Mathematical model of platelet turnover in thrombocytopenic and nonthrombocytopenic preterm neonates

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NEONATAL THROMBOCYTOPENIA is a hematological disorder, and ~22–35% of all neonates admitted to the neonatal intensive care unit suffer from this condition (22, 27). Premature very low birth weight (VLBW, ≤0.67 kg) neonates are more often affected, as the incidence of thrombocytopenia is inversely proportional to gestational age. Traditionally, the definition of thrombocytopenia in neonates of all gestational ages is the same as in adults, namely a platelet (PLT) count of <100,000/mm³ based on fetuses in the second trimester having PLT counts within the normal adult range (13).

The underlying general mechanisms responsible for neonatal thrombocytopenia are similar to adults, namely, increased PLT destruction, decreased PLT production, PLT sequestration, or a combination of these processes (27). However, it is more difficult to identify causes of neonatal thrombocytopenia as the diagnosis can be challenging owing to the dynamic nature of newborn development and technical difficulties associated with performing certain diagnostic tests in neonates, such as bone marrow biopsy and/or radiolabeled PLT kinetic studies. Furthermore, for both ethical and practical reasons, clinicians are limited by less frequent blood sampling (due to the very small blood volume or preterm infants) and as a result must also rely on indirect measurements of PLT production and consumption, such as the immature PLT fraction (IPF), a measure of newly produced PLTs in the blood (27).

Currently, the only viable treatment available for neonatal thrombocytopenia is PLT transfusion. Consequently, a majority of neonates affected by thrombocytopenia receive prophylactic PLT transfusions (22, 27). This practice of frequent PLT transfusions puts neonates at risk of experiencing transfusion-related complications, including viral or bacterial infections, transfusion-related lung injury, circulatory overload, or immune-mediated transfusion reactions (30). Despite the common practice of PLT transfusion therapy in sick neonates, there is no standard-of-care consensus on PLT count thresholds for initiating therapy (18).

Thus the purpose of this study is to improve the understanding of progression of neonatal thrombocytopenia during the first 4 wk of life, with the primary objectives of 1) developing a mathematical model that characterizes IPF and PLT kinetics in preterm VLBW neonates, 2) evaluating covariates influencing model parameters regulating PLT kinetics, and 3) determining the role of phlebotomy and growth in influencing PLT kinetics.

MATERIALS AND METHODS

Study subjects. Data were obtained from a clinical study in the neonatal intensive care unit at the University of Iowa Children’s Hospital (Iowa City, IA). Study procedures have been previously described (14). The present study is a secondary data analysis conducted using serial IPF and PLT counts from EDTA whole blood samples obtained from VLBW preterm neonates during the first 4 wk of life. IPF and PLT measurements were performed on a Sysmex XE-2100 instrument, which operates in a linear range from 0–5.0 × 10⁹ PLT/μL, and coefficients of variation were less than 15%. Study inclusion criteria included treatment with expectation of survival and moderate-to-severe respiratory distress requiring ventilation. Exclusion criteria included known hematological conditions (except for anemia associated with phlebotomy blood loss and prematurity), alloimmune hemolytic anemia, diffuse intravascular coagulation, and subjects receiving erythropoiesis-stimulating agents. The study was...
PLT dynamic model. Transit compartment models have been used to describe the time course of PLT in adults (17) to emulate distributions. The first transit compartment for IPF is described by the equation:

$$\frac{dIPF_1}{dt} = \text{Input} - \frac{n_{IPF}}{\tau_{IPF}} \cdot IPF_1 - \frac{D_{IPF}}{n_{IPF}} \cdot \delta_{PHL}(t)$$

with IPF, as the cell count in the first compartment, \(\tau_{IPF}\) represents the mean transit time for IPF, and \(n_{IPF}\) is the number of IPF transit compartments in the model. The \(\delta_{PHL}(t)\) represents cell loss due to phlebotomy. The amounts of IPF lost to phlebotomy (\(D_{PHL}\)) were calculated as the product of the volume of blood removed and the IPF concentration.

