22.5. The mean number of RBC transfusions per infant was 3.3 ± 2.7. Average volume of blood received per infant was 46.9 ± 46.6 mL (51.0 ± 44.8 mL/kg). Late RBC transfusions, defined as transfusions after 3 weeks of age, were given to 40.5% of the infants (n = 47). Only 12.9% of infants (n = 15) did not receive any transfusions during the hospital stay.

Hematologic indices were measured, on average, 10.8 ± 2.3 times in each infant. Hematologic indices at admission and at the end of r-HuEPO therapy and at discharge are shown in **Table II** and **Table III** (available at www.jpeds.com), respectively. Changes in the hematologic measures during hospitalization are shown in **Figure 2**. Infants experienced an initial drop in Hgb and Hct after birth, followed by a slight increase in both measures during r-HuEPO therapy. The mean corpuscular volume decreased steadily from birth and was not altered during r-HuEPO therapy. Reticulocyte count and red cell distribution width increased after the beginning of r-HuEPO therapy and reached a peak at 30-32 week PMA, before steadily decreasing thereafter. The platelet count started low and steadily increased during r-HuEPO therapy.

The first set of iron studies were drawn before r-HuEPO was started, and the majority of infants had been transfused before the first set of iron studies. On average, each infant had 6.5 ± 1.5 sets of iron measurements. Iron indices before beginning r-HuEPO and iron supplementation and at the conclusion of high iron supplementation and the last values obtained during r-HuEPO therapy are given in **Table II** and **Table III**, respectively. Trends in iron indices during r-HuEPO therapy are shown in **Figure 3**. Serum iron was low at the initiation of iron supplementation and steadily increased thereafter, whereas serum ferritin had an initial increase before the start of r-HuEPO and steadily decreased over time during r-HuEPO therapy. Transferrin saturation showed an initial decrease, followed by a steady increase during r-HuEPO therapy. Similarly, both transferrin and TIBC showed an initial decrease, followed by an increase, before plateauing around 30 weeks PMA, followed by an increase after the conclusion of r-HuEPO, whereas serum iron, serum ferritin, and transferrin saturation exhibited their greatest rate of change around this time. Serum ferritin was <75 ng/mL in 60.2% of the infants, 76-400 ng/mL in 33.7%, and >400 ng/mL in 6.1% at the conclusion of r-HuEPO therapy at a PMA of 35 ± 1 weeks.

In an effort to establish the factors influencing serum ferritin levels, quartiles based on serum ferritin at the conclusion of r-HuEPO therapy were determined. Thirty-three infants did not have iron measurements at a PMA of 35 ± 1 weeks, and were excluded from all serum ferritin quartile analyses. The characteristics of infants with serum ferritin in the lowest quartile (ie, those at risk for tissue iron deficiency), middle 2 quartiles and the highest quartile (ie, those at risk for iron overload) were compared (**Table IV**). Total iron intake, total amount of packed RBC transfused, TIBC, and transferrin concentration were statistically different between the quartile groups. A post hoc analysis confirmed that the lowest serum ferritin quartile had statistically higher amounts of total iron intake, TIBC, and transferrin, and lower volume of packed RBC transfused compared with the highest serum ferritin quartile (all $P \leq .01$) (**Table IV**). Repeating the analysis using physiologic threshold of ≤75 ng/mL and >75 ng/mL yielded similar results to quartile-based analysis with the exception of total iron intake, which was no longer different between the groups (**Table V**; available at www.jpeds.com). Results from testing the overall effects of serum ferritin quartile

