Population pharmacodynamic analysis of erythropoiesis in preterm infants for determining the anemia treatment potential of erythropoietin

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ANEMIA IS A FREQUENT COMPLICATION in very low birth weight (VLBW) premature infants (birth weight <1,500 g) that is usually referred to as anemia of prematurity (14). There are two main causes of anemia of prematurity: insufficient erythropoietin (EPO) production and heavy blood loss resulting from frequent laboratory blood sampling (53). Previous studies have shown that during the first 4 wk of life in preterm infants with a gestational age <28 wk or birth weights <1 kg, approximately twice the volume of red blood cells (RBCs) transfused relative to the volume removed for laboratory blood testing (50). Thus the effect of blood sampling is substantial and must be considered in the management of anemia and in the assessment of erythropoiesis in preterm infants. Anemia of prematurity is also exacerbated by other factors such as shortened RBC life span and the rapid expansion of blood volume with growth (32).

For many years, RBC transfusion was the only effective treatment for severe anemia of prematurity. In 1987, the first clinical trial of EPO demonstrated that EPO treatment was demonstrated to reduce the need for RBC transfusions in adults with end-stage renal disease (18). Subsequently, there has been a series of reports demonstrating variable results regarding the benefit of EPO in the treatment of anemia of prematurity. Several trials demonstrated that EPO treatment resulted in a significant reduction in RBC transfusions (4, 31, 39, 46). However, others reported that the effect of EPO treatment was insufficient in reducing RBC transfusions to be of clinical significance in the treatment of anemia of prematurity (5, 25, 38). These differences may be due to inconsistent transfusion guidelines, treatment protocols, and/or interpatient variability in EPO responsiveness (9). Also, since none of the study designs considered the complex pharmacokinetics/pharmacodynamic (PK/PD) of EPO, this likely resulted in empirical EPO doses and dosing schedules that were suboptimal.

The objective of the present study was threefold: 1) to describe erythropoiesis pharmacodynamics (PD) as a function of plasma EPO concentration using a mechanistic population PK/PD model, 2) to identify covariates that help explain the variability in the response to EPO in preterm infants, and 3) to estimate the capacity to produce additional Hb when stimulated by EPO aimed at reducing RBC transfusions. This study builds on a previous PK/PD study of preterm infants (21). In the previous study (involving 14 subjects), parameters, namely, blood volume and Hb production rate, were scaled to the body weight to account for growth in preterm infants. We observed a wide interindividually variability in the PD parameters in this study group (21). The proposed mechanistic PK/PD model for the present study, which included a larger study population ($n = 27$), was formulated to account for the additional important clinical variables affecting erythropoiesis. These included transfusions, phlebotomies, shortened RBC life span, and blood volume expansion during growth. Explaining the variability in the response to endogenous EPO was accomplished by identifying factors influencing the variability of EPO estimated PD parameters in preterm infants.

Glossary

- $a$: Time between the erythroid progenitor cell stimulation by EPO and the first appearance of Hb in the circulation
- $b$: Time between the erythroid progenitor cell stimulation by EPO and their removal from circulation by senescence
- AIC: Akaike’s information criterion
- BIC: Bayesian information criterion
- CRP: C-reactive protein
- C_{EPO}: Plasma EPO concentration
- EC_{50}: EPO concentration that results in Hb production rate that is 50% of the scaled $E_{max}$

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Subjects

Pregnant women who presented to labor and delivery at <29 wk gestation and infants born at <29 wk gestation and intubated were eligible for enrollment. Infants that presented with hematological disease (except for anemia of prematurity), received RBC transfusion prior to enrollment, or received erythropoiesis stimulating agents were excluded from the study. There were a total of 162 mothers or infants who met the eligibility criteria. Many of those eligible were not approached for the following reasons: 1) they had already been approached for another clinical study with similar eligibility criteria and not approached for this protocol (n = 39); 2) only two research subjects were allowed to be studied at one time due to the significant workload imposed on the clinical laboratory for weighing all clinical samples (n = 62); 3) the infant had received a blood transfusion before consent was obtained (n = 13); and 4) the infant was not approached due to staff availability (n = 5).

A total of 45 families were approached (28%), 11 before delivery and 34 after delivery. Consent was obtained from 33 families (73%), 10 families refused (22%), and 2 infants were transfused before consent was obtained (4%). Six women who were consented antenatally delivered at >29 wk and became ineligible.