The process of thrombopoiesis (Input) is described by the empirical function:

$$\text{Input} = R_0 + \alpha \cdot k \cdot t \cdot e^{-k \cdot t}$$

with \(R_0\) as a basal zero-order production rate constant, \(\alpha\) is the magnitude of the empirical function, and \(k\) is a first-order rate constant controlling the rise and fall of PLT production. Subsequent transit compartments can be defined by the general mass balance differential equation:

$$\frac{dIPF_i}{dt} = \frac{n_{IPF}}{\tau_{IPF}} \cdot (IPF_{i-1} - IPF_i) - \frac{D_{IPF}}{n_{IPF}} \cdot \delta_{PHL}(t)$$

with \(i\) representing individual IPF compartment numbers. The rate of change of the first transit compartment for PLT is described by the equation:

$$\frac{dPLT_1}{dt} = \frac{n_{PLT}}{\tau_{PLT}} \cdot (PLT_{i-1} - PLT_1) + \frac{D_{PLT}}{n_{PLT}} \cdot \delta_{TF}(t)$$

with PLT_1 as cell count in the first compartment, \(\tau_{PLT}\) is the mean transit time for PLT, and \(n_{PLT}\) is the number of PLT transit compartments. The \(\delta_{TF}(t)\) represents transfusion gains, and the amount of PLT lost to phlebotomy (\(D_{PLT}\)) was calculated in the same manner as for IPF. The rate of change of additional transit compartments for PLT are thus defined by the general equation:

$$\frac{dPLT_i}{dt} = \frac{n_{PLT}}{\tau_{PLT}} \cdot (PLT_{i-1} - PLT_i) + \frac{D_{PLT}}{n_{PLT}} \cdot \delta_{TF}(t) - \frac{D_{PLT}}{n_{PLT}} \cdot \delta_{PHL}(t)$$

IPF and PLT concentrations were set as being equal to the sum of the respective compartments divided by the apparent volume of distribution, \(V_{\text{total}}(t)\).

The initial conditions for IPF and PLT compartments were fixed to the observed values on day 0 divided by the number of respective transit compartments and corrected for \(V_{\text{total}}(t)\). The total distributional blood volume, \(V_{\text{total}}(t)\), was assumed to be proportional to body weight and was calculated as (14):

$$V_{\text{total}}(t) = m(t) \cdot V_N$$

with \(V_N\) as the body weight normalized blood volume (97.7 ml/kg) and \(m(t)\) as body weight that was included in the data set as a time-dependent covariate. For subjects receiving PLT transfusions, the fixed distribution volume (Eq. 6) allowed for the estimation of the recovered PLT “dose” from the transfusion or \(D_{TF}\).

Data analysis. The transit compartment models were simultaneously fitted to all IPF and PLT count data using the nonlinear mixed effects modeling module in ADAPT5 (version 5.035) (7). Parameter estimates were obtained using the maximum likelihood expectation maximization algorithm. Interindividual variability was described by a log-normal parameter distribution:

$$P_i = \theta_1 \cdot e^{\eta_{P_i}}$$

with \(P_i\) as the individual parameter for the \(i\)th individual, \(\theta_1\) is the typical value of the population parameter, and \(\eta_{P_i}\) is the interindividual variability, which is log normally distributed with a mean of zero, and a variance of \(\omega_{P_i}^2\) for the \(i\)th individual. The interindividual variability on all parameters was initialized at 40%, and only the diagonal of the covariance matrix was obtained in the final model. Residual variability was described by a proportional error model defined as

$$\text{Var} = (\sigma_1 \cdot Y)^2$$

in which \(\text{Var}\) is the variance, \(\sigma_1\) are the variance model parameters, and \(Y\) represents the model predicted concentrations for IPF or PLT. The value for \(\sigma_1\) was fixed to 2 during the model fitting process. Model building and performance were judged based on modeling fitting criteria, such as Akaikie information criterion, precision of estimated parameters, and visual inspection of individual observed versus predicted model outcomes and standardized residual plots.

Covariate analysis. A standard approach was used to identify whether subject characteristics could explain interindividual variability in estimated model parameters. Individual parameter estimates were plotted against potential covariates, including body weight, IPF counts on day of birth, PLT counts on day of birth, and presence of thrombocytopenia (i.e., PLT count < 150,000/\(\mu\)l) to inspect for any trends or potential covariate relationships. Covariates were included in the model based on improvement in the objective function value using the standard forward inclusion and backward elimination procedure.

Internal model qualification. The predictive performance of the model was evaluated using a visual predictive check. The population kinetic model was used to simulate 10,000 hypothetical patients with NONMEM version VI (double precision; Icon Development Solutions, Ellicott City, MD) (3). The distribution (median and 5th and 95th percentiles) of the simulated concentration time curves was compared with the observed IPF and PLT concentration values in the original data set.