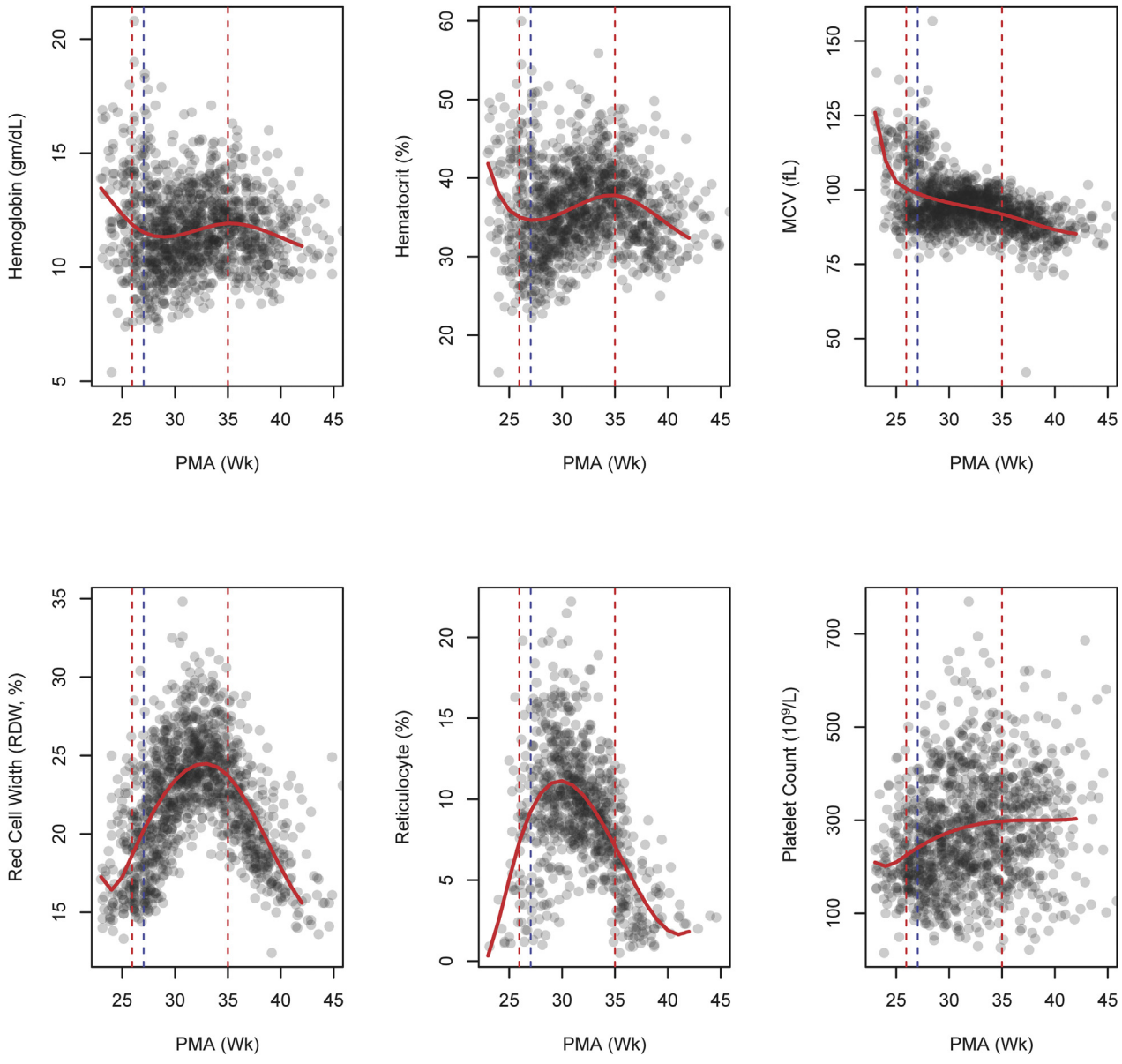

Figure 2. Scatter plot with nonparametric trend line for hematologic indices. The *red dashed vertical lines* show the start and end of r-HuEPO therapy. The *blue dashed vertical line* represents start of iron supplementation.

groups on BPD are provided in **Table VI** (available at www.jpeds.com). After adjusting for sex, gestational age, birth weight, growth rate, and total iron intake, serum ferritin quartile failed to reach significance. Similarly, no significant association was detected between serum ferritin quartile and ROP after adjustments (**Table VII**; available at www.jpeds.com).

Discussion

In our study, despite multiple packed RBC transfusions and standardized high-dose iron supplementation, the majority of ELGANs on r-HuEPO were found to be iron deficient to a degree where the developing brain is placed at risk as indicated by a serum ferritin of <75 ng/mL.[22-24] A parallel increase in Hgb and reticulocyte count indicates that prioritization of supplemented and stored iron towards erythropoiesis was likely responsible.[19,25] Inadequate absorption and/or use of orally supplemented iron also may have contributed as iron stores are better preserved with intravenous iron supplementation than oral iron supplementation during r-HuEPO therapy in preterm infants.[26] A serum ferritin of <75 ng/mL in the neonatal period is associated with abnormal short- and long-term neurodevelopment.[22-24,27] Thus, the beneficial effect of r-HuEPO on hematology may be offset by its potential to cause brain iron deficiency and adverse neurological effects.