Study Procedures

The details of the clinical and laboratory study procedures, including RBC transfusions and laboratory analyses, have been previously described for the first 14 infants. Data included as covariates in the current PK/PD modeling are summarized in Table 1.

Hb Mass Balance Model

The PD model for the effect of EPO on Hb production and disposition is depicted in Fig. 1. The amount of Hb present in the circulation represents a combination of the Hb produced endogenously, Hbendo(t), and that transfused, Hbtran(t):

\[ Hb(t) = Hb_{endo}(t) + Hb_{tran}(t). \]  

The Hb amount present in the systemic circulation was converted to a total number of RBCs, RBC(t), by dividing Hbendo(t) and Hbtran(t) by their corresponding mean corpuscular Hb, MCHendo, and MCHtran, respectively, as follows:

\[ RBC_{total}(t) = \frac{Hb_{endo}(t)}{MCH_{endo}} + \frac{Hb_{tran}(t)}{MCH_{tran}}. \]

The Hb transfused was assumed to have an equal representation of RBCs of all ages up to the life span of transfused RBC (τtrans). It was assumed that blood transfused was from donors whose Hb was produced at a steady-state rate with a fixed RBC life span (7). This assumption is supported by previous survival studies in human adults by which it was demonstrated that the decline in labeled adult autologous and donor transfused RBCs was linear.
(i.e., exhibited constant breakdown rate) (30, 35). The change in the amount of transfused Hb for the ith transfusion, resulting from an amount of Hb transferred at time \( t'_{\text{trans}} \) was described by a zero-order process as displayed in Eq. 3:

\[
\frac{dHb_{\text{tran}}(t)}{dt} = \sum_{i=1}^{m} dHb_{\text{tran}}(t)/dr, \tag{4}
\]

where \( m \) is the number of transfusions.

The model used to describe Hb production assumed that Hb production was stimulated by EPO through a stimulation function, \( f_{\text{stim}} \) (Eq. 5). To account for infant growth, stimulation function was scaled to the body weight to the power 3/4 (2). Before birth \((t < 0)\), the stimulation function was assumed to constant, \( k_{\text{prod \_in utero}} \), scaled to the body weight. Stimulation function after \((t \geq 0)\) was related to plasma EPO concentrations by an \( E_{\text{max}} \) model, which resulted in the following Hb production stimulation function:

\[
f_{\text{stim}}(t) = \begin{cases} \frac{k_{\text{prod \_in utero}}}{E_{\text{max}} + C_{\text{EPO}}} \cdot \left(\frac{w(t)}{w_0}\right)^{3/4} & \text{if } t \leq 0 \\ \frac{k_{\text{prod \_in utero}}}{E_{\text{max}} + C_{\text{EPO}}} \cdot \left(\frac{w(t)}{w_0}\right)^{3/4} & \text{if } t > 0 \end{cases} \tag{5}
\]

where \( k_{\text{prod \_in utero}} \) is the Hb production rate constant before birth, \( w(t) \) is the body weight, \( E_{\text{max}} \) is the maximum Hb production rate, which is scaled to the body weight by a 3/4 power function, \( E_{\text{50}} \) is the EPO concentration that results in Hb production rate that is 50% of the scaled \( E_{\text{max}} \), and \( C_{\text{EPO}} \) is plasma EPO concentration.

The Hb production model describes that in the absence of phlebotomy, produced RBCs are removed only by senescence according to a fixed life span (28). Accordingly, the change in the amount of Hb produced endogenously is described by

\[
\frac{dHb_{\text{endo}}(t)}{dt} = f_{\text{stim}}(t - \alpha) - f_{\text{stim}}(t - b) \tag{6}
\]

with the initial condition \( Hb_{\text{endo}}(0) = Hb_0 \cdot V_{\text{total}}(0) \), where \( \alpha \) is the time between the erythroid progenitor cell stimulation by EPO and the first appearance of Hb in the circulation, and \( b \) is the time between the erythroid progenitor cell stimulation by EPO and their removal from circulation by senescence. The RBC life span is the difference \( b - \alpha \). The Hb parameter represents baseline Hb concentration at birth, and \( V_{\text{total}}(0) \) is the blood volume at birth.

The parameter for Hb production before birth \( (k_{\text{prod \_in utero}}) \) was estimated by solving Eq. 7:

\[
Hb_0 \cdot V_{\text{total}}(0) = \int_{t \leq 0} k_{\text{prod \_in utero}} \cdot \left(\frac{w(t)}{w_0}\right)^{3/4} \cdot dt, \tag{7}
\]

where the only unknown \( k_{\text{prod \_in utero}} \) and the values of other parameters are the individual parameter estimates. According to the mass balance principle, the change in the total amount of Hb in the circulation is the summation of the change in Hb transfused and the increase in Hb produced endogenously:

\[
\frac{dHb(t)}{dt} = \frac{dHb_{\text{endo}}(t)}{dt} + \frac{dHb_{\text{tran}}(t)}{dt}. \tag{8}
\]

The EPO plasma concentrations were nonparametrically represented using a linear spline function. The infant postbirth body weight was represented by a fourth-order polynomial fit to the observed body weight data to interpolate between body weight observations and provide a smooth function of total blood volume. A fourth-order polynomial derived using MATLAB (version 7.8.0) was used because it captures the daily change in weight (Eq. 9). To account for the in utero growth in calculating Hb production rate when \( t \leq 0 \), a power function was fitted to mean body weight from 22 to 32 wk of gestation (3):

\[
w(t) = w_0 \cdot \left(\frac{t + GA}{GA}\right)^{3.45} \quad \text{for } t < 0
\]

\[
\alpha_0 + \alpha_1 \cdot t + \alpha_2 \cdot t^2 + \alpha_3 \cdot t^3 + \alpha_4 \cdot t^4 \quad \text{for } t \geq 0
\]

where GA is the gestational age, and \( t \) is the time relative to the time of birth, where \( \alpha_0, \alpha_1, \alpha_2, \alpha_3, \) and \( \alpha_4 \) are constants.

Correction for Hb phlebotomy loss. The above derivation does not take into account Hb removed from circulation by phlebotomy. We accounted for the Hb removed from circulation by phlebotomy as previously described (21, 45). The details of correction for phlebotomy are described in APPENDIX A.

Finally, the estimated amounts of Hb in the circulation were converted into the observed concentrations by dividing by the estimated total blood volume, \( V_{\text{total}}(t) \). To account for blood volume expansion as a result of growth, total blood volume was adjusted in a direct linear fashion with body weight:

\[
V_{\text{total}}(t) = w(t) \cdot V \tag{10}
\]

where \( V \) is the body weight normalized blood volume.

Covariate analysis. To assess whether covariates influenced the pharmacodynamics of EPO, the parameter-covariate relationship was explored. First, the model described in Eqs. 1–10 was fitted to the data without including a parameter-covariate relationship. This step was conducted using a nonlinear mixed-effects modeling approach. Second, the relationship between the estimated individual parameters (estimated in the previous step) and covariate (Table 1) was modeled using multiple linear regression with stepwise addition (52). Third, covariates identified in the multiple linear regression as being statistically significant \((P < 0.05)\) were included in the mixed-effects model using a stepwise addition procedure. Finally, covariates that resulted in a drop in minus two times the log-likelihood value \((-2LL)\)
of more than 3.84 were included in the final model to describe the parameter-covariate relationship. The $-2\Delta L$ is approximately $\chi^2$ distributed. A difference in $-2\Delta L > 3.84$ is significant at the 5% level (1 degree of freedom) if nonlinearity and heteroscedasticity in the model are accounted for (51). The third and fourth steps are two steps utilizing a single approach. In the third step, the concentration-time data were not considered in evaluating the significance of covariates. In the fourth step, covariates and concentration-time data were considered in evaluating the impact of incorporating covariates on both decreasing the interindividual variability observed with the estimated parameters and the ability of the model to describe the concentration-time relationship.

**Interindividual and Residual Error Model**

A log-normal distribution was used to describe parameters distribution:

$$\ln(P_i) = \ln(P) + \eta_i$$

where $P_i$ denotes the $i$th individual’s parameter value and is a function of $P$, the population value of the parameter in preterm infants, and $\eta_i$ is the individual random effect (a zero-mean random variable with the variance $\omega^2$), which accounts for the difference between the population parameter value and the individual value.

A proportional error model was used to describe the residual error of the data. The residual error was estimated for Hb and RBCs separately. Correlations between parameters were tested on the basis of graphical inspection of correlation in the empirical Bayesian estimates.

**Data Analysis and Model Evaluation**

Modeling was performed using the stochastic approximation expectation maximization algorithm (SAEM) for nonlinear mixed-effects models. The SAEM algorithm is implemented in the MATLAB language in the software MONOLIX (version 3.1R2 for Windows; http://software.monolix.org/sdoms/software/) with MATLAB (version 7.8.0). To improve speed of the algorithm, a numerical solver for ordinary differential equations, written in C, was interfaced to MATLAB and employed. The interface was implemented in Sundial’s TB MATLAB toolbox (Sundials version 2.4.0; https://computation.llnl.gov/casc/sundials/download/download.html). Model selection was based on standard errors, Bayesian information criterion (BIC), and graphic assessment from MONOLIX output (43). Standard error estimates were obtained by linearization of the Fisher information matrix of the nonlinear mixed-effects model (6).

The of the seven model-estimated parameters, i.e., namely, $V$, $MCH_{end}$, $P_{51}$, $\text{EC}_{50}$, $\alpha$, and $\text{EC}_{50}$, four were constants based on the following literature values: $\tau_{\text{norm}}$ (70.8 days), $F_T$ (0.875), $a$ (0.448 days), and $b$ (42.9 days).

**RESULTS**

A rich data collection design was employed providing a database composed of 27 patients contributing a total of 2,554 Hb concentrations, 568 RBC counts, and 1,510 EPO concentrations. The subject clinical and laboratory demographic characteristics are summarized in Table 2.

The Hb mass balance model fit along with plasma EPO concentration and body weight for three representative subjects are shown in Fig. 2. The model captures the general behavior of the Hb and RBC concentration data. The pharmacodynamic model parameters are summarized in Table 3.

The life span of transfused RBCs (70.8 days) was taken as the average of two different techniques, namely, biotin labeling (mean estimated life span = 85.2 days) (6) and the decline in fetal Hb percentage (mean estimated life span = 56.4 days) (47). The life span of endogenously produced RBCs was also fixed to 42.5 days on the basis of previous mean estimates using $^{51}$Cr RBC labeling (8). In addition, the time between Hb production stimulation by EPO and appearance of produced Hb in the circulation was set equal to 0.448 days on the basis of previous estimates (42).

Covariate screening process identified GA to be an important covariate affecting the value of $E_{\text{max}}$. The relation between GA and $E_{\text{max}}$ was described by Eq. 12. The values of gestational age were normalized by geometric mean, namely, $\text{GA}_{\text{mean}}$, for infants enrolled in the present study ($\text{GA}_{\text{mean}}$ = 26.7 wk). $E_{\text{max}}$ is the population parameter value with a gestational age equal to GA, $E_{\text{max}}^0$, is the population parameter value for infants with a gestational age of $\text{GA}_{\text{mean}}$. The value of $\beta$ of 10.2 with a standard error of 3.3 indicates a positive significant relationship between $E_{\text{max}}$ and GA ($P < 0.01$).

$$E_{\text{max}} = E_{\text{max}}^0 \cdot \left( \frac{\text{GA}}{\text{GA}_{\text{mean}}} \right)^\beta$$  \hspace{1cm} (12)

To test the influence of gestational age on the clinical outcomes of anemia of prematurity, the value of gestational age was plotted against the amount of Hb transfused over the first month of life. A negative correlation between gestational age and amount of Hb transfused was observed (Fig. 3). A mechanistic explanation for this observation is presented in the DISCUSSION.

The plots of the observed vs. population-predicted concentrations as well as the observed vs. individual-predicted concentrations showed a good visual agreement between predicted and observed data (Fig. 4).

**DISCUSSION**

In this study, erythropoiesis dynamics in anemic preterm infants was described using a mechanistic population PD model based on the physiological role of EPO as the primary factor stimulating erythropoiesis. Covariate analysis suggested that erythropoietic efficacy of EPO was increased for preterm infants with greater gestational ages. EPO was incorporated in the model as the main predictor of Hb production rate. Hb production rate and estimated blood volume were scaled to body weight as it changed within our preterm infant study population. Allometric scaling in the present study was based on standard approaches described in the literature (2). We also accounted for other important factors influencing the clinical outcomes of anemia of prematurity.
variables, including Hb gain and loss via transfusions and phlebotomy, respectively.

During human growth, several changes in the regulation of the erythropoietic system take place. For example, there is a switch in the major site of EPO production from the liver (fetus) to the kidney (adult) (19, 20). In addition, during early development there is a switch in organs producing erythrocytes, i.e., from the embryonic yolk sac to the fetal liver, and finally to the bone marrow (16). RBCs arising from the yolk sac are returned to “primitive” RBCs. In humans, blood islands appearing in the yolk sac membrane at about 16 days postconception give rise to primitive RBCs, which are EPO independent (16, 48). Yolk sac erythropoiesis begins to regress by the 10th week of gestation and ceases by the 16th week. Primitive erythroblasts are characterized by a large size with an estimated mean corpuscular volume of 250 fl/cell. Primitive erythroblasts have a shortened life span compared with fetal and adult RBCs (40).

The liver serves as the dominant site of red cell production from the 9th to the 24th week of gestation. These fetal liver-derived definitive erythroblasts are smaller than yolk sac-derived primitive megaloblasts and contain one-third the amount of hemoglobin (29). Hepatic erythroid progenitors can differentiate in vitro with EPO alone, in contrast to bone marrow-derived BFU-Es (burst forming unit-erythroblasts), which require EPO plus IL-3 (15, 16). Bone marrow erythropoiesis begins around the 13th week of gestation to become, and remain, the predominant erythropoietic organ by the fetal 24th week of gestation (29).

Two observations demonstrate the importance of gestational age in the treatment of anemia of prematurity. First, the erythropoietic efficacy of EPO was proportional to the gesta-

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Table 3. Final parameter estimates of the erythropoiesis pharmacodynamics model in preterm infants

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population Parameter</th>
<th>%RSE</th>
<th>(\omega^2)</th>
<th>%RSE</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(V), ml/kg</td>
<td>0.91</td>
<td>6</td>
<td>0.30</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>MCHendo, pg/cell</td>
<td>35.7</td>
<td>2</td>
<td>0.07</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>MCHtran, pg/cell</td>
<td>27.0</td>
<td>2</td>
<td>0.08</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Hb(_0)</td>
<td>13.6</td>
<td>3</td>
<td>0.14</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>(E^{\max}), (g·day(^{-1})·kg(^{-1}))(^{3/4})</td>
<td>0.43</td>
<td>21</td>
<td>0.73</td>
<td>15</td>
<td>0.0018</td>
</tr>
<tr>
<td>(\beta)</td>
<td>10.2</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC50, mU/ml</td>
<td>35.9</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual error, Hb</td>
<td>0.09</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual error, RBC</td>
<td>0.09</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary parameters</td>
<td>Mean</td>
<td>SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\alpha(_{\text{prod}})), (g·day(^{-1})·kg(^{-1}))(^{3/4})</td>
<td>0.40</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Secondary parameters are parameters that were not directly estimated. Values of secondary parameters were calculated from directly estimated parameters. See Glossary and text for definition of terms.
ional age. Second, the amount of Hb transfused over the first postnatal 30 days was inversely related to the gestational age (Fig. 3). Both observations can be attributed to the fact that in this patient population, RBCs are produced in two organs, namely, the liver and bone marrow. As noted above, the liver is the dominant site of RBC production from the 9th to the 24th weeks of gestation. Bone marrow erythropoiesis begins around the 13th week of gestation to become the predominant erythropoietic organ after the 24th week of gestation and remains so throughout the remainder of fetal life (29). Taking the above findings together, bone marrow may have more potential than the liver for producing RBCs. Compared with preterm infants with earlier gestational ages, infants with large gestational age have a larger fraction of RBCs produced by the bone marrow, which may result in more total potential for producing RBCs.

Physiological parameters are expected to be similar to the values reported in the literature. Accordingly, previous blood volume estimates determined using $^{131}$I-labeled albumin over the first 2 wk of life in premature infants was found to be 95 ml/kg compared with 91 ml/kg estimated in the present study (49). The MCH$_{endo}$ parameter (Table 3) estimated by this study were also comparable to previous literature values of 27–41 pg (33). As expected, the MCH$_{tran}$ estimates were lower than MCH$_{endo}$ because of the larger size of erythrocytes produced by preterm infants. The estimate of MCH$_{tran}$ (Table 3) was also within the reference range of 26–32 pg (34).

The degree of RBC removal from the circulation immediately after birth due to RBC damage was estimated previously to be 2–3% per day of the Hb amount present in the circulation (22). This corresponds to a Hb destruction rate of 0.221–0.331 g/day at the time of birth with the use of our population estimates of blood volume and Hb baseline values and assuming a VLBW infant average birth weight of 0.891 kg. Scaling this birth weight to the power 3/4 transforms the published values to 0.240–0.361 (g·day$^{-1}$·kg$^{-1}$)$^{3/4}$, which agrees with the value for $k_{prod}$ of 0.40 (g·day$^{-1}$·kg$^{-1}$)$^{3/4}$ determined by our population model-based analysis.

It is acknowledged that in addition to gestational age, other markers may be important predictors of response to recombinant human EPO (rhEPO). However, since this analysis was done retrospectively, we were not able to examine all plausible predictors of rhEPO responsiveness. There are many conditions reported as being associated with rhEPO resistance, namely, inflammation, oxidative stress, and iron deficiency (24, 41). C-reactive protein (CRP), IL-6, soluble IL-2, and elastase plasma levels had been described as inflammatory markers for rhEPO responsiveness in hemodialysis patients (11, 13). Iron deficiency markers include mean cell hemoglobin, red cell distribution width, and soluble transferrin receptor (s-TfR) serum levels (12). Similar to inflammatory markers,

![Fig. 3. Amount of Hb transfused over the first month of life vs. gestational age for the 27 study subjects.](image)

![Fig. 4. Observed Hb concentrations and RBC counts (top and bottom, respectively) vs. population-predicted and individual-predicted concentrations of Hb and RBC (left and right, respectively).](image)
differences in the level iron deficiency markers between rhEPO responders and nonresponders has been observed in hemodialysis patients. The investigation of these markers may help in identifying additional factors that contribute to the erythropoiesis process in preterm infants.

There are several limitations of the present analysis. One is that the literature estimates used for the average life spans of endogenously produced RBCs and exogenously transfused RBCs are accurate. To obtain direct determinations of RBC survival for both of these RBC populations, different study procedures are needed, i.e., those in which both types of RBCs are labeled and followed sequentially. The measurement of RBC life span is beyond the scope of the present study.

Another limitation is the unverified assumption that Hb production rate correlates with body weight to the 3/4 power. Although it might be desirable to investigate other possibilities, it is impractical and not feasible to investigate every possibility given the long computer time required. Nonetheless, it is noteworthy that the overall performance of the presented model was examined by comparing several of the estimated values reported in the literature. Specifically, the values reported for blood volume, MCHendo, MCHtr, and \( k_{\text{in utero}}^{\text{prod}} \) were used as estimates in the present analysis. This provides some assurance that the present model is physiologically relevant to the preterm infants studied.

A third limitation of the present analysis is it does not specifically model the effect on EPO production of local tissue oxygenation or clinically observed saturation values (1, 17, 37). That would have enabled an analysis of the relationship between oxygen saturation and EPO production. However, such an analysis is beyond the scope of the present analysis. Instead, our analysis focuses on the resulting EPO levels and how that variable influences the erythropoiesis. This is done by representing the EPO levels as a linear spline function that is used as a pharmacodynamic forcing function in the model. In the present analysis, transfused RBCs were assumed to have a constant rate of senescence. This assumption is supported by previous studies reporting a linear decline in RBC survival in adult humans (30, 35). Another possible factor that was not considered in the present analysis is storage time. However, Luten et al. (30) compared the survival of RBCs stored for a relatively brief period of 0–10 days with the survival of RBCs stored for the longer duration of 25–35 days. They concluded that there was no significant difference in the long-term survival between the two RBC groups. This same conclusion was reached by Moroff et al. (36) in human adults following gamma irradiation.

In addition to anemia of prematurity, several types of anemia are associated with a high variability in the response to rhEPO, including: anemia due to chronic kidney disease and anemia associated with cancer treatment (10, 23). The high variability in response to rhEPO contributes to the controversy in the efficacy of rhEPO in the management of anemia of prematurity.

In the present model EPO concentration responses are represented as forcing functions in the PD transductions through the use of a fixed linear spline function for each subject. Accordingly, the model indirectly accounts for a possible negative feedback control of production of EPO by the circulating Hb mass, a mechanism that has been suggested previously (1, 37).

The present study involves 14 infants who were included in our previous study (21). The current study expands the number of infants to 27 and applies a mixed-effects modeling approach. In addition, the effect of different covariates on the erythropoiesis dynamics was also explored. The nonlinear mixed-effects modeling approach makes use of data across subjects while simultaneously including all relevant data in the analysis. Therefore, nonlinear mixed-effects modeling improves the power to identify an appropriate structural model and provide more accurate parameter estimates (27). In our previous work (21), in some subjects we were unable to detect nonlinearity in Hb production rate function (“PD transduction function”) using an individual fitting approach. Accordingly, a linear Hb production rate function (Eq. 13) was previously applied in such infants, based on Akaike’s information criterion (AIC) (21). As a result, the individual (non-population-based) approach to modeling the data may result in simplistic models that exclude important mechanistic components. This is because a single subject’s data, in contrast to all data considered in a combined way in the population analysis approach, may not contain sufficient information to identify important structural components of the true model. For example, the range of EPO levels observed in some of the subjects was in the linear range of the PD transduction function (the \( E_{\text{max}} \) model) and thus did not allow nonlinearity in the PD to be properly determined. This problem is avoided in the present mixed-effects modeling approach, thereby improving the derived parameter estimates. Based on AIC, the nonlinear Hb production rate function (Eq. 5) was preferred over a linear approximation (Eq. 13).

\[
f_{\text{stim}}(t) = \begin{cases} k_{\text{in utero}}^{\text{prod}} \cdot \left[ w(t) \right]^{3/4} & \text{if } t \leq 0 \\ \alpha \cdot C_{\text{EPO}} \cdot \left[ w(t) \right]^{3/4} & \text{if } t > 0 \end{cases}
\]

Several technical difficulties were encountered in the process of implementing the current model in a population framework using the MONOLIX software. First, it was challenging to interface user-written software to permit implementation of the complex phlebotomy correction and the use of linear spline and polynomials for nonparametric function representations. To resolve this challenge, we implemented the model in MONOLIX, which allows a MATLAB scripting interface. Doing so, however, resulted in a very long run time (i.e., 7–8 days). This problem was overcome by using an ordinary differential equation solver written in C and interfaced to MONOLIX via MATLAB. This decreased the run time by 75% (~2 days).

**Clinical Implications of the Analysis Results on Treatment of Anemia**

Results of the present analysis indicate that preterm infants produced RBCs at a rate that was 38.3% (range 27.2–57.0%) of the maximum production rate during the first month of life. This percentage was calculated using the average EPO concentration over the first month of life (mean of 22.3 mU/ml with a range of 13.4–47.54 mU/ml) and the population parameter value of \( EC_{50} \) of 35.9 mU/ml (Table 3). This indicates that preterm infants have the potential to produce additional RBCs that could decrease the need for RBC transfusion.

The difference in the gestational age (24–29 wk) showed an approximately sevenfold difference in the value of \( E_{\text{max}} \). This
conclusion was based on transforming the range of gestational age to a range of $E_{\text{max}}$ values based on the mathematical relationship described in Eq. 12. Using the estimated value of $E_{\text{max}}$ of 0.43 (g·day$^{-1}$·kg$^{-1}$)$^{3/4}$, based on the results from Eq. 12, the population value of $E_{\text{max}}$ for preterm infants with a gestational age of 24 wk was 0.147 (g·day$^{-1}$·kg$^{-1}$)$^{3/4}$. Similarly, the population value $E_{\text{max}}$ for preterm infants with a gestational age of 29 wk was 1.01 (g·day$^{-1}$·kg$^{-1}$)$^{3/4}$. This result indicates that gestational age needs to be considered when determining the dose of rhEPO to be given.

The amount of Hb removed for clinical sampling was only 48% of the total amount of Hb transfused. This finding is in agreement with other reports from the literature (49). The present PD model is highly relevant in that it encompasses phlebotomy loss as a key model component. Thus the present PD model permits the exploration of therapeutic approaches in which future decreases in phlebotomy loss (e.g., as a result of advances in noninvasive and nanotechnology laboratory testing) can be included in simulation studies focused on preventative measures to reduce the severity of anemia of prematurity.

**Perspectives and Significance**

The present PK/PD model has several important implications. First, the approach and analysis tools developed and applied can be used as an analysis framework for investigating the erythropoietic treatment potential of the expanding spectrum of new erythropoiesis stimulating agents. Second, the model can, through clinical trial simulations, be used to determine an optimized EPO dosing schedule and to determine if individualized EPO dosing targeted to specific, covariate-identified subsets of VLBW infants may result in elimination of RBC transfusions. This feature facilitates selection of appropriate study designs to answer the question whether rhEPO is effective in reducing, and possibly eliminating the need for, RBC transfusions. Finally, the analysis approach proposed has the utility for exploring additional covariates that were observed in this study that may influence the erythropoietic treatment potential of the expanding spectrum of new erythropoiesis stimulating agents. Second, the present PD model permits the exploration of therapeutic approaches in which future decreases in phlebotomy loss (e.g., as a result of advances in noninvasive and nanotechnology laboratory testing) can be included in simulation studies focused on preventative measures to reduce the severity of anemia of prematurity.

**APPENDIX A: PHLEBOTOMY CORRECTION**

The phlebotomy correction term, $PC(t_{\text{start}}, t_{\text{end}})$, involves a start time, $t_{\text{start}}$, and an end time, $t_{\text{end}}$. The time interval over which phlebotomy loss affects the Hb mass balance depends on the life span of RBCs, including both transfused and endogenously produced RBCs. Assuming $m$ phlebotomies occurred between $t_{\text{start}}$ and $t_{\text{end}}$, the fraction of Hb remaining in circulation after the $i$th phlebotomy, denoted $F_p(i)$, occurring at time $t_p(i)$, is given by Eq. A1:

$$F_p(i) = 1 - \frac{A_p(t_p(i))}{Hb(t_p(i))}, \quad (A1)$$

where $A_p(t_p(i))$ is the amount phlebotomized at $t_p(i)$. $PC(t_{\text{start}}, t_{\text{end}}) = \prod_{i=1}^{m} F_p(i), \quad (A2)$

where $t_p(1) \geq t_{\text{start}}$ and $t_p(m) < t_{\text{end}}$. $F_p(i)$ is the fraction of Hb remaining immediately after the $i$th phlebotomy relative to the amount present immediately after the $i$th phlebotomy. The phlebotomy correction term over the time interval $t_{\text{start}}$ to $t_{\text{end}}$ [i.e., $PC(t_{\text{start}}, t_{\text{end}})$] is the multiplication of fractions remaining for all phlebotomies that occurred between $t_{\text{start}}$ and $t_{\text{end}}$ (Eq. A2). To correct for the amount of transfused Hb removed during subsequent phlebotomies, the phlebotomy correction was applied for the phlebotomies that occurred between the time of the previous transfusion, $t_{\text{trans}}$, and the current time, $t$. Phlebotomy correction for Hb produced was applied for the phlebotomies that occurred over the life span of produced RBCs. The following equations summarize the mathematical presentation of phlebotomy correction:

$$\frac{dHb_{\text{tran}}(t)}{dt} = \sum_{i=1}^{m} \frac{dHb_{\text{tran}}(t)}{dt}$$

and

$$dHb_{\text{end}}(t)/dt = f_{\text{prod}}(t-a) - PC(t - \tau_{\text{trans}}, t) \cdot f_{\text{prod}}(t-b), \quad (A4)$$

where $PC(t_{\text{trans}}, t)$ is phlebotomy correction between the time of the $i$th transfusion ($t_{\text{trans}}$) and the current time ($t$), and $PC(t - \tau_{\text{trans}}, t)$ is phlebotomy correction between $t - \tau_{\text{trans}}$ and $t$.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

M.I.S., D.N., and J.A.W. performed experiments; M.I.S., D.N., J.A.W., and P.V.-P. analyzed data; M.I.S., D.N., J.A.W., and P.V.-P. interpreted results of experiments; M.I.S., D.N., J.A.W., and P.V.-P. prepared figures; M.I.S., D.N., J.A.W., and P.V.-P. drafted manuscript; M.I.S., D.N., J.A.W., and P.V.-P. edited and revised manuscript; M.I.S., D.N., J.A.W., and P.V.-P. approved final version of manuscript; J.A.W. and P.V.-P. conception and design of research.

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