RESULTS

Study subjects. Subject demographics for the 27 preterm neonates are listed in Table 1. The mean gestational age was 26.7 wk, and the mean birth weight was 900 g. All of the infants had frequent physician-ordered testing with an average of 54.6 ml/kg of blood drawn during the first 4 wk of life. The

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Gestational age</td>
<td>26.7 ± 1.29 wk</td>
</tr>
<tr>
<td>Birth weight</td>
<td>900 ± 254 kg</td>
</tr>
<tr>
<td>PLT count</td>
<td>202 ± 55.0 × 10^4/(\mu)l</td>
</tr>
<tr>
<td>IPF count</td>
<td>16.1 ± 7.47 × 10^4/(\mu)l</td>
</tr>
</tbody>
</table>

Values are means ± SD. IPF, immature platelet (PLT) fraction. *Counts at birth.
eight subjects who received PLT transfusions received one to three transfusions over the course of study.

**PLT modeling.** The final model consists of a series of four transit compartments, with two compartments each for the mean transit times of IPF and PLT in the systemic circulation (Fig. 1). This model captured the nature of IPF and PLT profiles well, and model performance deteriorated with increasing numbers of compartments (data not shown). The model with two compartments each for IPF and PLT count had the best goodness of fit criteria and precision of estimated system kinetic parameters and hence was chosen as the final model. Two representative profiles on nonthrombocytopenic and thrombocytopenic neonatal subjects are shown in Fig. 2. Good model performance was confirmed using individual observed and model predicted IPF and PLT counts for all subjects and standardized residual plots over time or as a function of model predicted values (data not shown). The final estimated parameter values are listed in Table 2. The coefficients of variation on all kinetic parameters were 43%, except for the recovered PLT "dose" at transfusion (DTRF), suggesting reasonable precision of parameter estimates. The greater coefficient of variation percentage on DTRF might be due to the relatively few number of subjects receiving transfusions, variability in the number of PLTs transfused, and interindividual differences in posttransfusion PLT recovery. The typical values for mean transit times in nonthrombocytopenic subjects were estimated at 0.912 and 10.7 days for IPF and PLT, whereas these values were 0.429 and 2.56 days in thrombocytopenic subjects. Substantial interindividually variability was observed for kinetic parameters defining cell production (Table 2).

**Covariate analysis.** The covariate analysis revealed that transit times for IPF and PLT were influenced by the presence of PLT transfusions. Incorporating transfusion as a categorical covariate on mean transit times significantly improved the objective function value (*P* < 0.0005). The covariate relationships were described by the following equations:

$$\tau_{\text{IPF}} = \tau_{\text{IPF}}^{\text{TRF}} \times \text{TRF} + \tau_{\text{IPF}}^{\text{NO-TRF}} \times (1 - \text{TRF}) \quad (9)$$

$$\tau_{\text{PLT}} = \tau_{\text{PLT}}^{\text{TRF}} \times \text{TRF} + \tau_{\text{PLT}}^{\text{NO-TRF}} \times (1 - \text{TRF}) \quad (10)$$

where $\tau_{\text{IPF}}$ and $\tau_{\text{PLT}}$ are the IPF and PLT transit times, $\tau_{\text{IPF}}^{\text{TRF}}$ and $\tau_{\text{PLT}}^{\text{TRF}}$ are the IPF and PLT transit times for thrombocytopenic subjects, and $\tau_{\text{IPF}}^{\text{NO-TRF}}$ and $\tau_{\text{PLT}}^{\text{NO-TRF}}$ are the IPF and PLT transit times for nonthrombocytopenic subjects.

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**Platelet dynamic model**

**Fig. 1.** Schematic of final model of immature platelet fraction (IPF) and platelet (PLT) dynamics. IPF, and PLT, are individual transit compartments, and IPF and PLT concentrations are set to the sum of the 2 respective compartments divided by a distributional volume (dashed rectangles). First-order intercompartmental rate constants are designated as the number of transit compartments divided by the life span of the cells (2/τ). The input function and model equations are included under Platelet dynamic model in MATERIALS AND METHODS.

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**Fig. 2.** Representative profiles of IPF (top) and PLT (bottom) dynamics for a subject not receiving PLT transfusions (left) and a subject who received 3 PLT transfusions (right). Black circles represent observed data, black traces represent individual model predicted profiles, and peaks (bottom, right) indicate times of platelet transfusion. Conc, concentration.
times for nonthrombocytopenic subjects, and TRF is an indicator for time of PLT transfusion (i.e., TRF = 1 during transfusion and otherwise set to 0). The visual predictive check revealed a large interindividual variability in both IPF and PLT profiles (data not shown).