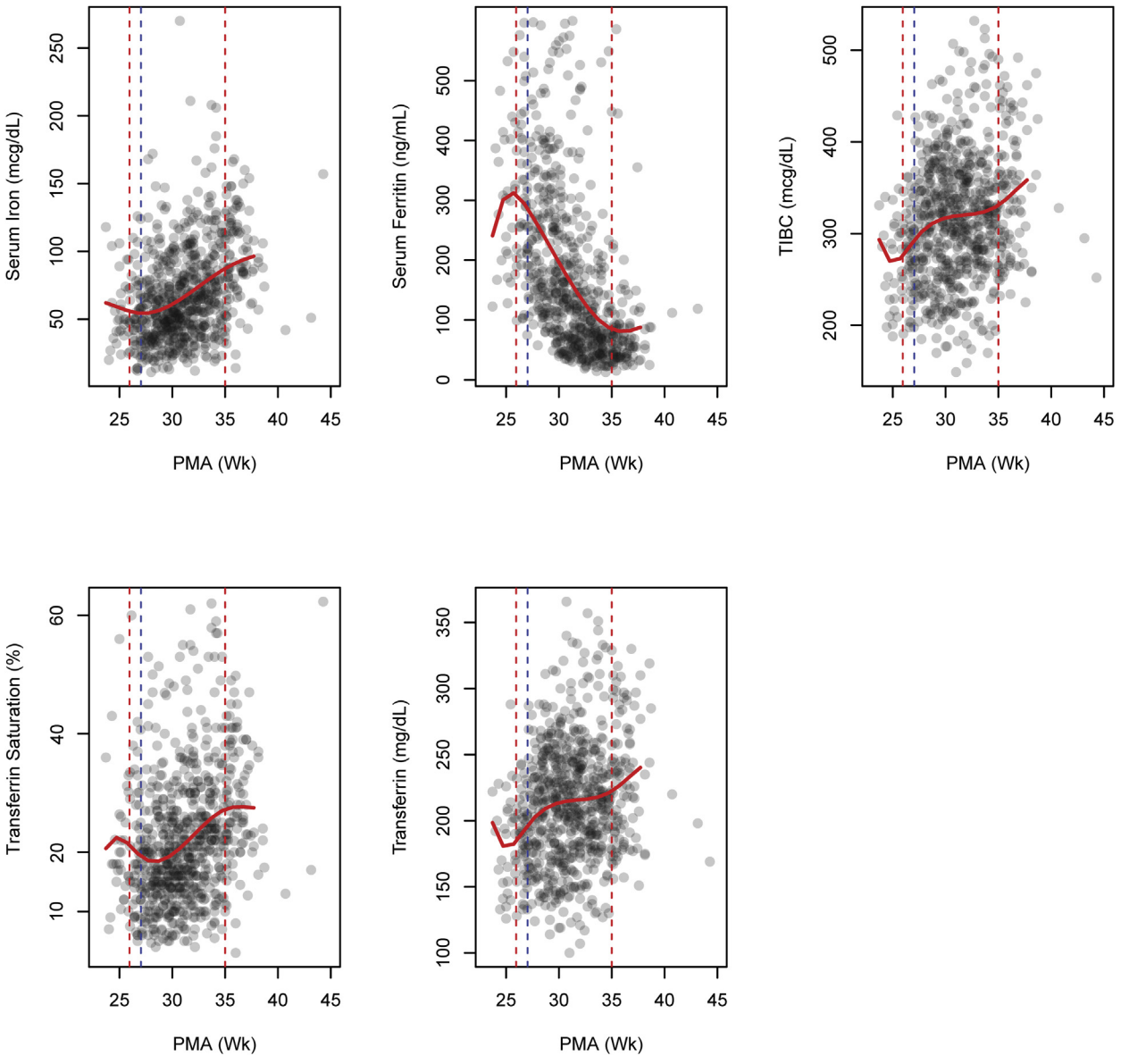

Figure 3. Scatter plot with nonparametric trend line for iron indices. The *red dashed vertical lines* show the start and end of r-HuEPO therapy. The *blue dashed vertical line* represents start of iron supplementation.