**DISCUSSION**

Although several algorithms have been developed for evaluating neonatal thrombocytopenia based on differentiating between early and late-onset thrombocytopenia (1, 16, 21, 22), treatment is limited given that PLT transfusions are the only currently available form of treatment. Neither the optimal PLT transfusion regimen nor the clinical impact of thrombocytopenia in neonates has been well defined. IPF has emerged as a potential biomarker in diagnosing and monitoring the treatment course of thrombocytopenia in this patient population (6, 23, 26). IPF refers to the fraction of PLTs in the circulation that are immature, previously also referred to as “reticulated PLTs.” Reticulated PLTs are newly formed PLT with residual megakaryocyte-derived RNA, which distinguishes them from mature PLT (4). The number of reticulated PLT (or the absolute IPF) usually reflects the rate of thrombopoiesis, increasing when PLT production rises and decreasing when production falls. Thus IPF was used as the PLT precursor pool for driving PLT kinetics in our mathematical model (Fig. 1).

One of the key indicators of thrombocytopenia is the mean transit time or life span for IPF and PLT. These two transit times reflect the state of cell maturation and the senescence systems. The average life span of a reticulated PLT is about 1 day in dogs (8) and that for the mature PLT is about 10 days in humans (9). Final estimated transit times parameters (Table 2) are in good agreement with these values in infants not receiving transfusions. Additionally, among thrombocytopenic subjects, both transit times were decreased markedly indicating accelerated PLT consumption (5). This also supports the decision to incorporate separate transit times for thrombocytopenic and nonthrombocytopenic patients in the current model (28).

Mathematical modeling of thrombocytopenia in preterm neonates poses various challenges such as large interindividual variability in temporal IPF and PLT profiles, lack of steady-state dynamics, and dynamic and physiological conditions, including laboratory phlebotomy loss and increasing body weight/blood volume with time. The two most common methods for modeling the dynamics of natural cells are using simple transit compartments and delayed differential equations. It is likely that high interindividual variability on production parameters also reflects the presence of different individual pathophysiological conditions in this patient population. Average rise in body weight for the current study population was 60%, with a maximum increase in one infant of 130% during the first month of life (Fig. 3). This provided the basis for including body weight and its corresponding blood volume as a time-dependent covariate in the model building process. Simulations of IPF and PLT profiles were conducted with and without dynamic volume as part of the model (Fig. 4). The dynamic blood volume clearly plays a role in determining the half-life of cells during the later stages of PLT kinetics. The visual predictive check confirmed the presence of large interindividual variability in model predictions (data not shown).

Neonates typically incur heavy blood loss due to phlebotomies during the first month of life. Phlebotomy in VLBW neonates is an important contributor to neonatal anemia (20). Thus phlebotomy losses were included in the model as negative bolus dosing events. Although including IPF and PLT loss due to phlebotomy did not provide significant improvement in the model-fitting criteria (data not shown), cell loss was retained in the final model to maintain mass balance. It is possible that the loss of PLT due to phlebotomy was contained within the natural noise of the data and hence made it difficult to assess its impact. Further research with a larger number of subjects (and potential biomarkers) is needed to assess the

**Table 2. Estimated PLT model kinetic parameters in neonates of very low birth weight**

<table>
<thead>
<tr>
<th>Parameter Description</th>
<th>n</th>
<th>Mean Value</th>
<th>SE (%CV)</th>
<th>IIV (%CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \tau _{IPF} ) (nontransfusion), day</td>
<td>19</td>
<td>0.912</td>
<td>19.3</td>
<td>18.2</td>
</tr>
<tr>
<td>( \tau _{PLT} ) (nontransfusion), day</td>
<td>19</td>
<td>10.7</td>
<td>9.2</td>
<td>38.5</td>
</tr>
<tr>
<td>( \tau _{PLT} ) (transfusion), day</td>
<td>8</td>
<td>0.429</td>
<td>35.2</td>
<td>13.9</td>
</tr>
<tr>
<td>( \tau _{IPF} ) (transfusion), day</td>
<td>8</td>
<td>2.56</td>
<td>10.3</td>
<td>40.5</td>
</tr>
<tr>
<td>( \alpha ), cells/day</td>
<td></td>
<td>1.21 \times 10^2</td>
<td>23.4</td>
<td>47.3</td>
</tr>
<tr>
<td>( k ), day(^{-1})</td>
<td></td>
<td>0.0258</td>
<td>42.1</td>
<td>71.9</td>
</tr>
<tr>
<td>( R_0 ), cells/day</td>
<td></td>
<td>1.78 \times 10^6</td>
<td>29.8</td>
<td>22.8</td>
</tr>
<tr>
<td>( D_{b,0} ), cells/day</td>
<td></td>
<td>7.82 \times 10^7</td>
<td>51.8</td>
<td>50.9</td>
</tr>
<tr>
<td>Slope of the variance model</td>
<td></td>
<td>0.411</td>
<td>5.81</td>
<td>—</td>
</tr>
</tbody>
</table>