The discharge Hct in our study of 34.9 ± 0.4% is comparable with previous studies of ELGANs treated with EPO.[19,28,29] The changes in Hgb, Hct, and reticulocyte count during r-HuEPO treatment in our study are also similar to the study by Ohls et al, where an increase in all 3 hematologic measures was observed.[19] Despite a positive effect on erythropoiesis, we did not see a decrease in the number of packed RBC transfusions. This suggests that transfusions were potentially given for reasons other than anemia (eg, for correcting hypotension). Our unit does not have transfusion guidelines and infants are transfused at the treating physician's discretion. This factor might have contributed to the higher transfusion rate in our cohort. Moreover, most transfusions were given during first week, before r-HuEPO was started; 85% of preterm neonates receive RBC transfusions during their hospital stay.[18,30-32] Our results are consistent with this finding. Likewise, the number of transfusions per infant in our study (3.3 ± 2.7) is comparable with previous studies (range, 2.1-4.3).[8,9,15] However, late transfusion (after 3 weeks) rate in our cohort (40.5%) is lower than the transfusion rates in ELGANs not receiving r-HuEPO therapy.[33] These results suggest that r-HuEPO therapy is effective in decreasing late RBC transfusions in ELGANs. However, without a non-r-HuEPO comparison group, this possibility remains conjectural. The lower rate of late transfusions also could be reflective of an overall clinical improvement by this age, leading to a lesser need for transfusions for nonhematologic reasons.

Table IV. Characteristics by serum ferritin quartiles

Characteristics	Lowest serum ferritin quartile	2 Median serum ferritin quartiles	Highest serum ferritin quartile	*P* value
Covariate	(n = 20)	(n = 40)	(n = 23)	
Male	12 (60.0)	16 (40.0)	13 (56.5)	.249
Gestational age (wk)	25.5 ± 1.4	25.8 ± 1.47	25.9 ± 1.47	.707
Birth weight (g)	748 ± 152.3	792 ± 169.9	798 ± 198.3	.579
Growth rate (g/d)	23.8 ± 3.07	24.4 ± 3.19	24.0 ± 4.6	.82
Total iron intake (mg)	693 ± 266.4	660 ± 253.0	449 ± 267.2*	.003
Total amount of blood (mL)	32.0 ± 33.0	45.5 ± 43.6	79.9 ± 63.8*	.004
Total mL/kg of blood	39.2 ± 35.6	55.3 ± 47.7	61.7 ± 46.5	.257
Hgb (g/dL)	11.9 ± 1.35	12.4 ± 1.68	12.4 ± 1.44	.592
Hct (%)	37.5 ± 3.58	38.9 ± 4.48	39.5 ± 4.78	.363
Serum iron (μg/dL)	91.0 ± 43.2	87.4 ± 42.1	86.0 ± 29.3	.909
TIBC (μg/dL)	353 ± 68.2	346 ± 59.9	295 ± 72.9*	.005
Iron saturation (%)	25.6 ± 11.2	26.4 ± 12.6	29.7 ± 10.6	.466
Transferrin (mg/dL)	237 ± 45.8	232 ± 40.2	198 ± 48.9*	.006
Serum ferritin (ng/mL)	30.4 ± 6.94	61.3 ± 15.3	253 ± 220.3	<.001

Values are mean ± SD for continuous variables, or number (%) for categorical variables, unless otherwise indicated.
Hematologic and iron values at a PMA of 35 ± 1 week.
*$P \leq .01$ compared with the lowest serum ferritin quartile.

Monitoring of the iron status and iron dose adjustment were rigorously adhered to during r-HuEPO treatment. Transferrin saturation was maintained in normal range during r-HuEPO therapy, indicating that we were successful in achieving the a priori goal of therapy. Transferrin saturation in the normal range indicates adequacy of iron for use. Transferrin saturation is an important iron status marker as it indicates current iron availability for use whereas serum ferritin is an indicator of stored iron. In our study, iron status markers indicated that supplemented and stored iron were actively utilized for erythropoiesis, yet 60.2% had evidence of iron deficiency with serum ferritin below 75 ng/mL during r-HuEPO therapy. A serum ferritin of <76 ng/mL in a cord blood sample of infants born at 38.2 ± 2.5 weeks was associated with an increased risk of long-term neurodevelopmental impairments.[22] The r-HuEPO dose used in the current study has been previously shown to lead to decreased serum ferritin, despite high-dose iron (8-16 mg/kg per day) supplementation.[28,29] In a previous study of <32 week preterm infants by Amin et al, a serum ferritin of <75 ng/mL was seen in 23% of the infants at 35 weeks PMA, a much lower value than the 60% in the present study.[14] However, unlike our study, r-HuEPO was not administered to these infants. On the other hand, more infants in that study had serum ferritin of >400 ng/mL than in the present study (19% vs 6%). A serum ferritin of >400 ng/mL is typically considered evidence of iron overload.[14] In the study by Amin et al, among infants who had received >3 transfusions, 50% had serum ferritin of >400 ng/mL.[14] Thus, r-HuEPO administration potentially decreases the risk of excess iron storage in ELGANs by diverting iron towards erythropoiesis.

Previous studies have reported iron supplementation at a dose ranging from 1 to 16 mg/kg per day during r-HuEPO treatment.[28,29,34] Infants in our study were supplemented as high as 12 mg/kg per day of iron, yet 60% had evidence of iron deficiency, suggesting that even higher doses may be necessary while on r-HuEPO therapy. However, a randomized trial demonstrated that oral iron supplementation at a dose of 16 mg/kg per day is not superior to a dose of 8 mg/kg per day for maintaining Hct and serum ferritin during r-HuEPO therapy at 900 U/kg per week (ie, the dose used in our study).[29] Additional studies are necessary to determine the optimal iron dose during r-HuEPO therapy. We did not measure biomarkers of iron toxicity in our study, but iron supplementation at a dose as high as 18 mg/kg per day have been used without evidence of iron toxicity.[35]

Comparison of infants in different quartiles of serum ferritin at the conclusion of r-HuEPO therapy showed that total iron intake, total amount of packed RBC, TIBC, and transferrin were different among the groups, an effect likely mediated by higher packed RBC volume and lower TIBC and transferrin concentration in the highest serum ferritin quartile group. In combination, these results may suggest the possibility of the presence of inflammation in this group. Without other laboratory measures (eg, C-reactive protein), this possibility remains conjectural, however. Moreover, lack of an association between serum ferritin and BPD and ROP, conditions associated with inflammation, also makes this possibility less likely. Of note, oral iron intake was not responsible for this effect because the total iron intake was lower in the highest serum ferritin group compared with the lowest serum ferritin group.

Studies have shown iron deficiency during the fetal and neonatal periods impairs short-term and long-term neurodevelopment with abnormalities in hippocampal-mediated memory function, delayed maturation of auditory brainstem response, and abnormal behavior, despite early recognition and treatment.[36-38] Conversely, some studies have reported an association between high serum ferritin levels and BPD and ROP, although there are no definitive studies confirming causation.[39,40] We also failed to find an association between higher serum ferritin and BPD and ROP in our study. Our data suggest that monitoring for iron deficiency during r-HuEPO treatment is more crucial than monitoring for iron overload. Monitoring serial changes in serum ferritin is useful for this purpose because a low serum ferritin is not seen in any conditions other than iron deficiency. However, serum ferritin could be elevated in certain conditions (eg, owing to inflammation) and cannot be used as a biomarker of iron status in those conditions. Transferrin saturation, a measure of iron bound to transferrin, and TIBC, the capacity of plasma proteins to bind iron, may be better markers of iron deficiency in these situations. A low transferrin saturation and increased TIBC are indicative of iron deficiency.[41,42] Similarly, increased red cell distribution

width and decreased reticulocyte Hgb are early indicators of iron-deficient erythropoiesis.[42,43] A recent study in neonatal rats has demonstrated that low reticulocyte Hgb is also an early biomarker of brain iron deficiency during phlebotomy-induced anemia.[44] A combination of iron markers may be useful for monitoring for iron deficiency and assess the adequacy of iron supplementation for erythropoiesis during r-HuEPO treatment.

This study assessed the effects of r-HuEPO only on hematologic and iron measures. Its effects on neurodevelopment were not determined. Given that iron plays a critical role in brain development, the finding of a lower serum ferritin in r-HuEPO treated infants is of concern. Conversely, prior studies have demonstrated that r-HuEPO treatment at a dose comparable with the one used in our study has beneficial effects on neurodevelopment.[16] Further studies assessing neurodevelopment in these infants would be appropriate. Other limitations of our study include lack of a non-r-HuEPO treated control group and the retrospective design. We were also unable to provide information on acute kidney injury as a clinical outcome in our cohort. Another limitation is that we were unable to compare hematologic and iron measures separately during parenteral and enteral iron administration periods. Finally, our protocol and monitoring strategy may not be generalizable, particularly when r-HuEPO is used for a shorter duration or is administered to infants with documented anemia.

In conclusion, our results demonstrate that r-HuEPO increases the risk of iron deficiency in ELGANs despite a biomarker-based iron dosing strategy. Preventive measures, such as minimizing phlebotomy losses may be beneficial for preventing or decreasing the severity of anemia of prematurity in this group. ■

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Reprint requests: Ashajyothi M. Siddappa, MD, FRCPCH, Department of Pediatrics, Hennepin County Medical Center, 701 Park Ave, Minneapolis, MN 55415. E-mail: jyoth001@umn.edu

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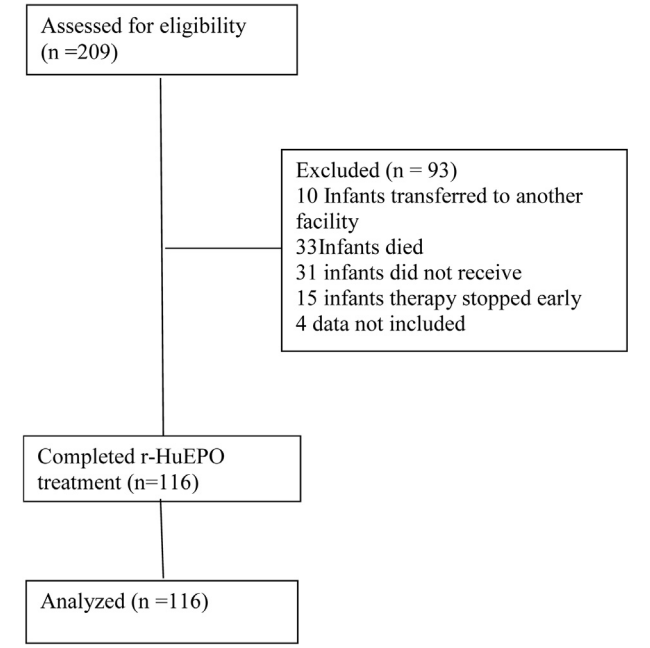

Figure 1. CONSORT flow diagram.

Table II. Hematologic and iron indices before r-HuEPO and at PMA 35 ± 1 week

Indices	N	Before EPO and high iron supplementation	PMA of 35 weeks	Mean change (95% CI)	*P* value
Hematologic indices					
Hgb (g/dL)	104	14.1 ± 0.2	12.1 ± 0.1	−2 (−2.5 to −1.5)	<.001
Hct (%)	104	42.2 ± 0.5	38.4 ± 0.4	−3.7 (−5.1 to −2.4)	<.001
MCV (fL)	104	113.6 ± 0.8	92.7 ± 0.5	−20.8 (−22.3 to −19.3)	<.001
Red cell distribution width (%)	103	16.4 ± 0.2	23.6 ± 0.3	7.1 (6.5 to 7.7)	<.001
Platelet count (10^9/L)	98	208.1 ± 6.3	278.4 ± 11.3	73.2 (50.2 to 96.1)	<.001
Iron indices					
Serum iron (μg/dL)	83	58.1 ± 2.7	87.9 ± 4.3	28.7 (17.0 to 40.4)	<.001
Serum ferritin (ng/mL)	83	283.8 ± 15.0	107 ± 16.1	−161.8 (−202.6 to −121.0)	<.001
TIBC (μg/dL)	83	285.2 ± 4.8	333.2 ± 7.6	48.1 (28.2 to 68.0)	<.001
Transferrin saturation (%)	83	20.7 ± 1.0	27.1 ± 1.3	6 (2.1 to 9.8)	.003
Transferrin (mg/dL)	83	190.8 ± 3.2	223.6 ± 5.1	33.1 (19.8 to 46.4)	<.001

MCV, mean corpuscular volume.
Values are mean ± SE, N at PMA 35 weeks unless otherwise indicated.

Table III. Hematologic and iron indices before r-HuEPO and before discharge

Indices	N	Before EPO and high iron supplementation	Last measurement before discharge	Mean change (95% CI)	*P* value
Hematologic indices					
Hgb (g/dL)	116	14.1 ± 0.2	11.5 ± 0.1	−2.6 (−3.0 to −2.2)	<.001
Hct (%)	116	42.2 ± 0.5	34.9 ± 0.4	−7.2 (−8.4 to −6.0)	<.001
MCV (fL)	116	113.6 ± 0.8	86.8 ± 0.7	−26.9 (−28.6 to −25.1)	<.001
Red cell distribution width (%)	116	16.4 ± 0.2	18.9 ± 0.3	2.4 (1.8 to 3.1)	<.001
Platelet count (10^9/L)	110	208.1 ± 6.3	318.9 ± 9.3	112.7 (92.7 to 132.8)	<.001
Iron indices					
Serum iron (μg/dL)	116	58.1 ± 2.7	95.3 ± 3.0	37.2 (28.8 to 45.5)	<.001
Serum ferritin (ng/mL)	116	283.8 ± 15.0	96.8 ± 10.2	−187 (−221.8 to −152.3)	<.001
TIBC (μg/dL)	116	285.2 ± 4.8	326.1 ± 6.1	41 (25.0 to 56.9)	<.001
Transferrin saturation (%)	116	20.7 ± 1.0	29.9 ± 0.9	9.1 (6.5 to 11.8)	<.001
Transferrin (mg/dL)	116	190.8 ± 3.2	218.8 ± 4.1	28 (17.2 to 38.7)	<.001

MCV, mean corpuscular volume.
Values are mean ± SE, unless otherwise indicated.

Table V. Study sample characteristics by serum ferritin quartiles based on physiologic threshold

Covariates	≤75 SF at 35 PMA (n = 50)	>75 SF at 35 PMA (n = 28)	*P* value
Male	23 (46.0)	15 (53.6)	.685
Gestational age (wk)	25.7 ± 1.43	25.8 ± 1.52	.925
Birth weight (g)	778 ± 160.2	787 ± 208.6	.825
Growth rate (g/d)	24.0 ± 3.12	24.5 ± 4.44	.617
Total iron intake (mg)	654 ± 237.9	602 ± 297.3	.398
Total amount of blood (mL)	40.1 ± 39.6	62.2 ± 50.7	.036
Total mL/kg of blood	52.4 ± 47.9	54.1 ± 40.0	.874
Hgb (g/dL)	12.3 ± 1.6	12.2 ± 1.54	.887
Hct (%)	38.6 ± 4.34	39.2 ± 4.87	.617
Serum iron (μg/dL)	86.3 ± 40.3	90.8 ± 38.1	.635
TIBC (μg/dL)	349 ± 63.2	316 ± 75.9	.047
Iron saturation (%)	25.4 ± 11.6	29.3 ± 12.1	.167
Transferrin (mg/dL)	234 ± 42.5	212 ± 50.9	.047
Serum ferritin (ng/mL)	44.8 ± 14.9	125 ± 57.8	<.001

Values are mean ± SD for continuous variables, or number (%) for categorical variables.
Hematologic and iron values at a PMA of 35 weeks (±1 week).
Five patients with an SF of >400 at 35 PMA were removed from this analysis.

Table VII. Effect of serum ferritin quartile on ROP

Covariates	OR (95% CI)	*P* value
2 Median serum ferritin quartiles (vs lowest quartile)	0.869 (0.484-1.559)	.637
Highest serum ferritin quartile (vs lowest quartile)	0.982 (0.559-1.726)	.95
Male sex (vs female)	1.178 (0.804-1.727)	.401
Gestational age (wk)	0.702 (0.618-0.798)	<.001
Birth weight (g)	0.995 (0.992-0.998)	<.001
Growth rate (g/d)	0.926 (0.83-1.033)	.169
Total iron intake (mg)	0.999 (0.998-1.001)	.288

Table VI. Effect of serum ferritin quartile on risk of BPD

Covariate	df	Deviance of residuals	*P* value
Serum ferritin quartile	2	4.46	.108
Sex	1	0.06	.814
Gestational age (wk)	1	18.09	<.001
Birth weight (g)	1	3.84	.050
Growth rate (g/d)	1	0.29	.592
Total iron intake (mg)	1	0.05	.819