IIV, interindividual variability; %CV, percentage of coefficient of variation.

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**Fig. 3.** Initial and final body weights from birth to 1 mo of age for all subjects.
physiological relevance of phlebotomy losses in treating thrombocytopenia.

Although the current model was able to capture PLT kinetics reasonably well, limitations in the data constrained the model in estimating some of the PLT production and loss associated parameters. For example, key biomarkers such as thrombopoietin (TPO) measurements were not available to incorporate into the model. TPO, also known as c-Mpl ligand, serves as the primary physiological growth factor for the megakaryocyte lineage (11). It is the most potent cytokine for stimulating the proliferation and maturation of megakaryocytes. This stimulation leads to an increase in megakaryocyte number, size and ploidy, which eventually results in increased pro-PLT formation and PLT release. Additionally, TPO is also a key contributor in the survival and proliferation of hematopoietic stem cells. Future models should include TPO measurements for investigating relationships between PLT count and TPO concentrations. Another model limitation is that we assume the life span of donor and recipient PLT are identical owing to problems with identifiability. Information from labeled PLT will be needed to distinguish specific life spans. During model development, we also considered random PLT destruction as a possible underlying mechanism for thrombocytopenia. To incorporate this aspect, a first-order rate constant of cell destruction was added to the model; however, the model failed to capture the time course of PLT concentrations and estimate this parameter with reasonable precision. Finally, although the estimated value for $D_{TRF}$ is consistent with the standard PLT recovery from transfusions for treating thrombocytopenia in VLBW infants, the low confidence in $D_{TRF}$ with its relatively high coefficient of variation percentage might be explained on the basis of factors such as body weight proportional dosing, large noise in the data set, and limited patient sample size.

PLT transfusion is the primary mode of treatment; however, neonates receiving PLT transfusions have a higher mortality than nonthrombocytopenic neonates (10, 15, 22). Moreover, lack of evidence-based guidelines for optimal transfusion “dose” and regimen have made it difficult to elucidate the clinical implications of PLT transfusion therapy. These issues highlight the urgent need to invest in alternative and more efficient therapies to treat neonatal thrombocytopenia. TPO serves as the primary physiological regulator of PLT production, and there is considerable interest in using TPO analogs as potential alternatives to PLT transfusions. Recombinant human TPO is associated with neutralizing antibodies; however, second generation analogs, known as TPO mimetics, do not share sequence homology with endogenous TPO and act by binding and activating the TPO receptor. Romiplostim (Nplate) and eltrombopag (Promacta) were approved by the United States Food and Drug Administration in 2008 for use in adults with immune thrombocytopenic purpura not responsive to conventional therapy. Interestingly, in vitro neonatal megakaryocyte receptors show an increased sensitivity to TPO compared with that in adults (13). Developmental differences in neonatal and adult PLT production and physiology remain to be resolved. Also, the nonhematopoietic effects of TPO have not been adequately investigated, particularly in neonates. The potential therapeutic benefits and safety concerns associated with TPO mimetics in treating neonatal thrombocytopenia are the subject of ongoing research (24). In addition to recapitulating IPF and PLT kinetics in this patient population, our model can be readily modified to include the pharmacological action of TPO mimetics through stimulation of PLT production (31). Thus the model should prove useful in evaluating patient specific factors that influence the time course of natural cell turnover and responses to transfusions and pharmacological interventions.

In summary, a mathematical model was successfully developed to capture PLT dynamics in 27 critically ill, ventilated, VLBW neonates. This model we have developed provides a beginning platform for studying underlying mechanisms of neonatal thrombocytopenia in a quantitative manner. Doing so will assist in identifying potential patient phenotypic characteristics that influence PLT kinetics. The model also has potential utility in evaluating TPO mimetic drug therapy in this patient population at high risk for thrombocytopenia.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES