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Optimization of anemia management in preterm infants

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OPTIMIZATION OF ANEMIA MANAGEMENT IN PRETERM INFANTS

by

Matthew Robert Rosebraugh

An Abstract

Of a thesis submitted in partial fulfillment
of the requirements for the Doctor of
Philosophy degree in Pharmacy
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Thesis Supervisor: Professor Peter Veng-Pedersen

ABSTRACT

Premature infants develop anemia in their first few weeks of life. This is the result of heavy laboratory blood loss, shortened red blood cell lifespan, low plasma erythropoietin levels and inadequate erythropoiesis. As treatment for clinically significant anemia, approximately 80% of very low birth weight infants weighing less than 1.5kg at birth and 95% of extremely low birth weight infants weighing less than 1.0kg at birth receive one or more red blood cell transfusions. To reduce or eliminate red blood cell transfusions is important because they are expensive and associated with complications including infection, fluid overload, electrolyte imbalance, transfusion related acute lung injury and exposure to plasticizers, lead, and other toxins.

The primary objective of this thesis is to examine erythropoietin (Epo) dosing, laboratory phlebotomy reduction and the use of restrictive red blood cell transfusion criteria to determine the potential to reduce or eliminate the need for red blood cell transfusions in preterm infants. In order to accomplish this objective, data were obtained from 27 preterm infants including: erythropoietin concentrations, phlebotomy volumes, transfusion information and multiple hematologic indices. The data were analyzed and modeled according to pharmacokinetic and pharmacodynamic principles to determine, through simulation studies, the potential for avoiding blood transfusions in preterm infants. Results from this research suggests that Epo administration, phlebotomy reductions and the use of restrictive blood transfusion criteria all have the potential to reduce the need for blood transfusions in preterm infants. Specifically, a combination of the three interventions was predicted to make blood transfusions unnecessary in all infants with a birth weight between 1.0-1.5kg, and 45% of infants with a birth weight of

<1.0kg. These findings are clinically important because avoiding transfusions may lead to better clinical outcomes. The results propose strategies to utilize in future clinical trials involving preterm infants.

The secondary objective of this thesis is to characterize the dynamic Epo receptor behavior in newborn sheep and determine a pharmacodynamic model which utilizes information from the Epo receptor dynamics. Results from this analysis show that the Epo receptor pool is an important predictor of red blood cell production. An Epo receptor based pharmacodynamic model is proposed that successfully predicted the red blood cell production in newborn sheep. Additionally, the optimal time for Epo administration was also determined in these newborn sheep based on the pharmacodynamic model. This optimal Epo administration time corresponded to approximately the time when the Epo receptor pool was the largest. Results from the Epo receptor based studies in newborn sheep suggest Epo clinical trials in preterm infants need to consider the dynamic Epo receptor behavior to produce the most optimal outcome.

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CERTIFICATE OF APPROVAL

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PH.D. THESIS

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To my beloved wife, Michelle and my parents

Tortured data will confess to anything.

Fredric M. Menger (1937), Candler Professor of Chemistry, Emory University

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A: disposition parameter for a two compartment model

α_j: time since transfusion j occurred

b: intercept for regression equation

β: disposition parameter for a two compartment model

BBB: blood brain barrier

β_k: time since phlebotomy k occurred

BFU-E: burst-forming unit erythrocyte

BV_{kg}: blood volume per kg of body weight

BW: body weight

C_A: actual capillary hematocrit value

CFU-E: colony-forming unit erythrocyte

Cl: clearance

$Cl_{n,C}$: calculated clearance for Epo dose n

$Cl_{n,R}$: reported clearance for Epo dose n

$Cl_P(t)$: volume of blood removed/time/blood volume at time t

Cl_R: receptor mediated erythropoietin clearance

$Cl_{R,SS}$: steady state receptor mediated erythropoietin clearance

$Cl_{L:}$ linear erythropoietin clearance

ΔHb: change in Hb concentration after the transfusion is complete

ΔHb_j: change in Hb concentration for transfusion j

ΔHb_k: change in Hb from the reduction of phlebotomy k

D(t): cumulative amount of blood removed from infant at time t

DPG: diphosphoglycerate

EC_{50}: concentration of Epo resulting in half the maximum Hb production rate

ELBW: extremely low birth weight (<1,000 g)

E_{max}: maximum achievable Hb production rate

$E_{max,SS}$: maximum Hb production possible at steady state

Epo: erythropoietin

EpoR: erythropoietin receptor

E_{ss}: Hb production rate at birth

E(t): effect at time t

F_j: fraction of the total blood volume removed in phlebotomy j

GM-CSF: granulocyte macrophage colony-stimulating factor

Hb: hemoglobin

Hb_A: hemoglobin amount

Hb_C: hemoglobin concentration

Hb_i: Hb measurement i

$Hb(t)_{Epo}$: hemoglobin amount produced from Epo at time t

$Hb(t)_{Epo,PHLE}$: hemoglobin amount produced from Epo with phlebotomies at time t

$Hb(t)_{phle}$: hemoglobin amount remaining from phlebotomy reduction at time t

$Hb(t)_{trx}$: hemoglobin amount remaining from transfused cells at time t

HCT: hematocrit

HCT_{trx}: hematocrit of blood transfusions

IL: interleukin

IV: intravenous

k:	number of phlebotomies after the RBCTx time t_0 but before current time
k_0:	zero order rate constant for *M* formation
k_1:	rate constant for Epo-EpoR binding
k_2:	first order feedback rate constant
k_3:	first order elimination rate constant for M
M:	intermediate variable used to account for the feedback
m:	number of phlebotomies that occurred after the phlebotomy but before t
M_D:	mean additive difference between capillary and venous hematocrit values
M_R:	mean ratio of venous hematocrit/capillary hematocrit
mRNA:	messenger ribonucleic acid
NICU:	neonatal intensive care unit
P_1:	maximum rate at which the Epo concentration declines
P_2:	Epo concentration at which Epo is eliminated at half of the maximum rate
PD:	pharmacodynamic
PINT:	preterm infants in need of transfusion
PK:	pharmacokinetic
$PHLE_V$	volume of phlebotomized blood
P_R:	percentage by which the phlebotomy removal was reduced
P(t):	continuous amount of blood removed from infant at time t
RBC:	red blood cell
RBCTx:	red blood cell transfusion
$R_i(t)$:	target Hb concentration at time t for subject i
SCF:	stem cell factor

$S_{Hb,i}$: simulated Hb profile for subject i at time t

SS: steady state

τ : red blood cell lifespan

τ_A: lifespan of transfused adult red blood cells

TACO: transfusion associated circulatory overload

TfR: transferring receptor

$\theta(t)$: percent error of the continuous bleed function at time t

τ_I: lifespan of infant red blood cells

t_{lag}: lag time between Epo-EpoR binding and Hb production

TRALI: transfusion related acute lung injury

TRX_V: volume of packed red blood cells transfused

T_0: time at which the most recent RBCTx occurred

V: volume of distribution

V_A: actual venous hematocrit value

V(t): volume of distribution of erythropoietin in sheep at time t

V_{Kg}: volume of distribution of erythropoietin in sheep per kg

VLBW: very low birth weight (<1,500 g)

W(t): weight at time t

CHAPTER 1. INTRODUCTION

1.1 Background

Erythropoietin (Epo) administration to preterm infants has been of clinical interest for many years as an alternative treatment for anemia. The rationale behind administering Epo to preterm infants is that these infants may be able to produce sufficient red blood cells (RBCs) in order to avoid blood transfusions which are associated with specific risks. In a comprehensive review of 27 clinical trials where preterm infants were given Epo the author concluded although Epo administration reduces the RBC transfusions and the total volume of RBCs transfused, that the reductions are of limited clinical significance (1). This conclusion can be challenged for several reasons. One problem with previous clinical trials is that they largely use a 'trial and error' method to determine clinically significant outcomes. Clinical trials have administered Epo intravenously (2, 3) and subcutaneously (4, 5) with Epo doses ranging from 70 U/kg/week (6) to 2100 U/kg/week (7). In addition, gestational age enrollment criteria for Epo clinical trials involving preterm infants have ranged from <=29 weeks to <=34 weeks (1). Inconsistent RBC transfusion criteria can also confound results from Epo clinical trials in preterm infants (8).

An additional reason why Epo clinical trials have been inconsistent is the large variability in pharmacodynamic (PD) response to Epo. Previous work has shown that there is a large variability in Epo PD parameters (9) , however, the reason for such variability remains unclear. Several covariates such as interleukins (10, 11), iron status (11), hepcidin (12) and other factors have been shown to influence erythropoiesis which

may explain the PD variability. Research that takes into account the complex pharmacokinetic/pharmacodynamic (PK/PD) may help elucidate why some infants respond well to Epo while others do not have sufficient erythropoiesis. Furthermore, future analysis utilizing population PK/PD modeling combined with extensive covariate information will provide valuable information for Epo clinical trials. The main application of such work is identifying optimized Epo dosing regimens that provide the best chance for infants to avoid transfusions as well as identifying specific infants that are likely to respond well to Epo therapy. This personalized medicine approach may lead to better anemia management in preterm infants.

1.2 Erythropoiesis and erythrocytes

Epo is a 30.4 kD glycoprotein hormone that contains 165 amino acids and is produced by peritubular fibroblast cells located in the kidney (13). Epo is produced by the kidney in response to decreased circulating oxygen in the blood which is sensed through hypoxia-inducible factor (14). Hypoxia-inducible factor is a transcription factor for the Epo gene which is digested by proteaosomes in the presence of oxygen which allows for Epo production in response to anemia (15). The primary function of Epo is to produce erythrocytes through erythropoiesis. Epo exerts its mechanism of action by binding to erythropoietin receptors (EpoR) located on erythroid progenitors burst-forming units (BFU-E) and colony-forming units (CFU-E) found primarily in the bone marrow (16). Once bound to the EpoR, Epo undergoes endocytosis followed by lysosomal degradation which leads to the differentiation of erythroid precursors into mature red blood cells (17).

Erythrocytes are produced primarily from the $CD34^{+}$ pluripotent hematopoietic stem cells located in the bone marrow which differentiate in the presence of cytokines(18). Following a path of differentiation into different cell types (Figure 1.1), progenitors BFU-E and CFU-E are the first cell lines that contain EpoRs (16). The bone marrow of the newborn infant is rich in such erythroid progenitor cells (19). CFU-E have been shown to respond strongly to Epo and contain many EpoRs while the BFU-E are greater in number but have less EpoR and are therefore less sensitive to Epo (18). Following the binding of Epo to an EpoR, a signal transduction pathway is initiated that begins to transform the erythroid precursor into a mature red blood cell (20). Following Epo-EpoR binding the first cell that can normally appear in circulation is the reticulocyte. Reticulocytes can be identified under a microscope by staining them with various stains that show ribosomal RNA inside of the cell. In recent years flow cytometry instruments have been used to identify reticulocyte percentages in a blood sample. Reticulocyte percentages are often used to diagnose anemia because values above normal correspond to a large amount of blood being replaced in the body (21). The reticulocyte will circulate the blood stream for a period of approximately 24 hours before becoming a mature erythrocyte (22).

In addition to EpoRs, CFU-E and BFU-E also contain transferrin receptors (TfR) which provide the cells access to iron which is required for synthesis of hemoglobin. TfRs are absent from mature red blood cells because there is no need to further synthesize hemoglobin and therefore no further need for iron. When transferrin is bound to the TfR the complex undergoes endocytosis and the iron is released into the cytoplasm of the cell. The TfR has been shown to be recycled once the iron has been released into

the cytoplasm of the cell and the transferrin is released from the receptor with iron no longer bound (23).

1.2.1 Erythropoietin resistance

There are many biological markers as well as other covariates which cause a subject to become less sensitive to erythropoiesis. The term often used for hyposensitivity to Epo is Epo resistance (16). A summary of the factors which have been shown to influence erythropoiesis are summarized in Table 1.1. Specifically, Epo resistance can be caused by factors associated with iron deficiency and inflammation in the body. Plasma concentrations of certain cytokines above the normal level have been shown to cause Epo resistance in hemodialysis patients e.g. interleukin-1 (IL-1), IL-2, IL-6, C-reactive protein and tumor necrosis factor (24). The exact mechanism by which the cytokines inhibit erythropoiesis is not entirely clear. However, IL-1, IL-2, IL-6, C-reactive protein and tumor necrosis factor have higher concentrations during inflammatory states which decreases the iron availability in the circulation due to increased uptake by phagocytes such as macrophages (25). IL-3 was found to significantly inhibit erythropoietin action in a mouse study due to its inhibition of endothelial cells which produce erythroid stimulating factors (26). The cytokine interferon-gamma (INF- γ) was found to prevent optimal red cell formation in patients with kidney failure (26).

In addition to the cytokines associated with erythropoiesis, iron deficiency is often stated as an inhibitor of erythropoiesis which requires iron for production of Hb. Accordingly, previous work has suggested that iron status is an important factor for the formation of new red blood cells (11). Van der Putten et al. demonstrated that iron

supply can be the limiting factor for Hb synthesis which cannot be corrected even with high concentrations of exogenous Epo (27). Hepcidin, a regulator of iron metabolism, has been proposed as a contributor to iron deficiency (28). Increased concentrations of hepcidin in the plasma will cause a decrease of the iron in the circulation, thus, decreasing the iron available for the synthesis of new Hb (27).

1.3 Epo pharmacokinetics

The pharmacokinetics of Epo has been studied extensively in adults. Most pharmacokinetic studies have observed a biexponential disposition curve (29-32). However, certain doses of Epo can cause saturation of the Epo receptors and nonlinear elimination is observed making the biexponential disposition curve inadequate (33, 34). Information regarding the *in vivo* clearance of Epo remains incomplete. Some studies have shown that Epo is cleared by the liver (35, 36) and the kidney (37) , however, their effects seem to be minimal (38). Receptor-mediated endocytosis in the bone marrow by erythroid progenitors followed by lysosomal degradation is the primary organ of Epo elimination from the body (17). Additional evidence for this elimination mechanism has been shown by studies investigating different degrees of bone marrow activity (39-41). Several preclinical and clinical studies have reported an increase in Epo clearance within one month following Epo treatment (2, 42-44) , while others found no statistical difference (30, 32). In a recent study by our group we reported up-regulation of EpoR mRNA levels of 4.97±3.92 times the baseline at 9 days following the induction of anemia (45). Although EpoR mRNA levels may not directly correlate with EpoRs, an increase in mRNA likely corresponds to an increase in the number of EpoRs. In the same study the Epo clearance was determined at multiple time points and was shown to significantly

correlate with the EpoR mRNA level. The combination of these two findings suggests that EpoRs increase following a period of anemia and that the Epo clearance is a sufficient surrogate marker for the EpoR level (45).

1.3.1 Epo pharmacokinetic parameters

Epo pharmacokinetic parameters have been previously determined in healthy adults (46) and in preterm infants (43, 47). A summary of pharmacokinetic parameters determined in preterm infants and healthy adults is shown in Table 1.2. The previously calculated parameters shown in Table 1.2 show a large variability suggesting there is a large intersubject variability. In addition, the clearance values given in Table 1.2 are body weight normalized because the absolute clearance value will increase as weight increases due to a larger receptor pool (48). Several studies which have determined Epo pharmacokinetic parameters have used a noncompartmental system analysis approach which avoids specific structural assumptions making the analysis more objective (43, 47, 49). Common Epo pharmacokinetic parameters are calculated according to the following equations assuming Epo is given as an IV bolus dose:

$$V = \frac{dose}{c(0)} \tag{1.1}$$

where, V is the volume of distribution, dose is the exogenous dose of Epo administered and c(0) is the initial concentration of Epo. In practice, the c(0) value is often obtained by back extrapolation from concentration vs. time data points.

The clearance values for Epo are often calculated according to the equation

$$Cl = \frac{dose}{\int_0^\infty c(t)dt} \tag{1.2}$$

The integral of the concentration versus time curve is often calculated using the trapezoidal rule or other numerical integration techniques. Although noncompartmental techniques can be used to determine the pharmacokinetic parameters for Epo, a pharmacokinetic model which can determine serial concentrations of Epo must consider the physiology and behavior of Epo.

1.3.2 Epo pharmacokinetic models

There have been several studies which have used pharmacokinetic models to describe the disposition of Epo in healthy adults (29, 49, 50) , animals (51-53) and preterm infants (43). Understanding the basis of previous pharmacokinetic models is important for understanding pharmacokinetic analysis in preterm infants.

Some of the pharmacokinetic models which described the disposition of Epo used a biexponential curve based on a two compartment model (29, 51). The equation used in the previous studies to describe the disposition of Epo is given below:

$$c(t) = A_1 e^{-\alpha_1 t} + A_2 e^{-\alpha_2 t} \qquad (1.3)$$

where, c(t) is the concentration of Epo at time t following an IV bolus administration and A and α are the disposition parameters calculated from the two compartment model given in the equation above. The model presented in Equation 1.3 is advantageous because it is simple and can describe the concentration of Epo when low doses are administered through the IV bolus pathway. However, a disadvantage of the model is that at higher Epo concentrations the Epo receptors become saturated and nonlinear behavior is observed for Epo disposition.

1.3.3 Ramakrishnan et al. model

In order to model the nonlinear behavior of Epo a model was developed as shown in the equation below (50):

$$\frac{dA_P}{dt} = -\left(\frac{V_{\max}}{k_m \cdot V_d + A_p}\right) \cdot A_p \tag{1.4}$$

where A_p is the amount of Epo in the body; V_{max} is the maximum elimination rate of the saturable elimination process; K_m is the plasma Epo concentration at which the elimination rate reaches half the V_{max} and V_d is the volume of distribution. This model describes the saturable receptor based elimination mechanism of Epo in contrast to the simple two compartment model given by Equation 1.3. However, the model does not account for the linear elimination pathway which was characterized experimentally by previous work (41).

1.3.4 Kato et al. model

Kato et al. presented a model consistent with the hypothesized elimination mechanism of Epo by assuming the disposition occurs through a linear and nonlinear elimination pathway (52). This model involved both first-order elimination and Michaelis-Menten type elimination of Epo from the central compartment. As described by the following differential equations:

$$\begin{aligned} \frac{dc_1}{dt} &= -(k_{12} + k_e + V_{\max}/(V_C(k_m + c_1)))c_1 + k_{12}c_2 \\ \frac{dc_2}{dt} &= k_{12}c_1 - k_{21}c_2 \end{aligned} \tag{1.5}$$

where, c_1 is the concentration of Epo in the central compartment; c_2 is the concentration of Epo in the peripheral compartment; K_m is the Michaelis-Menten constant; V_{max} is the

maximum elimination rate of the saturable elimination process from the central compartment; k_{12} and k_{21} are the first-order transfer rate constants between the central and peripheral compartments, respectively; k_e is the first-order elimination rate constant from the central compartment and V_c is the volume of distribution of the central compartment. Veng-Pedersen et al. later confirmed the physiology of the elimination of Epo by experimentally determining that Epo is cleared both by a receptor mediated (non-linear) and a non-receptor mediated linear pathway (41). One potential problem with the model presented by Kato et al. is that although the linear elimination pathway can be characterized, the physiology of the linear elimination pathway is still unknown.

1.3.5 Chapel et al. model

Previous work has shown that the Epo receptor state is an important consideration in the PK/PD modeling of Epo (41). A study which modeled the change in the EpoR level over time following the induction of anemia (48) showed that Epo clearance hit a minimum (below baseline)1.8 days after anemia induction and hit a maximum (above baseline) 8.5 days after anemia induction. This study by Chapel et al. was the first study to model the dynamic changes of the Epo receptor population in a PK model:

$$\begin{aligned} \frac{dEPOR}{dt} &= k_3 M - k_1 \cdot c_{EP} \cdot EPOR \\ \frac{dM}{dt} &= k_0 + k_2 (EPOR_0 - EPOR)_+ - k_3 M \end{aligned} \qquad (1.6)$$

where k_3 is a first order rate constant for Epo receptor formation from M; M is the amount of the unknown intermediate compound; k_1 is the first order rate constant for Epo binding to the Epo receptor leading to endocytosis; c_{EP} is the concentration of Epo; EpoR is the quantity of Epo receptors contributing to Epo's elimination; k_0 is the zero order rate

constant for the formation of M; k_2 is a first order feedback rate constant; $EpoR_0$ is the EpoR level at baseline (48). In this study it was assumed that the Epo clearance is directly proportional to the Epo receptor level. This assumption was validated by a previous bone marrow ablation study by our group (41). Thus, assuming the Epo clearance is proportional to the Epo receptor number, Equation 1.6 becomes:

$$\begin{aligned} \frac{dCl}{dt} &= k'_3 M - k'_1 \cdot c_{EP} \cdot Cl \\ \frac{dM}{dt} &= k_0 + k'_2 (Cl_0 - Cl)_+ - k_3 M \end{aligned} \qquad (1.7)$$

where, Cl(t) is a clearance function for Epo and Cl_0 is the clearance of Epo at baseline. Although Epo's mechanism of action is thought to be primarily through receptor binding (17), the chapel et al. model did not determine the receptor-based pharmacodynamics of Epo.

1.4 Epo pharmacodynamics

There have been several studies that have examined Epo pharmacodynamics in animals, adults and preterm infants (9, 54-60). Although it is beyond the scope of this introduction to examine all of the mathematics behind the different Epo pharmacodynamic models, these models can be discussed in terms of physiologic relevance, assumptions and weaknesses.

1.4.1 Loeffler et al model

Loeffler et al. presented a comprehensive physiologic based model of erythropoiesis in mice and rats (54). This model has several characteristics consistent with the current physiologic understanding of erythropoiesis. First, tissue oxygen pressure was used as a regulator for the production of erythropoietin consistent with the

known mechanism of action that Epo is produced by the kidney in response to decreased circulating oxygen in the blood sensed via hypoxia-inducible factor (14). Tissue oxygen pressure was used as a surrogate measurement for the circulating oxygen in a subject. Second, erythrocyte production in the model was regulated by the Epo concentration and the pool of CFU-E cells present in the bone marrow. Research has suggested that both BFU-E and CFU-E cells contribute to erythrocyte production, however, CFU-E cells have more Epo receptors and are hypothesized to be more sensitive to Epo than BFU-E cells (18). One main weakness of the Loeffler et al. pharmacodynamic model is the elimination of Epo was accounted for by a first order elimination process inconsistent with studies that have shown Epo to have nonlinear elimination by a saturable mechanism (33, 34, 41). Also, the model used a first order process to account for the removal of red blood cells. The first order elimination of red blood cells is inconsistent with the current understanding of a lifespan-based senescence of cells (9, 61, 62).

1.4.2 Woo et al. model

Woo et al. presented a pharmacodynamic model of Epo which utilized a target mediated drug disposition model (63). The primary mechanism of elimination of Epo is considered to be Epo receptor mediated endocytosis followed by lysosomal degradation (17). Epo binding to its receptor also leads to the erythrocyte precursor forming a mature erythrocyte. The Woo et al. study derived an Epo pharmacodynamic model which is consistent with the hypothesized mechanism of action of Epo. The model considers the senescence of red blood cells as surviving for a fixed lifespan before being removed from the circulation. The disposition for this model was described using the following equations:

$$\frac{dc}{dt} = k_{EPO} - k_{on} \cdot r \cdot c + k_{off} \cdot rc - (k_{el} + k_{pt}) \cdot c + k_{tp} \cdot \frac{a_T}{V_p} \qquad (1.8)$$

$$\frac{da_T}{dt} = k_{pt} \cdot c \cdot V_p - k_{tp} \cdot a_T \qquad (1.9)$$

$$\frac{dr}{dt} = k_{syn} - k_{on} \cdot r \cdot c + k_{off} \cdot rc - k_{\deg} \cdot r \qquad (1.10)$$

$$\frac{drc}{dt} = k_{on} \cdot r \cdot c - (k_{off} + k_{\text{int}}) \cdot rc \qquad (1.11)$$

with the following initial conditions:

$$c(0) = \frac{D_{IV}}{V_P} + c_0 \qquad (1.12)$$

$$a_T(0) = k_{pt} \cdot V_P \cdot \frac{c_0}{k_{tp}} \qquad (1.13)$$

$$r(0) = \frac{(k_{syn} - k_{EPO} + k_{el} \cdot c_0)}{k_{\deg}} \qquad (1.14)$$

$$rc(0) = \frac{(k_{EPO} - k_{el} \cdot c_0)}{k_{\text{int}}} \qquad (1.15)$$

where, c is the concentration of free Epo in the plasma; a_T is the concentration of Epo in the tissues; r is the number of Epo receptors; rc is the drug receptor complex; k_{Epo} is the zero-order production rate of endogenous Epo; k_{on} is the second-order rate constant; k_{off} is the first order dissociation rate constant of the rc complex; k_{el} is the direct elimination rate constant of Epo; k_{pt} and k_{tp} are the tissue distribution constants; V_p is the volume of distribution of Epo in the plasma; k_{syn} is the zero-order production rate of Epo receptors; k_{deg} is the first order degradation rate of the Epo receptor and k_{int} is the internalization and degradation of the Epo molecule.

The pharmacodynamic model for Epo was based on the Epo-Epo receptor bound complex (represented as rc in the study) which is consistent with the known mechanism of action of Epo. The current study modeled the pharmacodynamics according to the following equations:

$$\frac{dRET}{dt} = k_{in} \cdot S(t - T_{p1} - T_{P2}) \cdot S(t - T_{p2}) \cdot I(t - T_{p1} - T_{P2}) - k_{in} \cdot S(t - T_{p1} - T_{P2} - T_{RET}) \cdot S(t - T_{p2} - T_{RET}) \cdot I(t - T_{p1} - T_{P2} - T_{RET}) \quad (1.16)$$

$$\frac{dRBC_M}{dt} = k_{in} \cdot S(t - T_{p1} - T_{P2} - T_{RET}) \cdot S(t - T_{p2} - T_{RET}) \cdot I(t - T_{p1} - T_{P2} - T_{RET}) - k_{in} \cdot S(t - T_{p1} - T_{P2} - T_{RET} - T_{RBC}) \cdot S(t - T_{p2} - T_{RET} - T_{RBC}) \cdot I(t - T_{p1} - T_{P2} - T_{RET} - T_{RBC}) \quad (1.17)$$

where, the stimulation (S) and inhibition (I) functions are represented as:

$$S(t) = \left(1 + \frac{S_{\max} \cdot RC(t)}{SC_{50} + RC(t)}\right) \quad (1.18)$$

$$I(t) = \left(1 - \frac{I_{\max} \cdot \Delta Hb(t)}{IC_{50} + \Delta Hb(t)}\right) \quad (1.19)$$

where, RET is reticulocytes; RBC_M is the mature red blood cells; the T parameters are the lifespans of their representative cell population; ΔHb(t) is the change in the hemoglobin relative to the baseline value at time t; S_{max} is the maximum stimulation of responses by the Epo-Epo receptor complex (rc); SC_{50} is the RC values producing half of the maximum stimulation; I_{max} is the maximum amount of inhibition of stimulation; IC_{50} is the ΔHb where the inhibition is at 50% of the maximum inhibition. Equations 1.16 and 1.17 describe the changes in reticulocytes and red blood cells based on lifespan assumptions, production stimulation from Epo-Epo receptor binding and production

inhibition based on increased Hb concentration. Both of these equations are consistent with the hypothesized mechanism of action of Epo (17).

This pharmacodynamic model was tested in rats, monkeys and humans all under the assumption that there was no change in the production of Epo or changes in the Epo receptor pool available for binding. In reality, the Epo production rate constantly changes (14) as well as the Epo receptor pool making this a main weakness of the model (45, 48). Another assumption of this model is that the baseline Hb concentration must be known in order to derive the ΔHb(t) term used in Equation 1.19. In a case such as anemia of prematurity an infants initial Hb has a large variability making identification of their baseline impossible (64). Therefore, this model is not applicable to preterm infants who have changing Epo production and Epo receptor levels and an unidentifiable Hb baseline level.

1.4.3 Neelakantan et al. model

Neelakantan et al. published a pharmacodynamic model of Epo in sheep and proposed extending the model to determine suitable dosing in preterm infants (65). Five sheep were phlebotomized in this study and their Hb recovery and endogenous Epo concentrations were determined. Epo was not administered in this study. However, the pharmacodynamics could be determined from endogenous Epo concentrations, Hb concentrations and reticulocyte counts. Epo was used as a forcing function in the pharmacodynamic model given by the following equations:

$$f_{ac}(c_{EPO}(t)) = \left(\frac{E_{\max} \cdot c_{EPO}}{c_{50} + c_{EPO}(t)} \right) \tag{1.20}$$

where, f_{ac} is the transduction function leading to erythroid progenitor activation; c_{Epo} is the concentration of Epo; E_{max} is the maximum progenitor activation rate; c_{50} is the Epo concentration at which half the maximum progenitor activation rate is achieved. The f_{ac} leads to reticulocyte production according to the following equation:

$$R(t) = f_{ac}(t) * UIR(t) \tag{1.21}$$

where, R(t) is the response at time t given by reticulocyte counts; * denotes the convolution operator and the UIR (unit impulse response function) is given by:

$$UIR(t) = U(t-a) - U(t-b) \quad a < b \tag{1.22}$$

$$U(x) = \begin{cases} 1 & x \geq 0 \\ 0 & x < 0 \end{cases} \tag{1.23}$$

where, a is the time it takes for a reticulocyte to enter the blood stream following progenitor activation; b-a is the time for a reticulocyte to mature into a RBC. Values calculated for a and b were 0.971 and 4.71 days, respectively. The time it takes for a reticulocyte to enter the blood stream following progenitor activation (a) is consistent with a previous study which found 1.28 days (22).

Results from this model were able to successfully predict the reticulocyte responses in 5 sheep after phlebotomies. One main weakness of this model is that the change in receptor state was not considered which has been shown to be constantly changing in sheep during a period of anemia (48). Another problem with this model is it does not model the change in Hb of the sheep after the phlebotomy which is also important from a clinical viewpoint. The Hb concentration is often considered as an important criteria for initiating RBC transfusions in preterm infants (8).

One of the conclusions the author states in this study is that higher than normal Epo doses of 50-300 U/kg may be effective in treating anemia of prematurity because the Epo receptor pool is not saturated (65). This conclusion is consistent with the hypothesized mechanism of action of Epo (17), however, the dose range given for high doses of Epo (50-300 U/kg) are no longer considered high. Approximately 20 years ago when Epo clinical trials first began in preterm infants the dosing range of Epo was within the range stated by Neelakantan (6). More recently, studies have used Epo doses as high as 700 U/kg in preterm infants (7) suggesting higher Epo doses are needed to treat anemia of prematurity.

1.4.4 *Freise et al. model*

Determining the pharmacodynamics of erythropoietin in preterm infants is not a trivial process because of the numerous phlebotomies and red blood cell transfusions which occur in preterm infants. Any attempt to model the pharmacodynamics of erythropoietin without accounting for phlebotomies and transfusions would result in an incorrect estimation of the production rate. Recently, Freise et al. overcame these problems using a novel pharmacodynamic modeling approach in combination with endogenous Epo levels in preterm infants (9). This study used a simple E_{max} model which was parameterized as the following:

$$f_{prod}^{exutero}(t) = \frac{p_1 \cdot C_{EPO}(t)}{1 + p_2 \cdot C_{EPO}(t)} \qquad (1.24)$$

where f(t) is the ex utero Hb production rate at time t; $C_{Epo}(t)$ is the concentration of Epo at time t; $p_1 = E_{max}/ EC_{50}$; $p_2 = (EC_{50})^{-1}$; E_{max} is the maximum body weight scaled Hb production rate and EC_{50} is the plasma Epo concentration which results in 50% of E_{max}.

Equation 1.24 can determine the Hb production if there are no transfusions or phlebotomies present, however, there are multiple transfusions and phlebotomies in preterm infants in practice. In order to account for transfused blood without phlebotomies the following equation was used:

$$Hb'_{trans}(t) = -F_T \cdot \sum_{i=1}^{m} Hb_{Ti} \cdot f_{trans}(t - T_{Ti}) \qquad Hb_{trans}(0) = 0 \tag{1.25}$$

with

$$f_{trans}(t) = \begin{cases} \dfrac{1}{L_{trans}} & \text{for } 0 \le t < L_{trans} \\ 0 & \text{otherwise} \end{cases} \tag{1.26}$$

where $Hb'_{trans}(t)$ is the change in Hb from the transfused cells; F_T is the fraction of transfused cell surviving beyond the transfusion; m is the number of transfusions; HB_{Ti} is the amount of Hb administered by the ith transfusion; T_{Ti} is the time of the ith transfusion and L_{trans} is the lifespan of transfused red blood cells. In the presence of phlebotomies the Hb contribution from transfusions will be lower because some of the transfused blood will be removed through phlebotomies. To determine the transfused blood in the presence of phlebotomies, the following definition was made:

$$F_{Pj} = \frac{Hb_{total}(T_{Pj}) - Hb_{Pj}}{Hb_{total}(T_{Pj})} \tag{1.27}$$

where, F_{Pj} is the fraction of Hb remaining immediately after the jth phlebotomy relative to the amount of Hb present immediately before the jth phlebotomy; T_{Pj} is the time of phlebotomy j and HB_{Pj} is the Hb removed from phlebotomy j. With the definition from equation 1.27 the phlebotomy correction factor for multiple phlebotomies becomes:

$$\begin{cases} \prod_{j=k}^{n} F_{Pj} & \text{if } n \geq k \text{ and } T_{Pk} < t \\ 1 & \text{otherwise} \end{cases} \qquad (1.28)$$

where, k is the first phlebotomy after entry of the cells of interest into the systemic circulation; n is the last phlebotomy before the current time; and T_{Pk} is the time of phlebotomy k. This phlebotomy correction factor is multiplied by Equation 1.25 to give:

$$Hb'_{trans}(t) = -F_T \cdot \sum_{i=1}^{m} Hb_{Ti} \cdot f_{trans}(t - T_{Ti}) \cdot \prod_{j=k}^{n} F_{Pj} \qquad (1.29)$$

Equation 1.29 determines the contribution of transfused Hb to the infants overall Hb in the presence of phlebotomies.

In addition to the fact the Freise et al. study was the first to determine preterm infant Hb production with the correction of transfusions and phlebotomies, there are several important conclusions determined from the study (9). First, the Emax parameter was determined to be 0.566 g/day$\cdot$kg$^{3/4}$ while the average Hb production was determined to be 0.144 g/day$\cdot$kg$^{3/4}$. This suggests that preterm infants naturally produce a modest level of Hb which may greatly be increased with the administration of exogenous Epo. Second, the study was only able to determine nonlinear pharmacodynamic parameters in 6 out of the 14 subjects studied because of the low levels of endogenous Epo present. Future studies which administer exogenous Epo will be able to determine the nonlinear pharmacodynamic parameters in all infants. Finally, there was a large variation in the pharmacodynamic parameters which were determined in the study. This high level of variation suggests there may be covariates that determine an individual preterm infant's ability to respond to Epo. Identification of these covariates is potentially important for screening which preterm infants will respond the best to Epo therapy.

1.5 Allometric scaling from animal studies to human studies

Predicting PK/PD drug behavior in humans from animal studies is an important part of the drug development process and is often referred to as allometric scaling. Allometric scaling often considers factors such as weight, hepatic blood flow and renal blood flow. In addition, allometric scaling can help to identify appropriate animal models to use before human testing of specific drugs by comparing physiologic parameters.

A commonly referenced equation for simple allometric scaling was developed in the early 1980s by Boxenbaum et al. (66) and is given by:

$$\text{Physiologic parameter} = a \cdot W^{b} \tag{1.30}$$

where, a is an allometric coefficient with the proper units, W is the weight in kg of the subject and b is the allometric exponent. For example, if the physiologic parameter of interest is the clearance then Equation 1.30 can be rearranged to the equation shown below:

$$\log(Cl) = \log(a) + b \cdot \log(W) \tag{1.31}$$

Accordingly, a double log plot of the clearance and weight is linear. Woo et al. showed that Equation 1.31 describes Epo clearance (Figure 1.2) and Epo volume of distribution (Figure 1.3) among mouse, rat, monkey, rabbit, dog, sheep and human species (67). However, many studies have failed to predict an interspecies clearance relationship based on the simple allometric relationship given in Equation 1.30. For a preterm infant who weighs approximately 1 kg, this would put their clearance and volume of distribution at approximately 15 mL/h and 90 mL, respectively. These values are comparable to the

pharmacokinetic parameters given in Table 1.2, however, the clearance of Epo is highly variable due to the non-linear elimination.

Since the initial development of allometric scaling, more complex equations have been used to correlate animal pharmacokinetic parameters with human parameters. Factors used in these allometric scaling equations include maximum lifespan potential, brain weight, liver blood flow rate, molecular weight of the drug and the number of hydrogen bond acceptors on a drug molecule (68). Since the pharmacokinetic parameters of drugs are often dependent on multiple factors, investigators have started to use multiple regression analysis with the available data (69). A combination of using the proper allometric scaling equations with information regarding the physiologic activity of drugs will lead to the best possible allometric scaling.

1.6 Anemia of prematurity

1.6.1 *Development of anemia of prematurity*

Preterm infants develop anemia in the first few weeks of life for a number of reasons: (a) the first month of life for the infant is a period of rapid growth which means a sufficient amount of blood must be produced in order to maintain a "steady state" hemoglobin level (70), (b) in the fetus and newborn, Epo is produced primarily by the liver which is relatively insensitive to hypoxia compared to the kidney (71, 72) since the liver is less sensitive to anemia, preterm infants are unable to respond as well to anemia, (c) erythropoiesis is suppressed immediately following birth due to an oxygen rich postnatal environment which suppresses the transcription of hypoxia-inducible factor (73), (d) preterm infants have a number of iatrogenic phlebotomies which can significantly contribute to the development of anemia (74), (e) pathological conditions

such as include hemorrhage, infection/sepsis, inadequate nutrition intake (especially iron insufficiency) and cardiorespiratory disease can contribute to anemia of prematurity (75). In addition to the previously mentioned causes of anemia of prematurity, it has been suggested that infants having a lower gestational age at birth develop more severe anemia. A study recently published shows the effect that gestational age has on hemoglobin levels in the first month of life (Figure 1.4) (64). Figure 1.4 shows that infants with a gestational age of 29-34 weeks have a lower average Hb than infants with a gestational age of 35-42 weeks for the first 28 days of life. This suggests that the infants born with a lower gestational age develop more severe anemia. In fact, this study tried to include an additional average Hb figure for infants born at less than 29 weeks gestational age, however, this was not possible due to the necessity of red blood cell transfusions in most infants (64).

Anemia of prematurity is exacerbated in the preterm infant because they have a different type of Hb than adults which cannot unload oxygen as well to tissues as adult Hb. In the fetus, by 12 weeks gestational age fetal hemoglobin (Hb F) accounts for the majority of the hemoglobin. For the remainder of the gestational period the hemoglobin gradually switches from Hb F to adult hemoglobin (Hb A). For a normal term birth (approximately 40 weeks gestational age) there is an approximately equal amount of Hb F and Hb A present in the circulation (76), however, a premature infant will have a larger fraction of Hb F in the circulation. Hb F has a higher oxygen affinity than Hb A primarily because it is insensitive to the effects of 2,3-diphosphoglycerate (77). Since Hb F has a higher oxygen affinity than Hb A, Hb F will not unload as much oxygen to the tissues which can lead to hypoxia. In addition, research has shown that levels of 2,3-

diphosphoglycerate increase after birth which will exacerbate an infant's hypoxia if their hemoglobin is mostly Hb F (78). In fact, Delivoria-Paradopoulos et al. has shown that for preterm infants that oxygen unloading capacity increases with age for preterm infants (Figure 1.5) (78). The hemoglobin switch is especially important in a clinical setting because laboratory tests that show hemoglobin concentrations but do not show oxygen unloading potential and may be misleading. Future clinical tests that can directly provide information about anemia of prematurity are important for determining clinical interventions.

1.6.2 *Phlebotomies in preterm infants*

In the first few weeks of life preterm infants have a number of iatrogenic phlebotomies which contributes to their anemia (74, 79). One study reported that for 14 preterm infants (gestation age $\leq$ 29 weeks) an average of 143 $\pm$ 56.1 phlebotomies were taken in the first 28 days of life (9). The reasons physicians obtain blood samples from preterm infants include but are not limited to the following: arterial blood gas, antibiotic blood level, blood type, blood culture, complete blood count with white cell differential, glucose level, hemoglobin concentration, ionized calcium, lactic acid levels, electrolytes, methemoglobin level, phosphorus level, platelet count, bilirubin, theophyline levels, and thyroid function tests. Although the volume of blood obtained for each individual phlebotomy may not be high, the large number of phlebotomies taken from preterm infants leads to a large volume of total blood removed (9). In a review of anemia of prematurity, it was stated that drawing a 6 to 7 mL blood sample from a 1 kg infant with a total blood volume of 80-100 mL is equivalent to obtaining 450 mL of blood from an adult (79). In this first 30 days of life a 1 kg infant will have an average of 51.6 mL of

blood removed (9) corresponding to the equivalent of an adult donating nine 450 mL units of blood in one month. This blood removed from a 1 kg infant is equivalent to an adult donating blood approximately once every 3 days! In addition, many preterm infants have a birth weight as low as 500 g which further increases the influence of iatrogenic phlebotomies on their Hb concentrations and exacerbates their anemia.

1.6.3 *Therapy for anemia of prematurity*

There have been five main strategies that have been employed as therapy for anemia of prematurity: 1) iron supplementation, 2) delayed cord clamping 3) minimizing blood loss, 4) erythrocyte transfusions and 5) Epo administration. Anemia of prematurity is often described as nutritionally insensitive (80); however, iron supplementation has been shown to contribute to the production of hemoglobin (81). Specifically, research has shown that preterm infants who are insufficient in iron may inhibit the effect of either endogenous or exogenous Epo because iron is needed for synthesis of additional Hb (82). Therefore, it is common clinical practice to supplement preterm infants with iron to ensure they are iron sufficient.

When an infant is born they are still attached to the mother via the umbilical cord which is part of the placenta. Two clamps are normally attached to the umbilical cord and the cord is cut between the two clamps. Research has shown that a delay in placement of the two clamps between 30 and 180 seconds will lead to the infant having a significantly higher Hb value at birth (83). Although delayed cord clamping is not as clinically relevant in term infants, preterm infants can have significantly higher Hb levels with delayed cord clamping which may lead to reduced RBC transfusions. In practice, Rabe et al. reported fewer RBC transfusions in 3 separate studies investigating delayed

cord clamping in preterm infants (84), although, some studies utilizing delayed cord clamping have seen no reduction in RBC transfusions (85). Almost all studies investigating delayed cord clamping in preterm infants have shown that the initial Hb concentration is higher suggesting that the early need for transfusions in preterm infants can be significantly improved (86). This is especially true since the amount of blood phlebotomized from preterm infants is the greatest in the first few weeks of life (see Chapter 5). There are some risks associated with delayed cord clamping which make clinical practice still uncertain. Polycythemia and neonatal jaundice are the main side effects reported to be associated with delayed cord clamping although these side effects are more prevalent with delayed cord clamping over 60 seconds (83). Nicholl et al. concluded in a review article of delayed cord clamping that delayed cord clamping seems to have more benefits than risks in moderately premature infants; however, larger studies with extended follow-up periods are needed to properly assess the risk-benefit ratio (86).

In addition to delayed cord clamping, minimizing iatrogenic blood loss is a valuable strategy to alleviate anemia of prematurity. Several clinical studies have shown that physician ordered phlebotomies correlate to the volume of blood transfused (74, 87-89). This correlation has also been shown in a study where strict blood transfusion criteria were employed (90). Estimates of phlebotomy loss from preterm infants in the first 6 weeks of life range from 11 to 22 mL/kg per week which corresponds to 15 to 30% of the infant's total blood volume (79). As a result hospitals have been encouraged to limit preterm infant phlebotomies if possible. A method currently under investigation for reducing the volume of blood removed in neonates is by using blood monitors rather than blood analyzers. As defined by the Food and Drug Administration analyzers require the

permanent removal of blood while monitors do not (91). Accordingly, if blood monitors can accurately perform many of the tests that blood analyzers can then the blood volume removed from preterm infants can be greatly reduced. A blood monitor device was used in a clinical trial involving 93 preterm infants (birth weight ≤1000g) to determine the potential phlebotomy reduction (92). The blood monitor obtained samples from an umbilical artery catheter, analyzed the samples for sodium, potassium and hematocrit levels and returned the sample to the patient. Although this clinical trial was terminated prematurely, the results demonstrated a 33% reduction in RBC transfusions for the monitor group compared to the no intervention group in the first week of life (92). An additional blood monitor clinical trial in preterm infants (birth weight ≤1000g) demonstrated a 46% reduction in transfusions with a 30% production in phlebotomies for the first 2 weeks of life (93). The potential for blood monitors to further reduce the iatrogenic phlebotomies is much greater if additional analytes such as glucose, bilirubin, blood urea nitrogen and creatinine are analyzed by a blood monitor. It has been estimated that the theoretical phlebotomy reduction possible with blood monitors is as high as 80% (91) which would greatly reduce the need for blood transfusions in preterm infants. In the future, blood monitors, microsampling techniques as well as less invasive tests for diagnostic purposes may help to limit preterm infant iatrogenic blood.

Erythrocyte transfusions are the most common treatment for anemia of prematurity in a hospital setting (74). Transfusions in infants carry the risk of infection as well as transfusion associated circulatory overload (TACO) and transfusion related acute lung injury (TRALI). Studies have also shown that transfusions in infants may be associated with fluid overload, electrolyte imbalance, and exposure to plasticizers, lead,

and other toxins (1). Furthermore, a recent review article suggests that the development of necrotizing enterocolitis, a potentially fatal condition, is related to the timing of packed red blood cell transfusions (94). In addition to the risks associated with transfusions, it has been debated whether maintaining high infant hemoglobin (liberal transfusions) or low infant hemoglobin (restricted transfusions) leads to a better long term outcome for the patient (8, 95). The current transfusion guidelines for the University of Iowa Neonatal Intensive Care Unit are given in Table 1.4. In this transfusion criteria chart, it is suggested to administer blood transfusions to infants at hematocrit levels ranging from 20-40% based on factors which include respiratory support, mean arterial pressure (MAP), FIO_2 level and a number of other health related factors. For the purposes of administering blood transfusions the Hb is often referred to as the hematocrit level divided by 3 (9). In 1989, preterm infants received an average of eight to ten transfusions during their hospitalization while today they only receive two (87). This suggests that physicians today use much more restrictive transfusion guidelines due to the fact that there are more risks associated with more transfusions with no obvious long term benefits.

There have been two large clinical trials in which the clinical outcome in preterm infants was compared to liberal and restrictive transfusion practices (96, 97). The larger of the two (n = 451) clinical trials known as the preterm infant in need of transfusion (PINT) trial demonstrated there were no significant differences in outcomes between a liberally and restrictively transfused group of preterm infants (Table 1.3) (96). In contrast, a smaller clinical trial (n=100) found a higher risk of brain injury and death among the restrictive RBC transfusion group with different transfusion criteria than the

PINT trial (97). The explanation of the difference between these two clinical trials remains unclear. One possible explanation for the difference is the smaller trial maintained average Hb values of 8.3 and 11.0 g/dL for the restrictive and liberal groups respectively while the PINT trial maintained average Hb values of 10.1 and 11.2 g/dL respectively. Due to the contrasting results of these two clinical trials, the acceptable transfusion criteria for preterm infants remains uncertain (8).

In addition to the transfusion clinical trials, our group published a study which examined infants who were transfused liberally and restrictively with the outcome being adolescent neurologic ability (98). This study found that the infants transfused in a restrictive manner had better neurologic outcomes in their adolescent years suggesting that restrictive transfusion practices may have a better long term outcome. The hypothesized physiologic mechanism of this study is that infants transfused restrictively have a higher endogenous Epo level which causes neuroprotective effects. Combined evidence from the transfusion based research in preterm infants suggests that a restrictive transfusion guideline which maintains an average Hb of 10 g/dL is beneficial while an overly restrictive transfusion guideline is potentially harmful to preterm infants. More information is needed for the development of optimal transfusion guide lines in preterm infants.

Despite the restrictive transfusion guidelines employed in recent years, the goal of eliminating blood transfusions in preterm infants still has not been met. One reason is that clinical tests that determine the need for transfusions in preterm infants are not currently available. Common information currently used to determine the need for erythrocyte transfusions includes hemoglobin/hematocrit levels, respiratory support,

tachycardia, tachypnea, blood pressure, sepsis and weight gain (99). All of the preceding information relates to indirect measures of hypoxia and is therefore not the optimal criteria for deciding the manner in which to transfuse preterm infants. As a result, transfusion practices on similar infants have a high level of variability (90, 100, 101). In an attempt to reduce the risks associated with blood transfusions, there have been efforts made to collect and store cord blood for future transfusions, however, procedures for collecting and storing the cord blood are technically challenging (102).

1.7 Venous-Capillary hemoglobin difference

When assessing anemia in preterm infants as well as transfusion criteria, it is very important to know the accurate hemoglobin concentration (8). However, several studies have shown that the hemoglobin level obtained depends on the site from which the blood sample was taken (Figure 1.2). The common clinical practice among physicians is to take an infant blood sample from a central line (venous sample) when a central line is already in place and obtain a blood sample from a heel stick (capillary sample) if no central line is present. Most studies done in infants which have compared capillary and venous samples have shown that the capillary hemoglobin sample is significantly higher than the venous sample (103-108). These studies are most commonly done by simultaneously sampling from a central line and a heel stick and comparing the results. The hypothesized reason that the capillary samples have higher hemoglobin values than the venous samples is poor circulation and stasis in peripheral vessels (104). This hypothesis is further supported by studies showing that the warming of the infant's heel before the capillary sample is taken reduces the difference between the capillary and venous measurement by reversing the poor circulation (104, 106, 108). In order to

properly diagnose anemia in preterm infants, capillary hemoglobin values must be corrected.

The two common methods to correct for venous-capillary differences are to multiply capillary hemoglobin values by a ratio or to subtract a fixed value from the capillary hemoglobin concentration. The important difference between the two methods is that the ratio correction factor depends on the measured capillary hemoglobin value while the fixed difference correction does not. In the literature on the correction factor no argument has yet been made to support a certain correction factor. Linderkamp and associates showed the preterm infants (gestational age 26-30 weeks) have a significantly greater difference between capillary and venous samples than term infants (36-41 weeks) suggesting that infants with a lower gestational age have less circulation in their heel (104). Finally, studies have shown in preterm (105) and term infants (103) that the difference between the capillary and venous hemoglobin samples decreases with age. This suggests that an optimal venous-capillary correction factor would take into account both the gestational age and age of the infant. Currently no such correction factor exists.

1.8 Epo clinical trials

1.8.1 *Epo clinical trials conducted in preterm infants*

Physiologic based evidence provides rationale which supports administering Epo to preterm infants in the first few weeks of life. Preterm infants endogenous Epo levels drop significantly after they are born due to an oxygen rich postnatal environment (73). With the exogenous administration of Epo, the serum Epo concentrations in preterm infants rise well above the natural endogenous levels of approximately 30 mU/mL (9) to as high as 40,000 mU/mL for IV bolus doses of 2500 U/kg (47). The hypothesis for Epo

clinical trials is that administering Epo to preterm infants will result in sufficient Hb production to reduce or in some cases eliminate the blood transfusions needed clinically.

There have been more than 30 clinical trials in which Epo was administered to preterm infants with the objective being to reduce the number and volume of RBC transfusions or to determine the neuroprotective effect of Epo (1). Several of these clinical trials have been summarized in Table 1.5. Primary outcomes for Epo clinical trials include the use of one or more RBC transfusion (3-7, 109-117), the total volume (mL/kg) of blood transfused per infant (6, 112, 113, 117), the number of RBC transfusions per infant (3, 5, 112-120), the number of donors the infant was exposed to (3, 120), infant mortality during initial hospital stay (3, 4, 6, 7, 109, 111, 118, 119) and length of hospital stay (113, 119, 121). In addition, the main side effects analyzed for Epo clinical trials include retinopathy of prematurity (3, 7, 111, 113, 114, 118), sepsis (4, 5, 110, 111, 113, 114, 117, 122), necrotizing enterocolitis (3, 6, 7, 109-111, 113, 114, 117, 118, 122), intraventricular hemorrhage (3, 5-7, 110, 111, 122), periventricular leukomalacia (3, 7), bronchopulmonary dysplasia (3, 6, 7, 109, 113, 114, 117, 118, 122), neutropenia (109-113, 119) and hypertension (4, 6, 110, 111, 113). The main results in the Epo clinical trials in preterm infants are that Epo led to a significant reduction in the total volume (mL/kg) of blood transfused per infant, the number of transfusions per infant and the number of donors to whom the infant was exposed. Furthermore, the only side effect of Epo that was shown to be more prevalent in the Epo group than the placebo group was retinopathy of prematurity (stage >=3). Retinopathy of prematurity was only shown to have a higher prevalence in the Epo group in one study (114). As a consequence, retinopathy of prematurity is now closely monitored in clinical Epo trials.

Drawing conclusions regarding the efficacy of Epo reducing blood transfusions in preterm infants is difficult because of the different administration routes of Epo, doses of Epo, duration Epo was administered and the enrollment criteria for preterm infants in the study (Table 1.5). In a comprehensive review of the 27 clinical trials where Epo was administered to preterm infants the author concluded that although Epo reduced the volume of RBCs transfused and the overall number of transfusions administered, these results were of limited clinical importance (1). In addition, the small sample size in most Epo clinical trials makes it difficult to reach statistically significant endpoints. Having a large enrollment in these clinical trials is difficult because of consent issues and the paucity of patients born with a critical level of prematurity. Furthermore, the inability to blind many of the Epo clinical trials introduces bias into the clinical trials.

The trend in recent years has been to increase the Epo doses used in clinical trials in order to maximize the endogenous RBC production of preterm infants, however, these dose increases have been arbitrary and not based on PK/PD principles. In addition, most Epo clinical trials are now dosing through the subcutaneous route in order to maximize the use of EpoRs (48). Veng-Pedersen et al. determined that Epo is eliminated through two pathways in a bone marrow ablation study: a nonlinear Epo receptor mediated pathway which leads to erythrocyte proliferation and an uncharacterized linear pathway which does not lead to erythrocyte proliferation (41). Thus, increased utilization of the Epo receptor mediated elimination pathway will lead to greater RBC cell production and less wasted Epo. The gradual absorption of subcutaneous Epo likely allows for greater use of the receptor mediated pathway than IV bolus dosing because the Epo receptors are less likely to become saturated. Additional evidence was given for subcutaneous dosing

in a study which demonstrated more Epo is lost through renal wasting when it is dosed through the bolus route (123). However, the analysis also needs to consider how the Epo receptor pool is changing as a function of Epo dosing.

In order to determine what future clinical trials are most likely to succeed, Epo dosing simulations based on PK/PD principles are needed to determine the potential to reduce or eliminate RBC transfusions in preterm infants. These simulation studies can suggest future clinical Epo trials with the best chance of eliminating RBC transfusions in preterm infants.

1.8.2 *Neuroprotective properties of Epo*

Although Epo has been shown to have very few side effects in preterm infants, a potential benefit of Epo that is still under investigation is its neuroprotective properties. A study published in 2005 administered large (1500U/kg) doses of Epo to adults and examined the resulting Epo concentrations in the cerebral spinal fluid (124). This study effectively shows that Epo is capable of crossing the blood brain barrier (BBB). The mechanism in which Epo crosses the BBB has not yet been identified but has been shown to be a first-order non-saturable process (124).

Epo has been shown to exert its neuroprotective effects by several mechanisms shown in the literature, although, Epo has only been indirectly shown to be neuroprotective in infants. Epo raises the blood hemoglobin (Hb) concentration which increases the oxygen carrying capacity the blood. This restored oxygen supply ameliorates attention difficulties and psychomotor slowing, improves memory capacities and normalizes neuroendocrine functions (125). Additionally, Epo can act as a neuroprotective factor directly in the brain by protecting against glutamate induced cell

death in a dose dependent way (126). Furthermore, a molecular biology study demonstrated Epo's neuroprotective properties by cross talk between the signaling pathways of janus kinase-2 and nuclear factor-kb (127). Other hypothesized mechanisms in which Epo can aid in the developing brain include neurogenesis, decreased neuronal apoptosis, decrease in inflammation, decreased nitric oxide-mediated injury and increased protective effects on glia (128).

With the evidence stated in the previous studies it is reasonable to assume that Epo may have some neuroprotective properties in infants, however, there are few studies that show the direct neurologic benefit of Epo in preterm infants. More recently research has shown Epo leads to better clinical outcomes in infants with hypoxic ischemic encephalopathy (129) and intraventricular hemorrhage (130). A study recently published in our group examined infants who were transfused liberally (at high Hb concentration) and restrictively (low Hb concentration) with the outcome being adolescent neurologic ability (98). Results from this study showed the restrictively transfused infants had significantly higher scores in verbal fluency, visual memory and reading. One possible explanation for the increased scores is that the restrictively transfused infants had higher endogenous Epo concentrations causing neuroprotection and leading to better neurologic outcomes at the age of adolescents. This study indirectly demonstrated that Epo may have neuroprotective effects on preterm infants. Additional long term studies are needed to better assess the potential neuroprotective aspects of Epo in preterm infants. Specifically, investigators are encouraged to follow up on subjects who received Epo therapy as infants and assess cognitive ability. Neuroprotective effects of Epo could have

a major influence on the risk-benefit ratio when considering administration of the drug to preterm infants.

1.9 Personalized medicine

Personalized medicine is based on the principle that it may be possible to optimize drug therapy for individual patients based on information regarding genetics and numerous other covariates. One particular area where personalized medicine has been effective is anticancer therapy (131-134). One study reported that different types of advanced cancer, when treated with available drugs, have a variation in response rate from 10 to >90% (134). Specifically, a common biological marker used for predictive therapy in breast cancer is the estrogen receptor which is used in selecting patients for endocrine hormone therapy (133). The rationale that the estrogen receptor was involved with tumor growth is based on the fact that the growth of some breast cancer tumors is based on the endogenous estrogen concentration; therefore, anti-estrogenic therapy may correlate with reduced tumor growth (132). A meta-analysis of randomized clinical trials showed that anti-estrogenic therapy was more effective in women with breast tumors rich in estrogen receptors than tumors not rich in estrogen receptors (135). This meta-analysis demonstrates the importance and utility of personalized medicine.

In addition to biological markers suggesting if an individual patient should receive a treatment, biological markers have led to predictions of safe and effective doses of specific drugs. Individualized doses of the anticancer drug paclitaxel have been determined based on covariates such as age, gender, bilirubin levels and single nucleotide polymorphisms in genes encoding for metabolizing enzymes (131). The pharmacokinetic profile of paclitaxel can vary significantly from patient to patient and excessive

concentrations of paclitaxel have been associated with neutropenia, peripheral neuropathy and decreased survival (136). Therefore, it is important to individualize paclitaxel therapy in order to avoid excessive concentrations. An analysis of the aforementioned covariates associated with paclitaxel elimination has led to individualized patient therapy which has maximized the benefits of paclitaxel while minimizing the toxic side effects (131).

1.9.1 Personalized medicine for Epo therapy

Epo is known to have a large intra-individual variability in pharmacodynamics among adults who are indicated for Epo therapy (137). Therefore, a personalized medicine approach to Epo therapy is desirable to maintain Hb concentrations within a target range. A personalized medicine approach has previously been used for Epo dosing in renal dialysis patients by the use of artificial neural networks (ANNs) (137-139). The principle behind artificial neural networks is that complex relationships can be determined between input and output using artificial neurons. The study design using ANNs normally consists of a learning phase, a cross-validation phase and a test phase. Often many different ANN are trained during the learning phase and the ANN that performs the best in the cross-validation phase is used for the testing phase of the study. A study published by Gabutti et al. used ANNs to predict the dose of Epo required in renal dialysis patients to obtain Hb levels in the target range (137). This study used data from 432 renal dialysis patients and segregated 50% of the data to the learning phase, 10% to the cross validation phase and 40% to the testing phase. Results from this study show that the ANN was able to predict an insufficient Epo dose in 48% of patients compared to 25% according to the nephrologists' opinion. The data input into the ANN

that was found to correlate significantly with the Epo dose was weight, ferritin, age, Epo administration route and presence or absence of cardiomyopathy. The main advantage of using ANNs in a personalized medicine approach is that they handle missing and incorrect data well and can describe non-linear relationships. The main disadvantage of ANNs is that their predictions give no insight into the physiologic mechanism of action by which Epo exerts its effect. ANNs could possibly be used in Epo clinical trials involving preterm infants to determine which infants will respond well to Epo therapy based on numerous covariates.

Although a personalized medicine approach has not been used for Epo therapy in preterm infants, sufficient evidence exists that suggests that such an approach is needed. Information regarding the complex erythropoietin receptor dynamics suggests that erythropoietin pharmacodynamics is highly variable and remains poorly understood (45, 48). Additional evidence regarding erythropoietin pharmacodynamics was recently published by Freise et al. in a study that determined preterm infants are capable of producing about 4 times as much Hb as they do naturally in the first month of life (9). However, there was a large variability in the parameter determined corresponding to the maximum Hb production possible in preterm infants suggesting that there are many unexplained factors that determine how much Hb preterm infants can produce. These unexplained factors could be covariates that include but are not limited to: genetic variation, Hb type profile, interleukin types and concentrations, ATP levels, disease states and general health characteristics such as birth weight and gestational age. If the aforementioned covariates can be determined in preterm infants in the first few days of

life, it may be possible to identify a select group of infants who will respond best to Epo therapy.

1.10 Epo population pharmacokinetics

One of the main areas of research which has led to the personalized medicine approach in recent years is population pharmacokinetics. Population pharmacokinetics examines the sources of variability in PK/PD in a target population group. There are numerous sources of variability among patients including demographic, biochemical pathophysiological and genetic factors. Although a population pharmacokinetic analysis has not been previously completed in preterm infants, numerous Epo population pharmacokinetic studies have been examined in adults (140-145). Consideration of these previous population pharmacokinetic models is important for design of preterm infant studies in the future. This is particularly true if, sources of Epo PK variability in adults are similar in preterm infants.

1.10.1 Jolline et al. model

Jolline et al. developed a population pharmacokinetic model in rats examining the influence of weight, sex and pregnancy status on the pharmacokinetic parameters (145). Pegylated Epo (PEG-Epo) was used in this study rather than recombinant Epo, however, the hypothesized mechanism of action is the same for Epo and PEG-Epo (145). The PEG-Epo was administered by an IV bolus dose and a two-compartment model with linear elimination from the central compartment was used to model the serum concentration of PEG-Epo leading to a biexponential disposition given by Equation 1.3.

The magnitude of the variability for the serum PEG-Epo concentration was modeled using an additive error model:

$$\ln c_{obs} = \ln c_{pred} + \varepsilon \qquad (1.32)$$

where, c_{obs} is the observed serum concentration at time t; c_{pred} is the corresponding model predicted concentration of serum PEG-Epo concentration and ε is the residual departure of the natural logarithm of the observed concentration from the predicted concentration. The interindividual variability in each pharmacokinetic parameter was given by the following equation:

$$In(P_j) = \ln(P^*) + n_j \qquad (1.33)$$

where, P_j is the pharmacokinetic parameter for the jth individual; P* is the population typical value for the pharmacokinetic parameter and n_j is the interindividual random effect which was assumed to follow a Gaussian distribution.

Results from this study showed that a twofold increase in the weight resulted in a 1.7 and 2.4 times increase in the absolute clearance (mL/h) and volume of distribution of the central compartment (mL), respectively. Since there was not a 1:1 correlation with increased weight to increased clearance this needs to be considered as a possibility in preterm infants. Unfortunately, the weight adjusted clearance and volume of distribution were not given in this study. In female rats there was an 11% drop in the volume of distribution of the peripheral compartment and there was no detectable difference observed for pregnancy status on pharmacokinetic parameters. Based on this result future Epo studies in preterm infants should consider sex differences in erythropoietic response. One weakness of the model used in this study is the two compartment linear model is not consistent with the non-linear behavior of Epo when receptor saturation

occurs (41). An additional weakness of this model is that translation of pharmacokinetic parameters from rats to humans can be difficult and may result in erroneous parameter estimation.

1.10.2 Chakraborty et al. model

Chakraborty et al. developed a population pharmacokinetic model for Epo in critically ill subjects (144). These critically ill subjects were admitted to the ICU for at least 3 days and were 18 years or older. Subjects received 40,000 U/week of Epo subcutaneously for as long as 4 weeks. The absorption and elimination of Epo were modeled according to the following equations:

$$\frac{dA_A}{dt} = -k_a \cdot A_A \tag{1.34}$$

$$(V/F) \cdot \frac{dc_s}{dt} = k_a \cdot A_A - (CL/F) \cdot c_s + k_{IN} \tag{1.35}$$

where, A_A is the drug amount at the subcutaneous site available for absorption; c_s is the serum Epo concentration; k_a is the first-order absorption rate constant; F is the relative bioavailability of a subcutaneous Epo dose; V/F is the apparent volume of distribution; Cl/F is the apparent clearance and k_{IN} is the endogenous Epo production rate. The interindividual variability in each pharmacokinetic parameter was given by Equation 1.33.

Results from this study showed that similar to the previous study the absolute clearance of Epo is dependent on the weight of the subject, however, all other covariates such as sequential organ failure assessment, previous RBC transfusions, age and gender were not significant predictors of the pharmacokinetic parameters. An important weakness of this model is that RBC transfusions were not accounted for in the model. It

is necessary to include RBC transfusions in the model because any blood transfusion would lead to a lower concentration of Epo. Although the numbers were not stated, it is reasonable to assume that several patients in this study received blood transfusions possibly confounding the results. One way to compensate for RBC transfusions is to use event processing modeling software such as WINFUNFIT (146) to perform separate integrations of the differential equations before and after an event such as a RBC transfusion. An additional weakness of this study is that the Epo doses were not adjusted according to the weight. This failure to adjust the Epo doses for weight could lead to significantly higher Epo concentrations in individuals who weigh less. In addition, if the Epo receptor pool is proportional to the weight then the subjects who weigh less are more likely to exhibit non-linear Epo behavior due to receptor saturation. This failure to adjust for doses may have caused a larger than expected interindividual variability.

1.10.3 Olsson-Gisleskog et al. model

Olsson-Gisleskog et al. performed a population pharmacokinetic meta-analysis of Epo in healthy subjects (141). One of the main advantages of this study is that more data was available for analysis using the meta-analysis method which can allow for detection of covariates which influence PK parameters. The disadvantage of the meta-analysis approach is the lack of homogeneity of the studies from which the data was taken. The pharmacokinetics of Epo after IV bolus administration were modeled using the following differential equations:

$$\frac{dA_2}{dt} = k_{32}A_3 - k_{23}A_2 - k_{20}A_2 - \frac{V_{\max}A_2/V}{k_m + A_2/V} \tag{1.36}$$

$$\frac{dA_3}{dt} = k_{23}A_2 - k_{32}A_3 \tag{1.37}$$

where, A_2 and A_3 are the amounts of Epo in the central and peripheral compartments, respectively; V is the volume of distribution in the central compartment; k_m is the concentration at which the saturable pathway operates at half the maximum rate; V_{max} is the maximum elimination rate of the saturable pathway; k_{20} is the linear elimination rate constant and k_{23} and k_{32} are intercompartmental rate constants. This pharmacokinetic model is consistent with the hypothesized elimination mechanism of Epo stated as receptor mediated binding followed by endocytosis and lysosomal degradation (41). The interindividual variability in each pharmacokinetic parameter was given by a modified version of equation 1.33 where the interoccasion random effect was included such that:

$$P_j = P^* e^{(n_j + \tau_k)} \tag{1.38}$$

where, τ_k is the interoccasion random effect.

Results from this study showed that females have a significantly lower (21%) baseline Epo value than males. This may suggest that females produce a different amount of endogenous Epo than males. The baseline difference should be examined for consistency when considering Epo data in preterm infants. In addition, this population pharmacokinetic analysis found that an increased age is associated with increased volume of distribution (1.1% increase per year) in the peripheral compartment. The authors stated in this study that the peripheral compartment refers to drug distribution in the bone marrow or blood cells although no evidence was given for this explanation. This finding may suggest that higher doses of Epo are needed in older subjects to reach the concentrations of Epo necessary in the bone marrow. Perhaps one weakness of the model

presented in this study is that the model did not account for the endogenous Epo production. A strength of this model is that the baseline endogenous Epo concentration was accounted for, however, details regarding how the endogenous Epo was modeled are limited. In addition, it was impossible to confirm the endogenous Epo model due to the inability to distinguish the endogenous and exogenous Epo. Future studies using Epo labeling methods will be able to obtain data to accurately model the endogenous Epo level.

1.11 Dosing optimization

The principle of dosing optimization is that if a fixed amount of drug is available to administer to a subject over a specified time period, how should that drug be administered to produce the maximum effect? For example, consider a drug with linear elimination and pharmacodynamics given by a simple E_{max} model. For this situation, it is not advantageous to increase doses when the effect is already close to the maximum effect because this will result in using more drug and getting little added effect. Accordingly, for different optimization studies the point of diminishing returns must always be considered. In addition, the administration route is also an important consideration for dosing optimization. In a situation where a low but constant drug concentration is needed an IV infusion could be considered. On the other hand, if higher plasma drug concentrations are needed an IV bolus dose could be considered. Covariate information obtained from population pharmacokinetic studies such as demographic, biochemical pathophysiological and genetic factors can help identify pharmacokinetic variability used for dosing optimization studies. This population pharmacokinetic information can be used for simulation studies to determine the optimal route of drug

administration, time of administration and dose. Dosing optimization is an area high interest in pharmaceutical development because approximately 90% of drugs fail during clinical development and clinical trials are associated with a high cost (147). Successful dosing optimization could lower costs by saving drug and producing clinically relevant outcomes. In the future, consideration of the intra and interindividual variability in drug response could lead to dosing optimization on the individual level which is the ultimate goal of personalized medicine.

There has been some research in which the authors examined dosing optimization. A recent study used dosing optimization techniques to test 6 simulated doing regimens of aprotinin in neonates undergoing heart surgery in order to maintain a predefined therapeutic concentration (148). The study identified the dose of aprotinin most likely to give a drug concentration within the therapeutic range (Figure 1.7). In an additional study, the drug ivabradine was investigated using clinical trial simulation in order to determine how to proceed with a phase III clinical trial (149). Results from the dosing optimization determined a recommended dose of ivabradine and the number of subjects per group needed to demonstrate a decrease in chest pain. Additional studies are beginning to use other factors for optimization such as study length (150).

1.11.1 Bayesian dose optimization

In addition to dosing optimization being used to maintain therapeutic drug concentrations, Bayesian dosing optimization has been used to adjust dosing regimens based on the available data from an individual patient (151). The Bayesian individualized dosing optimization has been accomplished using the MM-USCPACK software developed at the University of Southern California (152). The software

developed uses preexisting population pharmacokinetic models estimate the PK parameters in an individual subject. Although a single drug concentration from a subject can be used to estimate PK parameters from a preexisting model, multiple drug concentrations improve the precision and accuracy of the PK parameter estimates.

This individualized dosing optimization methodology has been successfully used in HIV therapy (151). One HIV infected subject showed impaired clearance of the anti-retroviral drug efavirenz and the dosing optimization software suggested reduction of the dose from 350 mg to 200 mg. At a follow up appointment the individual subject had efavirenz concentration within the therapeutic range. The same study also found similar results in a subject with higher than expected clearance (151). One problem with the individualized dosing optimizing is performing statistical analysis to determine success. Since each dosing optimization is a case by case basis, statistically analysis cannot be completed, however, in the 4 case studies used for the HIV dosing optimization study no adverse side effects were reported in the patients once the optimized dosing change was implemented.

Although dosing optimization has been done for clinical trial simulations, dosing optimization involving numerical optimization with pharmacodynamic modeling has not previously been done. Previous clinical research on Epo administration in preterm infants has used a "trial and error" approach to test for clinical improvements. As a result, an individual approach is necessary that accounts for the complex receptor-based PK/PD. A PK/PD model that takes into account the dynamic clearance of Epo and the factors that influence its pharmacodynamic response is needed for an optimization of Epo dosing in preterm infants.

1.11.2 Epo dosing optimization in preterm infants

Epo dosing optimization in preterm infants has not previously been examined; however, many covariates have been identified from a comprehensive review of the literature which may predict Epo responsiveness and the degree of anemia suggesting how to proceed with optimized Epo dosing. Large variability in the pharmacokinetics (47) and pharmacodynamics (9) of Epo in preterm infants suggests population pharmacokinetics combined with covariates may help more accurately determine PK/PD information for an individual infant. Reviewing all covariates which can potentially impact erythropoiesis and anemia is important to develop an optimized dosing regimen.

Interleukins, IL-1, IL-2, IL-3, IL-6 and biomarkers C-reactive protein, IFN-γ, tumor necrosis factor, hepcidin and ferritin have been previously discussed as influencing erythropoiesis (Table 1.1). In addition to these factors, fetal hemoglobin and 2,3 diphosphoglycerate (2,3 DPG) have been shown to decrease the oxygen delivery to the tissues in preterm infants (77). Although fetal hemoglobin and 2,3 DPG may not inhibit erythropoiesis, they may significantly exacerbate anemia of prematurity. From reviewing the population pharmacokinetic studies one can see that weight, age and sex can influence pharmacokinetic parameters. Specifically, population pharmacokinetic studies have shown that the clearance and volume of distribution of Epo increase with weight (144, 145). Olsson-Gisleskog et al. demonstrated the females have a lower baseline Epo level than males (141) and Chakraborty et al. suggested that the volume of distribution of Epo can change based on the age of the subject (144). Other factors which have been speculated to alter erythropoiesis but have not been directly shown in the literature are gestational age (64) and respiratory status (96). Infants with a lower gestational age have

been shown to have lower Hb concentrations over the first 28 days of life suggested their erythropoiesis may be inhibited (64). Respiratory status (i.e. using a respirator or supplemental oxygen) is used in deciding when to transfuse preterm infants (96) suggesting that this factor could also influence erythropoiesis. Furthermore, a large amount of covariate information is obtained from the hospital during a preterm infant's care (Table 1.6). As many of these covariates as possible should be considered and examined for significance when designing optimized dosing regimens for preterm infants.

1.12 Hypothesis and specific aims

Overall Hypothesis: The management of anemia of prematurity can be improved by Epo administration as well as phlebotomy reduction and using restrictive transfusion criteria.

Specific aim 1: Provide experimental evidence that RBC production is dependent on the Epo receptor level.

Hypothesis 1: The Epo receptor pool will be associated with an increased RBC production under anemic conditions.

Specific aim 2: Determine the optimal time to administer Epo in newborn sheep based on a dynamic Epo receptor PD model

Hypothesis 2: The optimal time to administer Epo to anemic sheep will be when the Epo receptor pool is the largest based on a modeled receptor feedback mechanism.

Specific aim 3: Determine if elimination or reduction of RBC transfusions in preterm infants is possible by optimized Epo dosing simulation studies which utilize the complex Epo PK/PD.

Hypothesis 3: The elimination of RBC transfusions is possible with optimized Epo dosing in a select group of infants who are able to produce more RBCs and have less blood removed for clinical laboratory testing.

Specific aim 4: Determine if elimination or reduction of RBC transfusions in preterm infants is possible by simulations of reducing blood removed for clinical laboratory testing.

Hypothesis 4: The elimination of RBC transfusions is possible with simulated phlebotomy reduction in a select group of infants who received fewer transfusions in the first month of life. This elimination of RBC transfusions will not be as substantial as provided by simulated optimized Epo dosing.

Specific aim 5: Determine if elimination or reduction of RBC transfusions in preterm infants is possible by a simulation of optimized Epo administration, laboratory phlebotomy reduction and use of restrictive RBC transfusion criteria.

Hypothesis 5: The elimination of RBC transfusions is possible with simulated combination therapy in a larger number of infants than with Epo simulation or phlebotomy reduction alone. This reduction in RBC transfusions will likely lead to a better clinical outcome for preterm infants.

1.13 Outline of thesis

Chapter 2 explores how the EpoR state in sheep is related to the amount of Hb that each sheep is able to produce. This suggests future studies which examine the EpoR state of infants are necessary for predicting erythropoietic response. Chapter 3 further elucidates the EpoR dynamics empirically examined in chapter 2 and presents a receptor based model which can predict erythropoietic response based on the current EpoR level.

Chapter 3 suggests that the timing of Epo administration is important due to the receptor dynamics.

Chapters 4-6 all involve a group of 27 preterm infants who were extensively studied for this research. Chapter 4 simulates Epo administration in this group of preterm infants based on their pharmacokinetic and pharmacodynamic parameters. This chapter determines an optimal Epo dosing regimen for these 27 preterm infants as well as estimating the potential for reducing blood transfusions. Chapter 5 examines the fact that phlebotomies play an important role in exacerbating anemia of prematurity. This chapter determines the potential for reducing blood transfusions if the phlebotomized blood is theoretically reduced by a certain percent. Finally, chapter 6 focuses on reducing blood transfusions in preterm infants with simulated phlebotomy reduction, Epo dosing and the use of a restrictive RBC transfusion criteria. Chapter 6 presents simulations of combination therapy as a best case scenario for reducing or eliminating RBC transfusions in preterm infants.

Table 1.1 List of biological markers shown to influence Epo responsiveness

Biomarkers Influencing Epo Responsiveness	Reference
IL-1	152
IL-2	11
IL-3	26
IL-6	11
C-reactive protein	11
IFN-γ	26
Tumor necrosis factor	153
Hepcidin	27
Ferritin	136

Table 1.2 Summary of Epo pharmacokinetic parameters in preterm infants and adults.

	Preterm infant (n=30) (47)	Preterm Infant (n=7) (43)	Healthy adult (n=10)(46)
Dose	500-2500 U/kg	10-500 U/kg	10-500 U/kg
Enrollment Criteria	≤ 30 Weeks	<1.25 kg	N/A
Elimination half-life (hr)	5.4-8.7	0.8-2.0	2.0-5.3
Volume of distribution (mL/kg)	89-115	74-93	44-50
Clearance (mL/hr/kg)	8.2-17.3	16.0-45.6	6.2-13.1

Table 1.3 Hemoglobin concentration when red blood cell transfusions are indicated according to the preterm infant in need of transfusion (PINT) study.

		Hemoglobin Concentration Low Threshold (g/dL)	
Age in Days	Blood Sampling Site	Respiratory Support	No Respiratory Support
1-7	Capillary	11.5	10.0
	Central	10.4	9.0
8-14	Capillary	10.0	8.5
	Central	9.0	7.7
≥15	Capillary	8.5	7.5
	Central	7.7	6.8
		Hemoglobin Concentration High Threshold (g/dL)	
Age in Days	Blood Sampling Site	Respiratory Support	No Respiratory Support
1-7	Capillary	13.5	12.0
	Central	12.2	10.9
8-14	Capillary	12.0	10.0
	Central	10.9	9.0
≥15	Capillary	10.0	8.5
	Central	9.0	7.7

Table 1.4 Red blood cell transfusion guideline for the University of Iowa neonatal intensive care unit.

	Subgroup A	Subgroup B	Subgroup C	Category II	Category III	Category IV
	(asymptomatic)	(symptomatic)	(pre-surgical)	(mild resp dis)	(mod resp dis)	(severe resp dis)
A. REQUISITE HEMATOCRIT	<20%	<25%	<30%	<30%	<35%	<40%
B. RESPIRATORY DISEASE		None to minimal		Mild	Moderate	Severe
Conventional ventilation	None	None	None	MAP ≤ 6 cm. water	MAP 6-10 cm. water	Yes
High frequency ventilation	None	None	None	MAP ≤ 8 cm. water	MAP 9-12 cm. water	Yes
CPAP	None	None	None	MAP ≤ 6 cm. water	MAP 6-10 cm. water	Does not apply
FIO2	≤0.25	≤0.25	≤0.25	>0.25 and ≤ 0.35	>0.35	>0.35
Nasal Cannula with 100% O2	≤1/16 L/min	≤1/16 L/min	≤1/16 L/min	1/8-1/4 L/min	>1/4 L/min	Does not apply
C. CLINICAL SIGNS	Absent	Any 1 of 4 signs listed below	Major surgical procedure	Often present	Often present	Sepsis, NEC, blood loss, etc.
	1) sustained tachycardia averaging > 180/min. for 24 hours based on the nursing record					
	2) sustained tachypnea >80/min. for >24 hours based on the nursing record					
	3) >10 apnea or bradycardia/8 hours, or > 2 apneic or bradycardic episodes requireing bag and mask ventilation					
	4) weight gain <10g.day x 4 days while receiving >100 kcal/kg					

Table 1.5 Summary of Epo clinical trials in preterm infants

Study	Epo Dose IU/kg/WEEK	Administration Time	Administration Route	Enrollment Criteria	N Total
Avent 2002	**1200**	**Day <=7-42**	**SC**	**900-1500g**	**93**
Soubasi 1995	**750**	**Day 0-28**	**SC**	**<=31 Weeks <=1500g**	**97**
Salvado 2000	**600**	**Day 8-36**	**SC**	**<1500g**	**60**
Haiden 2005	**2100**	**Day 0-59**	**IV**	**<800g, <=32 Weeks**	**40**
Soubasi 2000	**700**	**Day 7-?**	**SC**	**<=31 Weeks<=1300g**	**36**
Arif 2005	**400**	**Day 7-42**	**SC**	**<33 Weeks**	**292**
Meyer 2003	**1200**	**Day 0-21**	**SC**	**<33 Weeks, <=1700g**	**43**
Ohls 1995	**1400**	**Day 0-14**	**IV**	**>27 Weeks, 750g-1500g**	**20**
Maier 1994	**750**	**Day 3-42**	**IM**	**750-1499g**	**241**
Ohls 2001B	**1200**	**Day 2 -35 weeks GA**	**IV, SC**	**<32 Weeks, 401-1000g**	**118**
Maier 2002	**750**	**Day 3-63**	**IV, SC**	**500g-999g**	**145**
Romagnoli 2000	**900**	**Day 7-28**	**SC**	**<= 34 Weeks**	**230**
Obladen 1991	**70**	**Day 4-25**	**?**	**28-32 Weeks**	**93**
Ohls 2001A	**1200**	**Day 2 -35 weeks GA**	**IV**	**<32 Weeks, 401-1000g**	**172**
Yeo 2001	**750**	**Day 5-?**	**SC**	**<= 33 Weeks**	**100**

Table 1.6 Summary of covariate information available for preterm infants hospitalized for the first month of life

Neonatal information CPAP [a] Intubation Supplemental oxygen Chest compressions Race Steroids use Birth weight Zscore of the birth weight Apgar score (1 min) Apgar score (5 min) Gestational age Hemoglobin level at birth Length at birth Head size at birth Cord pH	**Neonatal information (continued)** First temperature taken ≤60miutes Auxiliary temperature Respiratory rate Blood pressure Heart rate Complete blood count results Caffeine use **Maternal information** Mother age Insulin dependent diabetes Hypertension Chorioamnionitis Rupture of Membranes Steroids prior delivery

a. CPAP: Continuous positive airway pressure

Figure 1.1 Erythrocyte formation pathway and biomarkers involved in cell differentiation

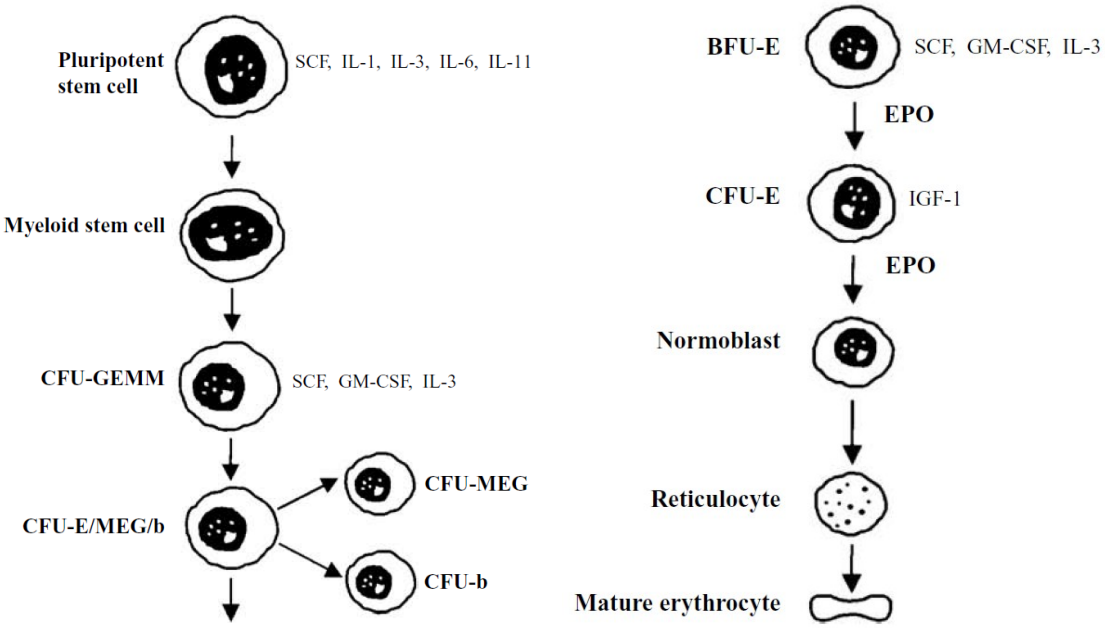


Figure 1.2 Allometric scaling of Epo clearance between species.

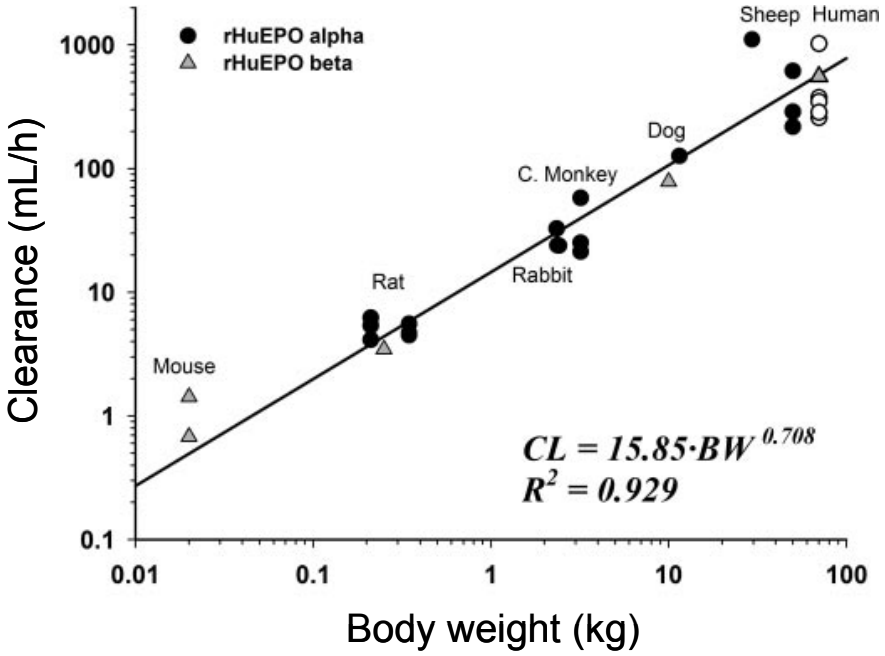


Figure 1.3 Allometric scaling of Epo volume of distribution between species.

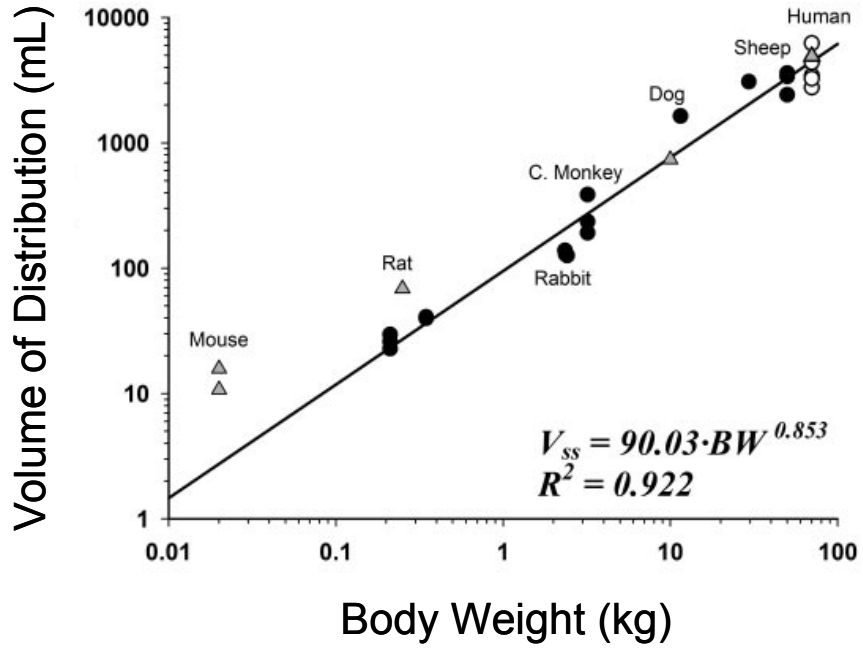


Figure 1.4 Average Hb values (±1 SD) of preterm infants with different gestational age over the first 28 days of life. Solid line represents the average Hb values and dashed line represents ±1 SD. These figures exclude infants who received transfusions. Left panel is infants with gestational age 35-42 weeks (n=41,957) and right panel is infants with gestational age 29-34 weeks (n=39,559)

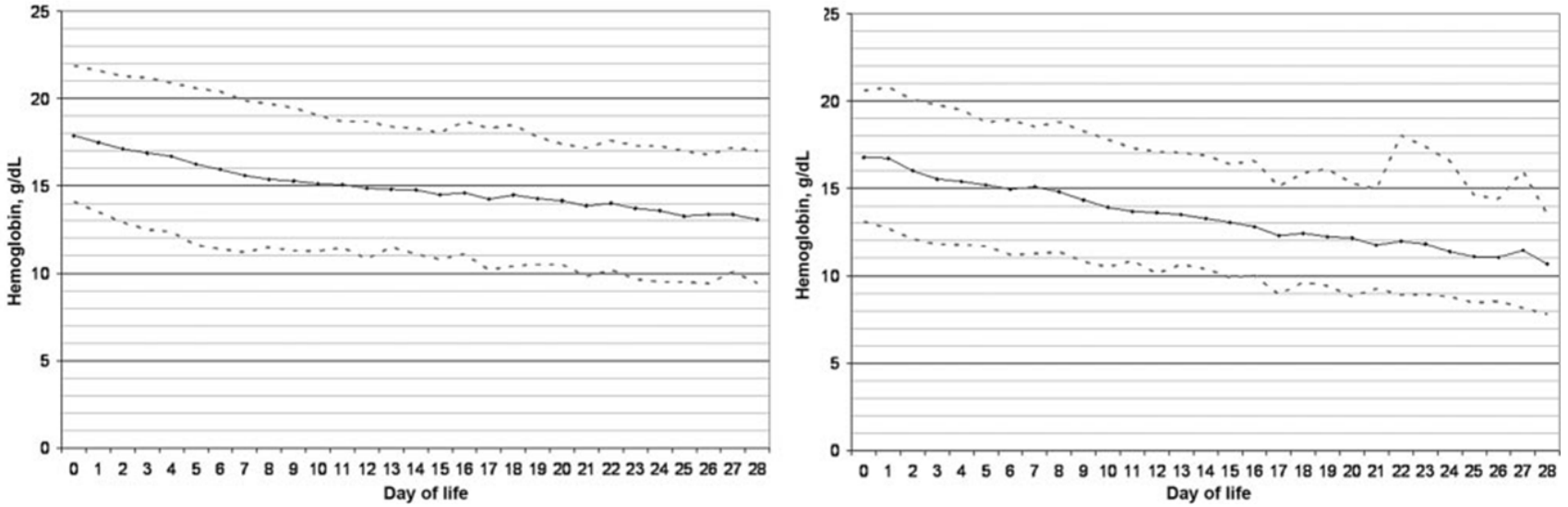


Figure 1.5 Oxygen unloading capacity for preterm infants at different postnatal ages. The double arrows represent the oxygen unloading capacity between a given arterial and venous partial pressure of oxygen in the blood.

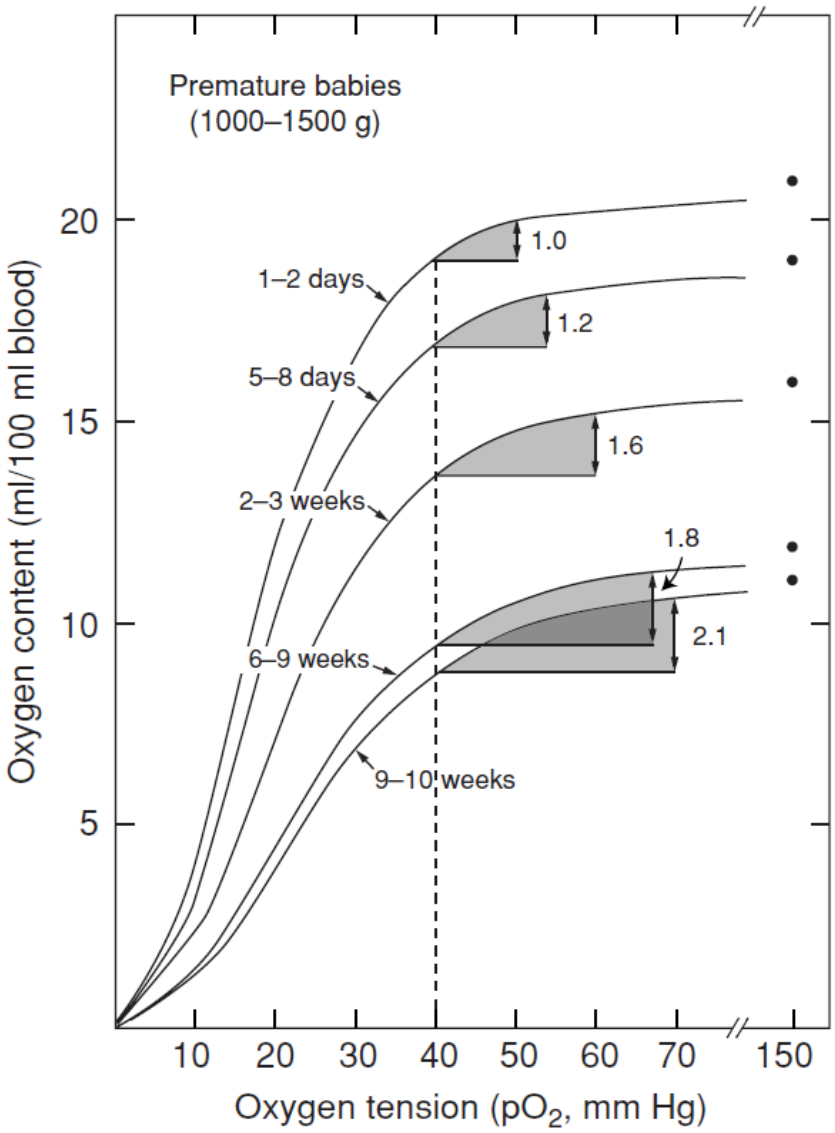


Figure 1.6 Hematocrit measurements for capillary and venous blood sampling in preterm infants

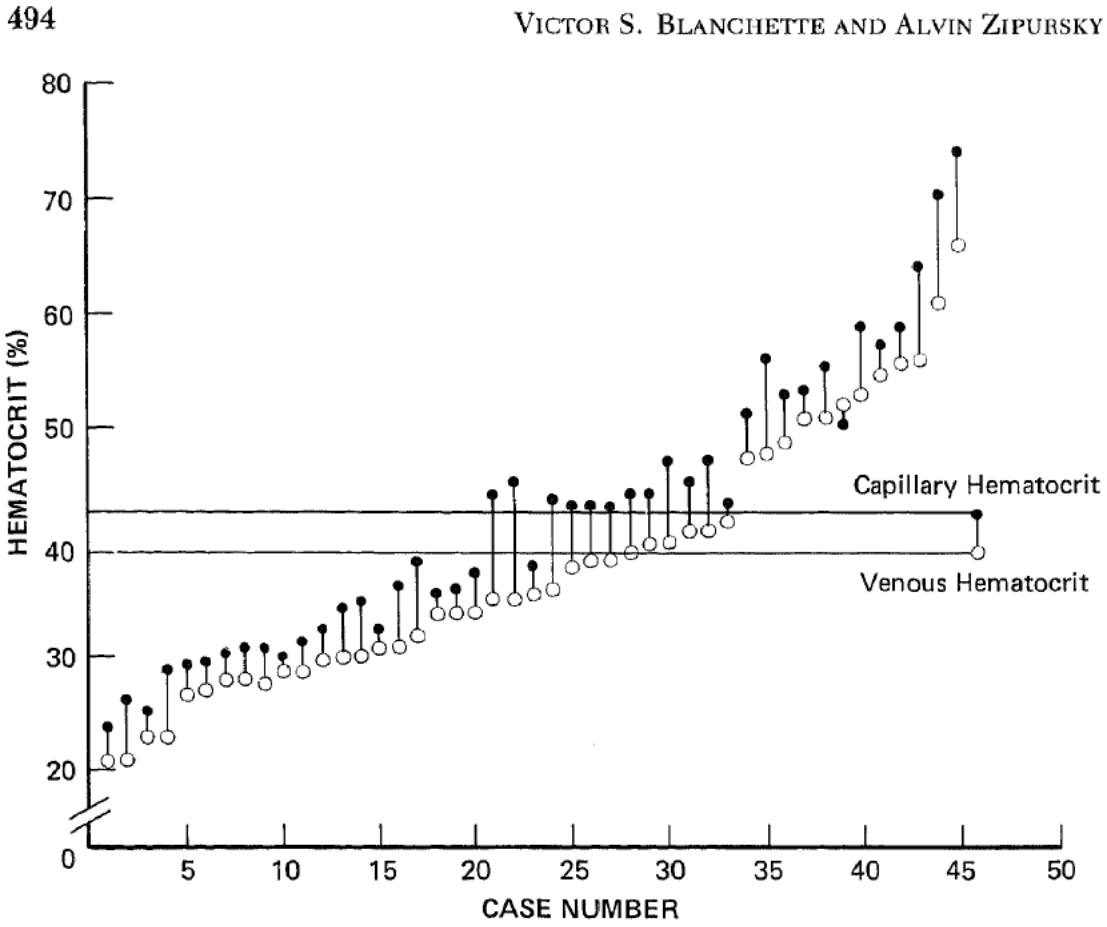
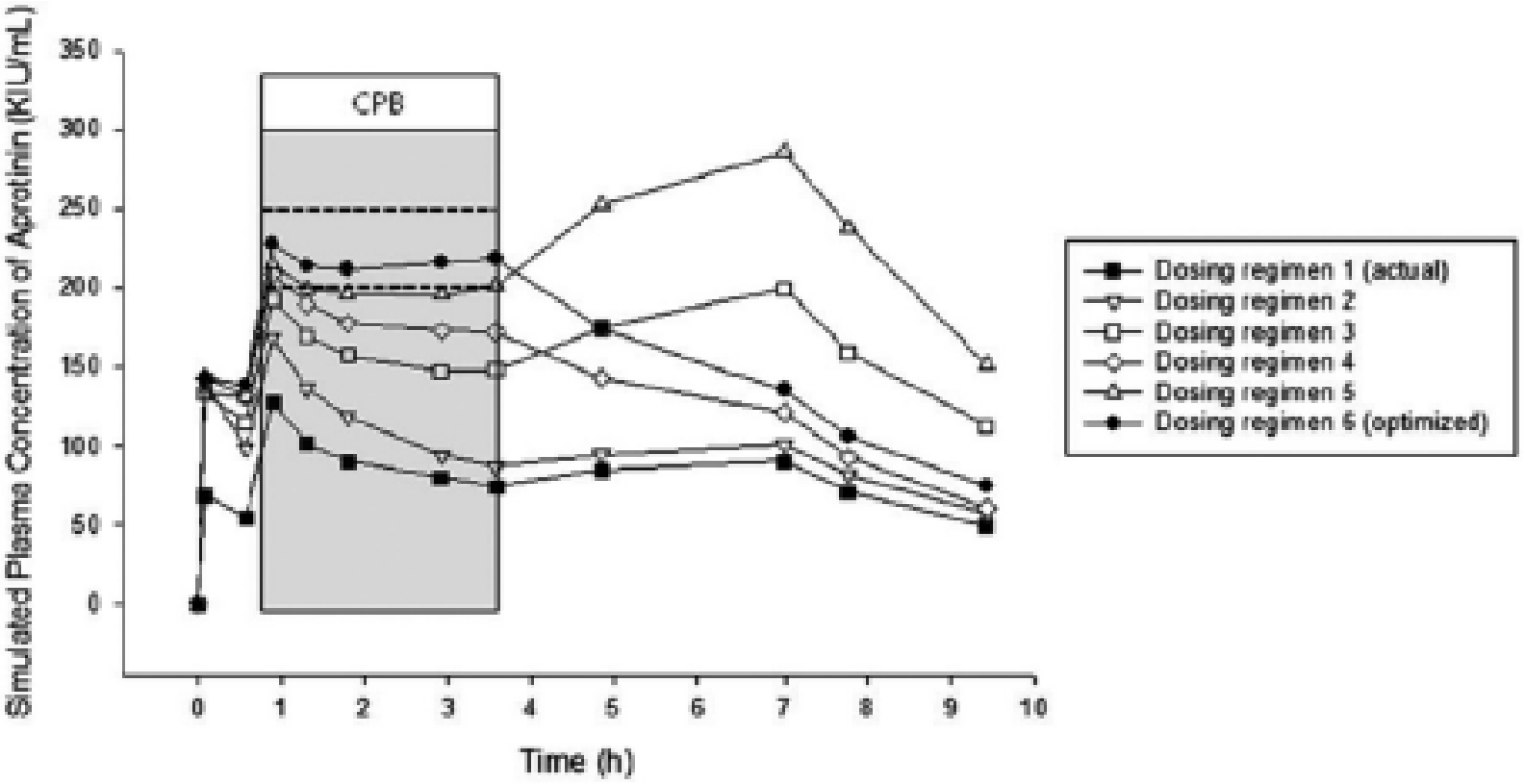


Figure 1.7 Simulated and actual aprotinin plasma concentrations. An optimized dosing regimen was simulated as shown by the dark circle line. The dashed lines represent the target drug concentration during cardiopulmonary bypass surgery.



CHAPTER 2. ERYTHROPOIETIN CLEARANCE DETERMINES HEMOGLOBIN PRODUCTION IN PHLEBOTOMIZED SHEEP

2.1 Abstract

Previous studies have reported a large variability in the pharmacodynamic response to erythropoietin (Epo) therapy. Since Epo produces red blood cells (RBC) by Epo receptor (EpoR) binding, the size of the EpoR pool is expected to be correlated to the pharmacodynamic hemoglobin (Hb) production. The purpose of this study is to experimentally demonstrate that the EpoR pool is related to anemic hemoglobin (Hb) production in newborn sheep by using Epo clearance as a surrogate measure of the EpoR pool. Twelve newborn sheep were phlebotomized to a Hb of 4.91±0.71 g/dL to induce anemia and their Hb recovery was monitored. The total endogenous Epo clearance of these sheep was determined at the first day of life and at week 3 of life. The clearance was determined using a tracer ^{125}I-rhEpo pharmacokinetic study. The clearance values in the newborn sheep were compared to their anemic Hb production. There was a significant correlation ($p<0.05$) between initial clearance and Hb production as well as between week 3 clearance and Hb production. There was also a significant correlation between weight adjusted initial clearance and weight adjusted Hb production. This study demonstrates that an increased Epo clearance correlates with an increased Hb production likely due to the EpoR pool. These findings may partially explain the large pharmacodynamic variability observed in preterm infants undergoing Epo therapy.

2.2 Introduction

Erythropoietin (Epo) is a 30.4 kD glycoprotein hormone which has been widely used in animals and humans for the treatment of anemia. Epo is released by the kidney and exerts its mechanism of action by binding to erythropoietin receptors (EpoR) located on erythroid progenitors burst-forming units and colony-forming units found primarily in the bone marrow (16).

Information regarding the *in vivo* clearance of Epo remains incomplete. Many studies have looked at the elimination of Epo in the liver (35, 36, 153) and the kidneys (37, 154) , however, their effect seems to be minimal (38, 67). Studies have shown that altered states of the bone marrow can significantly change the clearance of Epo suggesting that bone marrow is a significant source of Epo elimination (48, 155). Specifically, research has indicated that receptor-mediated endocytosis in the bone marrow by erythroid progenitors followed by lysosomal degradation is the primary method of Epo elimination from the body (17, 38).

Multiple studies have shown that the Epo clearance increases following a period of anemia (2, 42, 44, 48). In addition, recent work by our group has shown that the increase in the Epo clearance is correlated with an increase in the EpoR mRNA level in the bone marrow (45). Although the increase in EpoR mRNA level may not be directly proportional to the number of receptors, an increase in the Epo receptor mRNA level is likely correlated with an increase in EpoRs. While the dynamic clearance changes in Epo have been examined, additional experimental evidence is needed to show that Epo clearance is correlated with an increased Hb production in response to anemia. Investigation into this relationship could give additional validity in using Epo clearance

as a surrogate marker for EpoRs. The relationship between Epo clearance and Hb production may also suggest the importance for using Epo clearance as a covariate for pharmacodynamic analysis. Thus, the primary objective of this study was to demonstrate the relationship between the Epo clearance of the sheep and the Hb production under anemic conditions.

2.3 Materials and methods

All surgical and experimental procedures were approved prior to the study by the local animal care review committee. Subjects included 12 healthy newborn sheep that were studied for a period of 5 weeks. All animals were housed in an indoor light and temperature controlled environment. Jugular venous catheters were inserted into the sheep for undisturbed plasma sampling. In order to prevent infection, intravenous ampicillin (1 g) was administered for the first 3 days following insertion of the venous catheters. At week 3 a phlebotomy was preformed on all the sheep in order to reduce their Hb to 40% of their baseline value. The procedure for the exchange transfusion included removing blood from the venous catheter until Hb levels had fallen to 40% of the baseline Hb. While blood was being removed from the animal an equal volume of 0.9% NaCl was infused back into the animal in order to keep a constant total volume of blood in the animal. All lambs were supplemented with 12.5 mg/kg/day of oral ferrous sulfate in order to prevent iron deficiency. Hb concentrations were determined using the Sysmex XT-2000 veterinary flow cytometer (Sysmex Corporation, Kobe, Japan).

2.3.1 Clearance determination

In order to determine the Epo clearance in sheep, tracer pharmacokinetic studies were performed on the sheep during their first day of life and immediately (< 1 day) before the phlebotomy (week 3 of life). The pharmacokinetic studies consisted of administering a tracer dose (0.1 U/kg) of biologically active ^{125}I-rhEpo intravenously over less than 30 seconds. Following the administration of the tracer dose, 10-15 plasma samples were drawn over the next 7-8 hour period following Epo dosing. The concentration of ^{125}I-rhEpo was determined by an immunoprecipitation method as previously described (156).

The Epo plasma concentration data from a single intravenous ^{125}I-rhEpo tracer bolus dose was modeled using a biexponential function to determine the Epo clearance based on the dose administered and pharmacokinetic area-under-curve principles. The equation for the biexponential disposition curve is as follows:

$$c(t) = A_1 e^{-\beta_1 t} + A_2 e^{-\beta_2 t} \qquad (2.1)$$

where, c(t) is the concentration of Epo at time t following an IV bolus Epo administration and A and β are the disposition parameters calculated for a two compartment model given in the equation above. Equation 2.1 has been used previously to determine the Epo clearance in sheep with tracer doses of Epo administered (48). Curve fitting and parameter estimation were performed using WINFUNFIT a general nonlinear regression program evolved from the FUNFIT program (146).

2.3.2 Hemoglobin analysis

In order to determine each animal's recovery from anemia, the Hb production of each sheep was calculated for a five day period following the phlebotomy. This calculation was done by using a simple mass balance model as shown:

$$Hb\ Produced = (Hb\ End + Hb\ Senescence) - Hb\ Start \quad (2.2)$$

where, *Hb End* is the amount of Hb in the blood 5 days after the phlebotomy occurred; *Hb Start* is the amount of Hb in the blood immediately after the phlebotomy; *Hb Senescence* is the Hb amount removed from the circulation in the 5 days following the phlebotomy due to RBC senescence. The RBCs were assumed to have a uniform distribution of ages with an assumed lifespan of 120 days (67) and RBC senescence occurring through a zero order elimination given by:

$$Hb\ Senescence = \frac{Hb}{\tau} \cdot t \qquad t \leq \tau \qquad (2.3)$$

where, Hb is the amount of Hb immediately after the phlebotomy; τ is the lifespan of sheep RBCs fixed at 120 days and t time since the phlebotomy occurred. An additional assumption used for this model is that the newly produced red blood cells do not undergo senescence in the five day recovery period. The recovery period of five days was chosen because the animals will not likely reach their baseline Hb concentration in a five day period. Such a recovery of the Hb to a baseline level could confound statistical significance. Since only the Hb concentration data was available in the sheep, this value was converted to Hb amount according to the following:

$$Hb_C = \frac{Hb}{BV} \quad (2.4)$$

where, Hb_C is the Hb concentration and BV is the blood volume for sheep fixed to 110 mL/kg according to a previously reported value (157). All statistics were computed using the Compaq Visual FORTRAN IMSL library.

2.4 Results

All of the initial tracer Epo pharmacokinetic studies were completed in the sheep animal model in either their first or second day of life. The average weight of the sheep when the initial Epo pharmacokinetic study was conducted was 5.75±1.15 kg (mean ± standard deviation). The second Epo pharmacokinetic study was completed immediately before the phlebotomy occurred at day 21 of life. The average weight of the sheep when the second pharmacokinetic study was completed was 11.4±2.50 kg. The phlebotomy preformed reduced the sheep's Hb from 10.3±1.16 g/dL to 4.91±0.711 g/dL corresponding to a drop in Hb of 47.7±5.86%.

All of the Epo pharmacokinetic studies performed resulted in a biexponential disposition curve. Representative plots of the tracer Epo pharmacokinetic study are shown in Figure 2.1. The fitting for the tracer studies was completed using WINFUNFIT. The fitting as shown in Figure 2.1 was acceptable for all 24 tracer studies according to the correlation values (average R^2 value 0.99±0.01). The average initial clearance was 440±129 mL/hr (range 268-650 mL/hr) and the initial weight adjusted clearance was 84.7±19.7 mL/hr/kg (range 55-120 mL/hr/kg). The average clearance immediately before the phlebotomy was 731±240 mL/hr (range 465-1256 mL/hr) and the weight adjusted clearance was 70.0±15.2 mL/hr/kg (range 52-97 mL/hr/kg).

2.4.1 Hemoglobin production and Epo clearance

Following the phlebotomy there was an immediate increase in the sheep's Hb concentration for all 12 sheep followed by the Hb concentration returning to the pre-phlebotomy baseline. With red blood cell senescence taken into account, the average Hb

production for the 5 days following the phlebotomy was 5.40±2.04 g/day. The weight adjusted Hb production was 0.340±0.188 g/day/kg.

The clearance values were plotted against the Hb production (Figure 2.2) as well as the weight adjusted clearance against the weight adjusted Hb production (Figures 2.3 and 2.4). Simple two-tailed regression statistics were computed for all of the plots with the null hypothesis being that the slope is equal to zero and the alternative hypothesis being that the slope is not equal to zero. Statistical significance was reached ($p<0.05$) for figures 2.2 and 2.3 demonstrating a positive correlation between clearance and Hb production. Statistical significance could not be reached ($p = 0.11$) between the weight adjusted clearance before the phlebotomy and the weight adjusted Hb production (Figure 2.4).

2.5 Discussion

This study was able to correlate the Epo clearance and weight adjusted clearance with the Hb production of newborn sheep in response to anemia. Many research studies involving Epo have shown a large variability in pharmacokinetic (38, 67) and pharmacodynamic response (9). This study suggests that the Epo clearance may be able to partly account for some of the variability in anemic red blood cell production; however, reasons for the variability in the clearance have not yet been determined. Information about the Epo clearance may be used to develop future PK/PD models which may better account for the intra-subject variability in the production of Hb.

In this study the slope used to estimate the Hb production was calculated for five days following the phlebotomy. There were three reasons for using five days post phlebotomy as the calculation for the Hb production. First, most sheep had their Epo

concentrations return to baseline levels at approximately five days after the phlebotomy. With Epo levels at baseline it is difficult to determine differences in Hb production due to the modest amount of Hb being produced with baseline Epo levels. Second, if the Hb production is measured for an extended period of time following the phlebotomy then the Hb can return to baseline confounding the statistical significance. Third, the five day recovery period avoided the complication of RBC senescence of RBCs produced after birth.

The weight adjusted clearance immediately before the phlebotomy did not show a statistically significant correlation with the weight adjusted Hb production ($p>0.05$) (Figure 2.4). The reason this correlation was not significant is still unknown but could be attributable to the small number of animals ($n=12$) in the study. A follow-up study with a larger sample size is recommended to elucidate the reason why statistical significance was not reached.

2.5.1 Epo clearance as a measure of Epo receptor level

Epo clearance has previously been used as a surrogate marker for the Epo receptor pool (48). This relationship is consistent with the hypothesized mechanism of action of Epo which Sawyer et al. characterized as receptor mediated endocytosis followed by lysosomal degradation (17). In addition, our group showed in a recent publication that an up-regulation in Epo receptor mRNA level shows a statistically significant correlation ($p<0.05$) with an increase in the Epo clearance following a period of induced anemia (45). Although the Epo receptor mRNA level does not directly correlate with the number of Epo receptors, an increase in Epo receptor mRNA level is

likely associated with an increase in Epo receptors. This is an important finding to show with direct evidence that the Epo clearance and receptor level are related.

2.5.2 Linear clearance pathway

Previous research has shown that Epo is cleared through two pathways: a receptor mediated (non-linear) pathway which leads to RBC production and an additional unknown (linear) pathway (41). Although research has been described which has identified the receptor-mediated clearance pathway of Epo (17), understanding of the non-receptor mediated clearance of Epo remains incomplete. Our previous bone marrow ablation study in non-anemic lambs showed that the linear clearance pathway of Epo accounts for only 7.38%±2.7% of the total Epo clearance and that this clearance does not change under anemic conditions (41). The current study supports the low variability in the linear clearance pathway because if the linear clearance pathway was highly variable it would confound the statistical significance between the total clearance and the Hb production.

Identifying possible sources for the non-receptor mediated clearance mechanism of Epo is important for future characterization of the pathway. Some studies have shown that Epo is cleared by the liver (35, 36) and the kidney (37) , however, their effects seem to be minimal (38). Buhrer et al. determined the urinary losses of Epo in preterm infants confirming that clearance from the kidney is not a major contributor to Epo elimination (123). From previous research, it is well established that EpoRs are found in most tissues of the body and not just in the bone marrow (158-161). It is possible that these tissues contribute to Epo elimination in a linear manner without leading to erythrocyte production. Further research needs to be explored regarding the linear clearance pathway

to identify if it could change under different physiologic conditions. Physiologic changes in the linear clearance pathway could significantly change the Hb production in response to anemia.

2.5.3 Iron deficiency and Hb production

Endogenous iron is required for endogenous RBC formation from Epo stimulation of RBC precursors in the bone marrow (162, 163). Van der Putten et al. demonstrated that iron supply can be the limiting factor for Hb synthesis which cannot be corrected even with high concentrations of exogenous Epo (27). The common practice clinically is to supplement iron orally for patients in need of Epo therapy. The oral supplementation of iron has been previous used by kidney disease patients (162) and almost universally in preterm infants receiving Epo therapy (1). In adults, serum ferritin levels of less than 100ng/mL are often cited as iron deficient and iron supplementation should be implemented (162). There is currently uncertainty regarding iron supplementation in patients who are iron sufficient. Research in support of iron supplementation to subjects who are already iron sufficient suggests that smaller doses of Epo are needed to achieve the target if additional iron is supplemented (164). On the other hand, research has shown that excessive iron therapy can be linked to an increased risk of infection, inflammation and atherosclerosis (163). More research is needed to determine an optimal iron level for erythropoietic response that does not cause adverse side effects.

The current study supplemented iron (12.5 mg/kg/day of oral ferrous sulfate) in the sheep to insure that iron deficiency would not be a limiting factor in erythropoiesis. If any of the sheep were iron deficient this would confound the relationship obtained between the Epo clearance and the Hb production. Although the serum ferritin levels

were not directly measured in the sheep, all of the subjects recovered to baseline Hb levels after the major phlebotomy suggesting that the sheep were iron sufficient. Future studies which examine the ferritin level as an additional predictor of the Hb production are needed.

2.5.4 Application to human subjects

Epo has been frequently used in adults for the treatment of renal dialysis (165) patients, and patients undergoing chemotherapy (166). However, although preterm infants develop anemia early in life Epo therapy to counteract this anemia is not standard practice. The specific rationale for this research is that infants may be able to produce sufficient RBCs endogenously to avoid RBC transfusions which are associated with multiple risks. In a review article of 27 clinical trials where Epo was administered to preterm infants the author concluded the although Epo therapy reduced the number of transfusions and volume of blood transfused in infants, these findings were of limited clinical significance (1). One problem with finding clinically relevant endpoints when examining Epo clinical trials in preterm infants is that infants have a large variability in pharmacodynamic response to Epo therapy (9). The current study suggests that the Epo receptor pool is an important factor when determining the possible Hb production in sheep. Interestingly, there was a large range for the weight adjusted clearance at birth for the animals in the study (55-120 mL/hr/kg) and this value was able to predict the animal's response to anemia at 3 weeks of life. This suggests that if Epo receptor pool information could be obtained from preterm infants at birth, this may be an informative predictor of how they will respond to exogenous Epo therapy. In order to extend the current results to preterm infants, information must be obtained regarding the Epo

receptor state in preterm infants and currently such information is not available. This study used radiolabeled $^{I\text{-}125}$Epo to determine the Epo receptor state in sheep which cannot be given to infants due to ethical concerns. Future technology, such as a non-radioactive Epo label, would be valuable in elucidating Epo receptor information in infants in a non-invasive manner.

2.6 Conclusion

The Hb production in response to anemia was shown to significantly correlate with the Epo clearance in newborn sheep. Since Epo clearance has been used as a surrogate marker for the Epo receptor pool, the results suggest that the Epo receptor pool is an important predictor of Hb production under anemic conditions. Future Epo pharmacodynamic models developed in preterm infants which consider the dynamic Epo receptor pool should be able to better predict which infants are likely to respond well to Epo therapy and potentially avoid RBC transfusions.

Figure 2.1 Four representative plots of tracer Epo pharmacokinetic studies. Each clearance value was determined from a similar plot. Each plot follows a biexponential disposition.

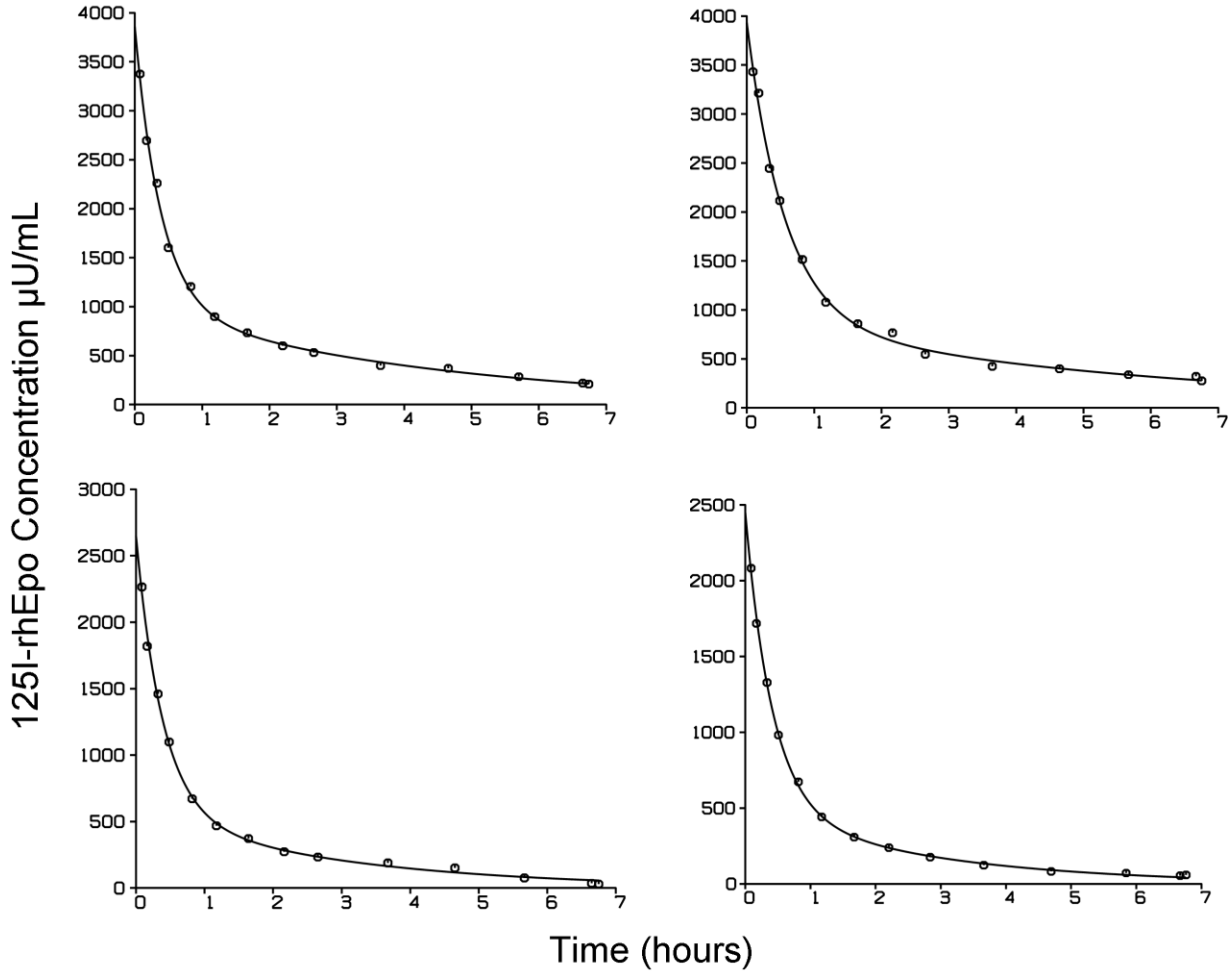


Figure 2.2 Post-phlebotomy Hb production plotted against Epo clearance values at birth (top) and immediately before the phlebotomy (bottom). Slope is significantly positive for both figures ($p < 0.05$)

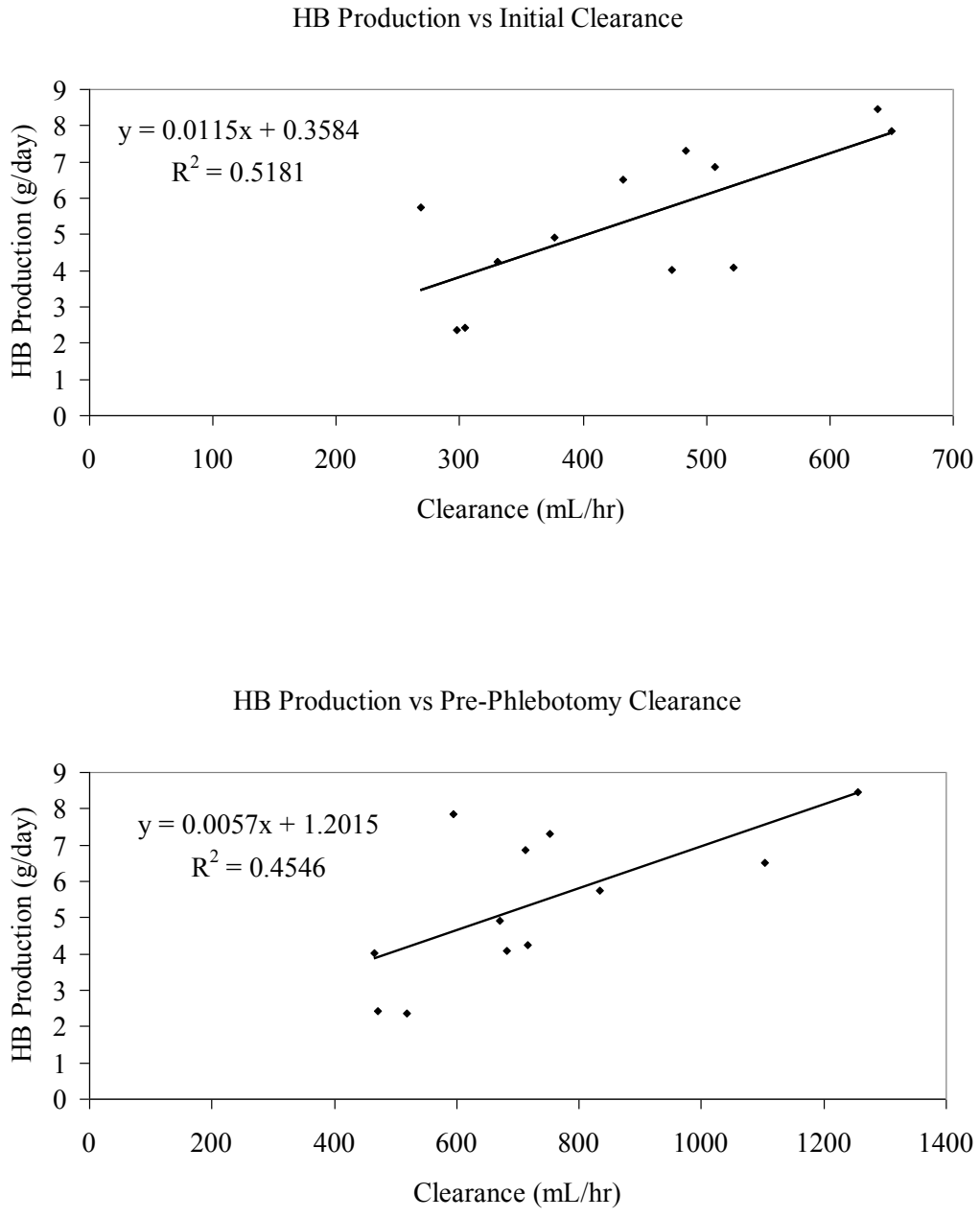


Figure 2.3 Post-phlebotomy weight adjusted Hb production plotted against weight adjusted Epo clearance values at birth. Slope is significantly positive ($p < 0.05$)

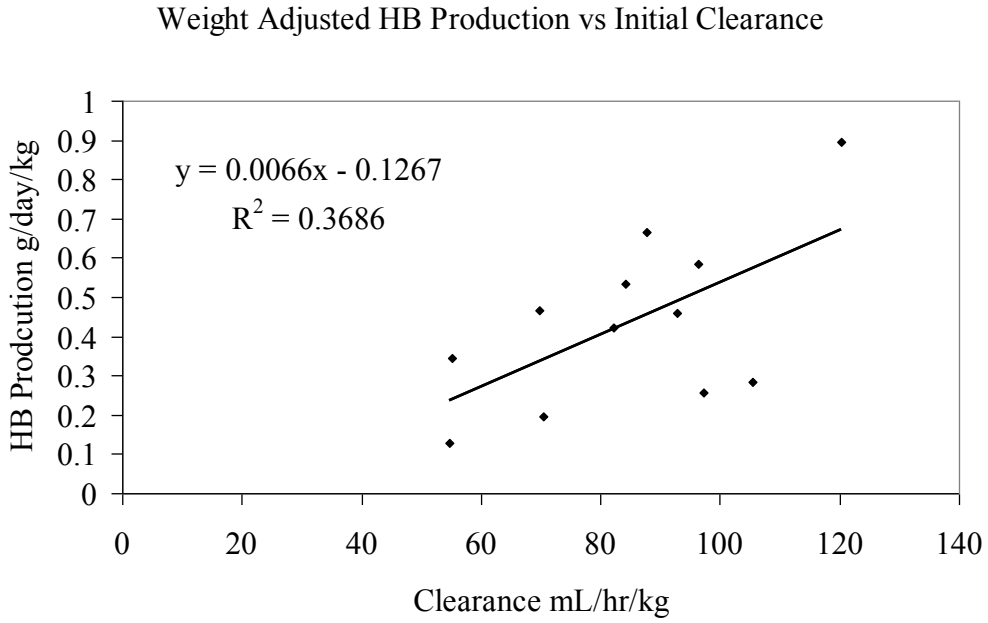
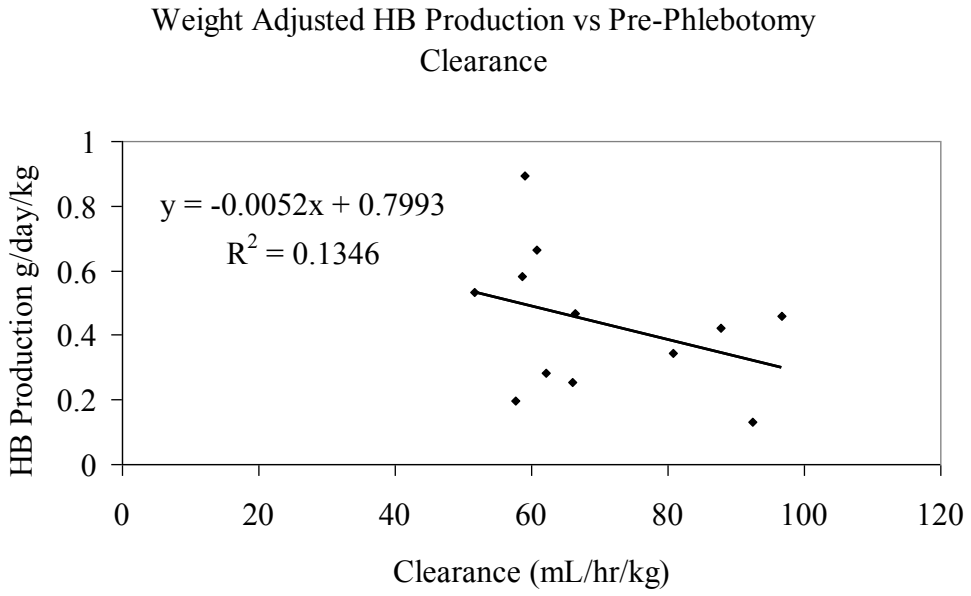


Figure 2.4 Post-phlebotomy weight adjusted Hb production plotted against weight adjusted Epo clearance values immediately before the phlebotomy. Slope is not significant ($p > 0.05$).



CHAPTER 3. RECEPTOR-BASED DOSING OPTIMIZATION SIMULATION OF ERYTHROPOIETIN IN JUVENILE SHEEP FOLLOWING PHLEBOTOMY

3.1 Abstract

The primary objective of this work is to determine the optimal time for administration of an erythropoietin (Epo) dose to maximize the erythropoietic effect using a simulation study based on a young sheep pharmacodynamic model. The dosing optimization was accomplished by extending a hemoglobin (Hb) production pharmacodynamic model, which evaluates the complex dynamic changes in the EpoR pool from the changes in Epo clearance. Fourteen healthy two month old sheep were phlebotomized to Hb levels of 3-4 g/dL. Epo clearance was evaluated longitudinally in each animal by administering tracer doses of ^{125}I-rhEpo multiple times during the experiment. Kinetic parameters were estimated by simultaneously fitting to Hb data and Epo clearance data. The phlebotomy caused a rapid temporary increase in the endogenous Epo plasma level. The Hb began to increase following the increased in the Epo level with a lag time of 1.13±0.79 days. The average correlation coefficient for the fit of the model to the Hb and clearance data was 0.953±0.018 and 0.876±0.077 respectively. A simulation study was completed in each sheep with fixed individual estimated model parameters to determine the optimal time to administer a 100U/kg IV bolus Epo dose. The optimal dose administration time was 11.4±6.2 days post phlebotomy. This study suggests that the Hb produced from Epo administration can be optimized by considering the dynamic changes in the EpoR pool.

3.2 Introduction

Erythropoietin (Epo) is a glycoprotein hormone responsible for regulating erythrocyte production. Epo exerts its mechanism of action by binding to erythropoietin receptors (EpoR) located on erythroid progenitors burst-forming units and colony-forming units found primarily in the bone marrow(16).

Information regarding the *in vivo* clearance of Epo remains incomplete. Some studies have shown that Epo is cleared by the liver (35, 36) and the kidney (37) , however, their effects do not contribute significantly to the total clearance (38). It has been hypothesized that in vivo desialidation is the rate limiting step for Epo metabolism by the liver (167). Receptor-mediated endocytosis in the bone marrow by erythroid progenitors followed by lysosomal degradation is the primary organ of Epo elimination from the body (17). Additional evidence for this elimination mechanism has been shown by studies investigating different degrees of bone marrow activity (39, 40). Several preclinical and clinical studies have reported an increase in Epo clearance within one month following Epo treatment (2, 42-44) , while others found no statistical difference (30, 32). An additional study modeled the change in the EpoR level over time following the induction of anemia (48). In a recent study by our group we reported up-regulation of EpoR mRNA levels of 4.97±3.92 times of baseline at 9 days following the induction of anemia (45). Although EpoR mRNA levels do not necessarily correlate directly with the number of receptors, an increase in the mRNA level is likely associated with an increase in the quantity of EpoR. An important aspect of Epo dosing is that receptor-mediated Epo clearance (Cl_R) leads to Hb production while the non-receptor mediated clearance (Cl_L) does not produce Hb. Accordingly, the greatest efficacy in Epo dosing is achieved

when the biggest fraction of the dose is eliminated via the erythropoietic elimination pathway. One study was able to characterize and quantify both types of clearances through a chemical bone marrow ablation method (41). With two different types of clearances, an optimal Epo dosing involves utilizing the receptor-mediated clearance pathway as much as possible and minimizing the fraction of Epo eliminated via the non-erythropoietic elimination pathway. Thus, the primary objective of this study was to determine the optimal Epo dosing time in neonatal sheep with phlebotomy-induced anemia considering the complex, receptor-mediated elimination mechanism of Epo. The dosing optimization was based on a mechanistic, pharmacodynamic model for predicting Hb production that considers the dynamic Epo-dependent changes in the EpoR pool that determines the erythropoietic efficacy.

PD analysis studies have modeled different variables in response to Epo's erythropoietic stimulatory effect. Prior studies have examined Epo's effect on reticulocytes (168) and Hb (169) individually, and reticulocytes and Hb together (170). An additional mechanistic PK/PD study took into consideration the Epo, reticulocyte, and EpoR levels to determine the Hb response to Epo (63). Although it is commonly stated that Epo's binding to EpoR accounts for erythrocyte production and target-mediated disposition, the quantity of EpoRs has not previously been used to predict Epo's pharmacodynamic response. Nonetheless, target-mediated drug disposition pharmacokinetics has been considered in different contexts previously (58, 171, 172). The modeling-based methodology utilized in this work is not limited to Epo but has been applied to other therapeutic agents with target-mediated disposition such as recombinant tumor necrosis factor (173) and selected angiotensin-converting enzyme (ACE) inhibitors

(174). The fundamental modeling objective was to determine the kinetic mechanism governing the complex relationship between the Epo concentration and the EpoR pool size, both of which are determinants of Hb production. The analysis we have chosen to employ proposes a feedback regulation mechanism for EpoR and is based on the assumption that the receptor-mediated Epo elimination pathway results in an Epo clearance that is linearly related to the quantity of EpoRs.

Epo therapy has been well established in renal failure patients (30) and patients undergoing chemotherapy treatment (175). However, the benefit of Epo therapy in anemic preterm infants is still under investigation. Clinical trials involving Epo administration in preterm infants have shown some significant results although the clinical significance is inconsistent (1). Young sheep have previously been used to model human anemia (34) and may provide guidelines for how to optimally administer Epo in preterm infants.

3.3 Materials and methods

All surgical and experimental procedures were approved prior to the study by the local animal care review committee. Fourteen healthy sheep ages 21-60 days were studied. All animals were housed in an indoor light and temperature controlled environment. Jugular venous catheters were inserted into the sheep for undisturbed plasma sampling. In order to prevent infection, intravenous ampicillin (1 g) was administered for the first 3 days following insertion of the venous catheters. Anemia was induced by an exchange transfusion using 0.9% NaCl. The procedure for the exchange transfusion included removing blood from the venous catheter until Hb levels had fallen to 3-4 g/dL. While blood was being removed from the animal an equal volume of 0.9%

NaCl was infused back into the animal in order to keep a constant total volume of blood in the animal. All lambs were supplemented with 12.5 mg/kg/day of oral ferrous sulfate in order to insure they were not iron deficient. Hb concentrations were determined using the Sysmex XT-2000 veterinary flow cytometer (Sysmex Corporation, Kobe, Japan).

To determine the Epo clearance, tracer doses of biologically active ^{125}I-rhEpo equivalent to 0.1 U/kg Epo were given intravenously over less than 30 seconds. Ten to 15 plasma samples were drawn over the next 7-8 hour period following Epo dosing. The concentration of ^{125}I-rhEpo was determined by immunoprecipitation as previously described (156). To evaluate the dynamic changes in the Epo clearance, ^{125}I-rhEpo clearance studies were completed before and after the phlebotomy. In addition to the tracer doses of Epo, Endogenous plasma Epo concentrations were measured at multiple time points during the study by using a radioimmunoassay as previously described (156).

3.3.1 Pharmacokinetic analysis

The Epo plasma concentration data from a single intravenous ^{125}I-rhEpo tracer bolus dose (0.1 U/kg) was fitted using a bi-exponential function to determine the Epo clearance based on the dose administered and the resulting area-under-curve. Curve fitting and parameter estimation were performed using WINFUNFIT, a general nonlinear regression program evolved from the FUNFIT program (146). The dosing optimization algorithms used with WINFUNFIT were programmed using FORTRAN.

3.3.2 Pharmacodynamic model for Hb response

A pharmacodynamic model was developed to predict the Hb response to induced anemia based on current understanding of the mechanism of Epo's action. Specifically,

the model assumes that Hb is produced only following the binding of Epo to its receptor, EpoR. It was also assumed that the receptor-based clearance of Epo (Cl_R) is proportional the EpoR pool size (48). Finally, it was assumed that prior to the start of the study the system was at "steady state" (SS), i.e., the Hb production was initially equal to the Hb removal resulting from the red blood cell (RBC) senescence. RBC senescence was accounted for in the model by assuming that no senescence occurred among newly produced RBCs following the phlebotomy within the time span of the experiment. This is justified based on the assumption of a 120 day lifespan of RBCs reported for sheep (62) which is longer than the approximately 30 days duration of the kinetic experiment. The model incorporated a lag time (t_{lag}) between the Epo-EpoR binding and the production of Hb that corresponded to the time from the binding of Epo to EpoRs in the bone marrow and the appearance of newly formed RBCs, i.e., reticulocytes in the peripheral bloodstream.

Modeling for the Cl_R is similar to our group's previous work (48) in that the feedback mechanism for the Cl_R is modeled through the use of an intermediate variable denoted M. The intermediate variable (M) in this model provides a mathematical method to account for the clearance rebounding above the baseline value. Two representative fitted Hb profiles with the Epo concentrations are illustrated in Figures 3.1 and 3.2. The following numerically integrated delay-type differential model equations were simultaneously fit in all animals to the Hb and clearance versus time data:

$$\begin{aligned} \frac{d(Hb)}{dt} &= E(t) - E(t-\tau) \\ \frac{dCl_R}{dt} &= k_3 \cdot M(t) - k_1 \cdot Epo(t) \cdot Cl_R(t) \\ \frac{dM}{dt} &= k_0 + k_2 \cdot (Cl_{R,SS} - Cl_R)_+ - k_3 \cdot M \end{aligned} \tag{3.1}$$

where *Hb* is the Hb amount; Cl_R: is the receptor-based clearance of Epo assumed to be proportional to the quantity of EpoR; $Cl_{R,SS}$ is "steady state" value of Cl_R; *M* is the intermediate variable used to account for the feedback; *Epo* is the Epo plasma concentration represented with a linear spline; τ is the lifespan of RBCs fixed to 120 days (62) *;* k_3 is a first order elimination rate constant for *M*; k_1 is the rate constant for Epo-EpoR binding; k_0 is the zero order rate constant for *M* formation and k_2 is a first order feedback rate constant. The + subscript denotes the truncation function, i.e.:

$$(Cl_{R,SS} - Cl_R)_+ = \begin{cases} Cl_{R,SS} - Cl_R & Cl_{R,SS} > Cl_R \\ 0 & otherwise \end{cases} \quad (3.2)$$

E(t) is the Hb production given by a modified Hill equation model :

$$E(t) = \frac{E_{\max}(t) \cdot Epo(t - t_{lag})}{EC_{50} + Epo(t - t_{lag})}$$
$$E_{\max}(t) = E_{\max,SS} \frac{Cl_R(t - t_{lag})}{Cl_{R,SS}} \quad (3.3)$$

, where t_{lag} is the lag time between Epo-EpoR binding and Hb's production; EC_{50} is the concentration of Epo resulting in half of the maximum Hb production rate; Emax(t) is the maximum Hb production possible at the current time. $E_{max,SS}$ is the initial SS value of E_{max} i.e. $E_{maxSS} = E_{max}(0)$.

In the presented differential equations *Hb* is Hb amount but the experimental data is Hb concentration. Accordingly, the output variable of the differential equation must be divided be V(t). Where:

$$V(t) = V_{Kg} W(t) \quad (3.4)$$

V_{Kg} is the volume of distribution per kg which is considered a constant and *W(t)* is the weight of the animal at time t. The weight function is represented by a linear spline fit to the weight data of each animal.

Previous literature has shown that the red cell lifespan (τ) for sheep is approximately 120 days (62). For the 14 sheep used in this study the average study time was 34.6±5.7 days. Therefore, the lifespan (τ) of RBCs is much longer than study length. Thus, the above differential equations become:

$$\begin{aligned} \frac{d(Hb)}{dt} &= E(t) - \frac{Hb_{SS}}{\tau} \\ \frac{dCl_R}{dt} &= k_3 \cdot M(t) - k_1 \cdot Epo(t) \cdot Cl_R(t) \\ \frac{dM}{dt} &= k_0 + k_2 \cdot (Cl_{R,SS} - Cl_R)_+ - k_3 \cdot M \end{aligned} \tag{3.5}$$

Subject to the following SS initial conditions:

$$\begin{aligned} E(0) &= Hb_{SS} / \tau \\ Cl_{R,SS} &= k_0 /(k_1 \cdot Epo_{SS}) \\ M_{SS} &= k_0 / k_3 \end{aligned} \tag{3.6}$$

The Epo concentration was used as a forcing function for the differential equations by representing the Epo response by a linear spline fit to the plasma Epo data. The tracer kinetic data enable the total clearance (*Cl*) of Epo to be obtained. Previous studies have shown that the total clearance includes a second linear clearance (Cl_L) that is assumed to be unaffected by anemia and independent of the Epo concentration (41). Accordingly, the total Epo clearance may be expressed as:

$$Cl(t) = Cl_L + Cl_R(t) \tag{3.7}$$

, where Cl_L is an estimated constant and $Cl_R(t)$ is the receptor-based clearance that changes according the dynamic changes in the receptor pool. The above total clearance equation and the numerically integrated differential equations define the model that was fit to the clearance and Hb data.

3.3.3 Dosing optimization

The objective for the dosing optimization was to find the optimal time to administer a single IV 100U/kg bolus Epo dose to anemic neonatal sheep based on a simulation study. In order to accomplish this, an additional differential equation was added to the model:

$$\begin{aligned}
\frac{d(Hb)}{dt} &= E(t) - \frac{Hb_{SS}}{\tau} \\
\frac{dCl_R}{dt} &= k_3 \cdot M(t) - k_1 \cdot Epo(t) \cdot Cl_R(t) \\
\frac{dM}{dt} &= k_0 + k_2 \cdot (Cl_{R,SS} - Cl_R)_+ - k_3 \cdot M \\
\frac{dEpo_x}{dt} &= \frac{-(Cl_L + Cl_R)}{V(t)} \cdot Epo_x
\end{aligned} \tag{3.8}$$

, where Epo_x is the exogenous Epo concentration resulting from an IV bolus injection, and $V(t)$ is the weight adjusted volume of distribution at time t. The objective function for the optimization is based on the total amount of post phlebotomy Hb produced to the end of the study when administering a fixed 100U/kg IV bolus dose of Epo at a certain time:

$$Hb\ Produced = Hb\ End - Hb\ Start + Hb\ Senescence \tag{3.9}$$

, where *Hb End* is the amount of Hb in the animal at the end of the study and *Hb Start* is the amount of Hb in the animal immediately after the phlebotomy; *Hb Senescence* is the Hb amount removed from the circulation due to natural erythrocyte senescence.

It is assumed that Cl_L is constant, that Cl_R is proportional to the EpoR pool, and that the Epo dosing does not significantly change the endogenous Epo production.

3.3.4 Simulation study

The simulation study involved a dose simulation for each of the 14 sheep with fixed individual PK/PD parameters that were determined individually from the model based on Equations 3.5. The individual E_{max}, EC_{50}, k_1, k_2, k_3, τ and lag time parameters were fixed for each individual dose simulation. The software developed for the analysis determined the post-phlebotomy Hb response for a sheep when a single 100U/kg IV bolus dose is given at a particular time. For each sheep and each simulated Epo dosing, the software determined the post-phlebotomy Hb produced based on Equation 3.9. The post-phlebotomy dosing time that produced the most Hb was considered to be the optimal dosing time. This optimal dosing time was determined by numerical optimization using the derivative free optimization algorithm developed by Nelder and Mead (176) commonly referred to as the simplex method. Similarly, the simplex optimization algorithm was also set up to determine the lowest post-phlebotomy Hb produced according to Equation 3.9 for a single 100U/kg IV Epo bolus dose. The dosing time with the lowest Hb produced was considered to be the worst possible dosing time.

3.4 Results

Study animals began with a mean pre-phlebotomy Hb of 10.5±2.22 g/dL, a mean starting weight of 14.4±6.26 kg and a mean ending weight of 21.8±7.07 kg. All animals were phlebotomized to a similar Hb value of 3.87±0.450 g/dL. Figures 3.1 and 3.2 show two representative fits of the proposed model to the clearance and the Hb data. As

expected, in all cases there was a marked increase in the plasma Epo concentration following the phlebotomy followed by an increase in Hb. Furthermore, every animal showed a decrease in Epo clearance immediately following the phlebotomy followed by an increase in clearance above each animal's baseline clearance once plasma Epo levels had declined to nearly normal levels. The sheep reached an average peak clearance of 110.3±25.90 mU/hr/kg at 5.57±2.12 days following the phlebotomy.

The simultaneous curve fitting was able to capture the behavior of the data for all animals. Although there was an age difference of up to 39 days for some animals, there was no statistical difference ($p>0.05$) in baseline clearance values or parameter values for the different age groups. Table 3.1 summarizes the parameters and goodness of fit values. The curve fitting showed a trend towards a rapid increase in Hb level of production followed by a more gradual increase in Hb concentration. In all cases the Hb levels continued to increase when plasma Epo concentrations were at baseline as a result of the EpoR pool size being above the baseline value. The average lag time for the study was 1.13±0.794 days. The average Epo "steady state" clearance value for all of the animals was 62.7±17.5 mL/hr/kg. The average maximum and minimum clearance value for all of the animals were 94.3±28.8 and 24.7±9.8 mL/hr/kg, respectively.

The simulation of exogenous Epo administration caused a variable increase in post-phlebotomy Hb production which was dependent on the time the Epo dose was administered (see Figure 3.4). A representative plot of this increased Hb production is shown in Figure 3.3 (top). These Hb profiles are based on the Epo profiles shown in Figure 3.3 (bottom). The two Hb profiles in Figure 3.3 are super imposable until the Epo dose is given. In all cases there were two local solutions for the dosing optimization

algorithm, which are summarized in Tables 3.2 and 3.3. Figure 3.4 shows the objective function values (Hb produced) as a function of the IV bolus dosing time. The plot demonstrates two local solutions found for a particular animal. For 11 out of the 14 animals the later dosing time was more optimal than the first. The average for the optimal dose administration time was 11.4±6.2 days post phlebotomy which corresponded to an average increase in Hb production of 21.1±9.7% over the worst possible dosing time. The average for the second local solution for the optimal dosing time was 4.6±4.4 days post phlebotomy which corresponded to an average increase in Hb production of 10.2±5.4% over the most unfavorable possible dosing time. The averages for the two optimal dosing times could be estimated in a different manner as dealt with in the discussion section. The average worst possible dosing time was 6.6±3.5 days post phlebotomy. The average Hb produced was 186.2±50.5g for the optimal dose time which was significantly larger ($p<0.05$) than for the worst possible dose time: 152.1±33.6 g.

3.5 Discussion

Judged from the goodness of fit parameters, the proposed kinetic model was able to accurately predict the Hb production in phlebotomized sheep based on the Epo concentration and Epo clearance values. Furthermore, the model was extended to determine the optimal time to administer an IV bolus Epo dose to anemic sheep. Since the Epo Cl_R is assumed to be proportional to the change in receptors and since the linear clearance is assumed to be constant (41), any change in the total clearance, Cl, is due to a change in the quantity of EpoRs. In addition, since the EpoRs are located primarily in the bone marrow the phlebotomy does not significantly change EpoR pool. Our group recently showed that EpoR mRNA is upregulated following the induction of anemia in

sheep which is consistent with the model used in this study (45). Since this study used the GAPDH gene as a control it can be suggested that the number of EpoRs per cell increased rather than the total number of cells. Another study recently released shows that the Epo receptors decrease following large plasma Epo concentrations demonstrating consistency with our model (177). This study, however, does not show EpoRs increasing above the baseline value, which is most likely due to the short time scale (5 hours) of the study. The present simulation study provides additional evidence that the increased total clearance of Epo is likely due to an increase in the number of EpoRs present in the bone marrow. The agreement of the present model provides additional indirect evidence for a receptor-mediated clearance mechanism for Epo. Specifically, the decrease in the total Epo clearance immediately following the endogenous Epo surge suggests that the EpoR is consumed in a receptor-mediated endocytic process.

There are several limitations of this study that may be improved based on future experimental work. One limitation of the current study is that an indirect method was used to determine the EpoR pool. Utilizing methodology which can determine the protein level of the EpoR may provide a more accurate representation of the EpoR pool. In addition, the lifespan (τ) of red blood cells is fixed to 120 days to account for senescence. In reality, the red blood cell lifespan could be variable throughout the course of the animal's life. Following labeled red blood cells until senescence occurs using labeling methods such as ^{51}Cr or biotin would help validate the lifespan assumption (178). Although the simulation study suggests a potential Hb production benefit based on considering the dynamic EpoR state, experimental work needs to be done to verify the

simulations. The study is also based on the assumption of an initial "steady state", which may not be true.

Recent work by others has shown the potential for scaling preclinical animal Epo studies to humans (179). Specifically, an Epo allometric scaling relationship has been determined between humans and sheep for volume of distribution and clearance (67). Thus, with a proper allometric pharmacodynamic scaling it should be possible to make use of the sheep model for making pharmacodynamic dosing predictions useful in planning mechanism-based human efficacy studies.

3.5.1 Dosing optimization solutions

The dosing optimization algorithm obtained two solutions for the optimal time to administer an IV bolus Epo dose. The average dose administration time was reported as the average for the best solution and the second best solution. In order to better analyze the optimal dosing times it is more informative to average the solutions by the early solution and the late solution. If the averages are computed in this manner, then the average early post-phlebotomy Epo administration solution is 2.3±1.0 days and the average late solution is 13.7±3.5 days post phlebotomy. The later dosing time was expected and is likely due to the EpoR state being upregulated around the optimal dosing time. The earlier solution for the dosing optimization was not expected at first. However, this solution may be explained by an earlier Epo dosing allowing the endogenous Epo to make use of the upregulated EpoRs resulting in a greater efficacy of the endogenous Epo.

The optimization algorithm found a 21.1±9.7% increase in the amount of Hb produced for the best dosing time above that of the worst dosing time. This percentage

corresponds to an average difference in Hb of 34.0 grams for the best dosing time compared to the worst dosing time. The estimate for sheep blood volume for our study is 98.1mL/kg. With this value we can estimate, based on the average weight of the sheep in this study, that a Hb difference of 34.0 grams would correspond to an improvement in Hb concentration of 1.57 g/dL.

3.5.2 The linear clearance mechanism

The understanding of the linear non-erythropoietic clearance component of Epo remains incomplete. Our previous bone marrow ablation study in non-anemic lambs showed that the linear clearance of Epo accounts for only 7.38%±2.7% of the total Epo clearance and that this clearance does not change under anemic conditions (41). Even though the total linear Epo clearance does not change under anemic conditions, the proportional contribution of the linear clearance to the total clearance does change. The linear clearance in the present study was shown to contribute an average of 26.8% when the EpoR were the most down regulated.

While this study does not attempt to identify a mechanism for the linear Epo clearance, its significant contribution to the total clearance when the EpoR are down-regulated merits further examination. From previous research, it is well established that EpoRs are found in all tissues of the body and not just in the bone marrow (158-161). It is possible that these receptors have a different Epo binding characteristics in mediating a linear (non-saturable) clearance of Epo (41). However, it is not known if these additional receptors are responsible for the linear Epo clearance.

3.5.3 Lag time in the present study

In the present study the lag time between receptor stimulation and the corresponding newly formed reticulocytes appearing in the circulation was calculated to be 1.13±0.794 days. Other studies have looked at the lag time between Epo presence and reticulocyte production. One study found this lag time to be 1.7 days in adult humans (180) while another study calculated the lag time as 0.873 days in young sheep (170). These results seem to be similar to the results found in this study although the variability on the lag time in this study was high. A recent publication has shown that under anemic conditions reticulocytes may be prematurely released into the blood stream (181). Early release of reticulocytes will lead to an earlier detection of new Hb in the blood stream which could contribute to study-to-study variability in lag time.

3.5.4 Application to other Epo stimulating agents

Results from this study show that there is a large variability on what Epo administration time is optimal (Tables 3.2 and 3.3). In addition to knowing the optimal Epo administration time, achieving peak Epo plasma concentrations is also important to produce the maximum possible effect. Achieving the peak plasma concentration at the optimal time in Epo is difficult because Epo has a short terminal half-life of approximately 4-8 hours (46, 47). Fortunately, there are several Epo stimulating agents (ESAs) which still interact with the Epo receptor resulting in erythrocyte production but have a longer terminal half-life. The ESA novel erythropoiesis-stimulating protein (NESP), pegylates human erythropoietin and continuous erythroid receptor activator (CERA) have been shown to have terminal half-lives of 24-26 hours (38), 28 hours (145) and 134 hours (182), respectively in humans. With the longer half-life of these ESAs it is

possible for their endogenous concentration to stay high around the optimal time to administer Epo. Of course, utilizing these alternative ESAs assumes that they have the same Epo receptor activation as Epo which can lead to the same level of erythrocyte proliferation. This assumption is likely not valid for the ESA NESP where research has shown that the Epo receptor affinity for Epo is 4.3 fold higher than for NESP (38). Whether this differential receptor affinity counteracts the extended half-life is debatable and currently under further investigation. Additional detailed PK/PD research is needed to properly assess the benefits of ESAs over conventional Epo therapy.

3.5.5 Potential clinical application

There have been a number of clinical trials where Epo has been administered to preterm infants with the goal of reducing or eliminating the need for blood transfusions. A review study done on 27 different Epo clinical trials with preterm infants concluded that although Epo administration reduces the use of RBC transfusions and the quantity of RBCs transfused, these reductions were claimed to be of limited clinical importance (1). However since these published clinical trials used a "trial and error" methodology that does not consider the complex PK/PD of Epo it is not clear how well they can be used as a guide to the real benefit of Epo when Epo is *optimally administered* to anemic infants.

The results from the current study show that the Epo receptor state plays an important role in Hb production. Furthermore, in the sheep studied the endogenous Epo drops sharply at a certain point (Figure 3.1 day 9). In a similar way, preterm infant's endogenous Epo concentrations drop sharply in the first few weeks of life (9). This similarity in the drop in Epo indicates a similarity may exist in the Epo clearance/ receptor pool dynamics in sheep and humans. Thus, the knowledge of Epo's efficacy

relative to the Epo receptor pool dynamics gained from sheep experiments may provide a guide for how to optimally administer Epo in anemic infants. The hypothesis based on this similar kinetic mechanism would be that a larger Epo receptor pool provides a greater potential for Hb production. Accordingly, the Epo dosing should optimally be "in sync" with the variable status of the Epo receptor pool so that the peak Epo concentration coincides with the maximally unregulated quantity of Epo receptors.

3.6 Conclusion

A substantial increase in Epo's efficacy is possible by a dosing optimization that considers the dynamic receptor regulation mechanism. The current work illustrates the importance of considering the EpoR state in pharmacodynamic models. This study was conducted under anemia induced by a major phlebotomy, but the basic approach may be extended to other anemic conditions for which similar large improvements in erythropoietic efficacy may be possible by considering the EpoR dynamics such as anemia of prematurity.

Table 3.1 Parameter value summary for pharmacodynamic model

Parameter	Mean Value	Mean STDEV
E_{max} (g/day)	0.333	0.412
EC_{50} (mU/mL)	578	1160
Lag Time (days)	1.13	0.794
K_3 (1/day)	0.807	0.359
K_1 (mL/mU/day)	9.75E-03	4.63E-03
K_2 (1/day^2)	16.3	10.9
Hb Correlation r	0.953	0.0180
Clearance Correlation r	0.876	0.0770

Table 3.2 Summary of dosing optimization for best possible solution

Subject #	Optimal Post-Phlebotomy Dose Time 1 (days)	Percent increase in objective function
1	1.40	23.0%
2	13.2	20.9%
3	2.20	24.3%
4	13.7	33.5%
5	14.1	30.0%
6	7.90	26.5%
7	13.0	31.3%
8	16.8	12.3%
9	18.1	26.0%
10	18.0	7.80%
11	19.1	6.50%
12	1.90	6.10%
13	12.9	15.6%
14	7.40	31.2%
Mean	11.4	21.1%
STDEV	6.20	9.70%

Table 3.3 Summary of second dosing optimization solution

Subject #	Optimal Post-Phlebotomy Dose Time 2 (days)	Percent increase in objective function
1	13.0	15.6%
2	4.10	13.7%
3	12.0	4.50%
4	2.00	14.8%
5	2.40	11.5%
6	1.50	12.2%
7	1.20	12.9%
8	2.10	3.30%
9	4.10	6.70%
10	0.900	6.80%
11	3.40	4.30%
12	12.8	4.30%
13	3.00	10.4%
14	2.50	21.7%
Mean	4.60	10.2%
STDEV	4.40	5.40%

Figure 3.1 Representative fit of pharmacodynamic model to Hb (top) and Cl data (bottom). Erythropoietin data represented with a linear spline function. (top) Hb: Hemoglobin; Epo: Erythropoietin; Cl: Clearance

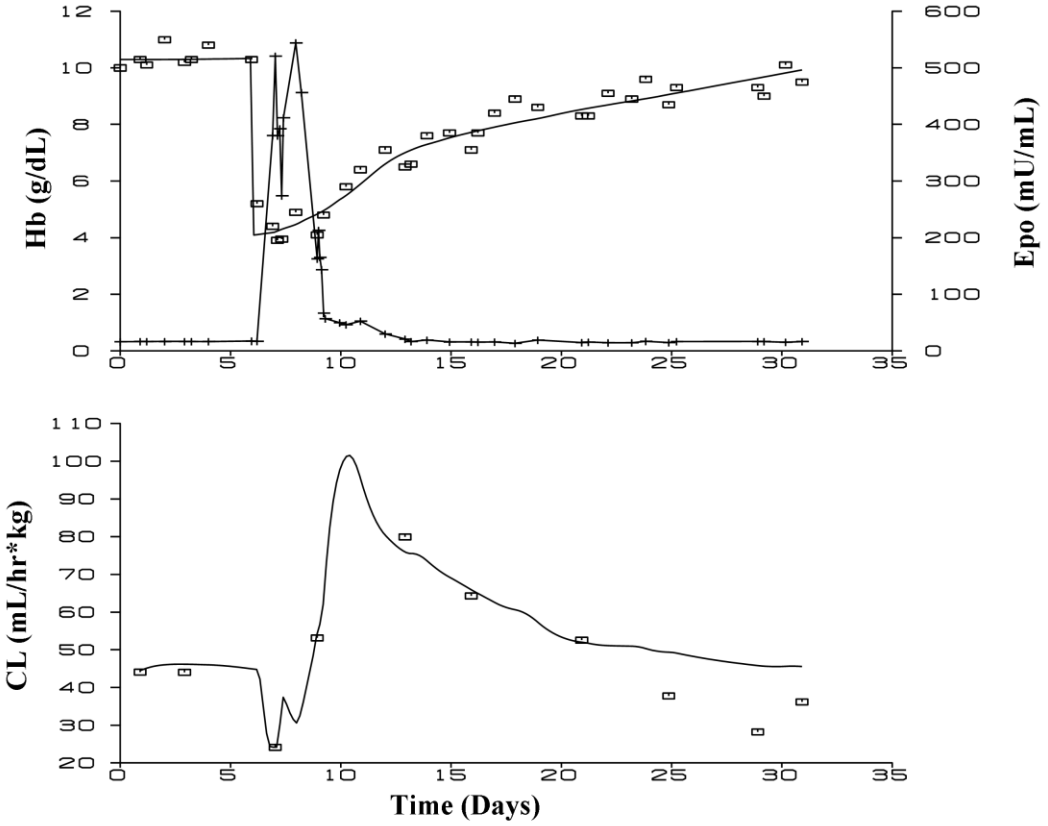


Figure 3.2 Representative fit of pharmacodynamic model to Hb (top) and Cl data (bottom). Erythropoietin data represented with a linear spline function. (top) Hb: Hemoglobin; Epo: Erythropoietin; Cl: Clearance

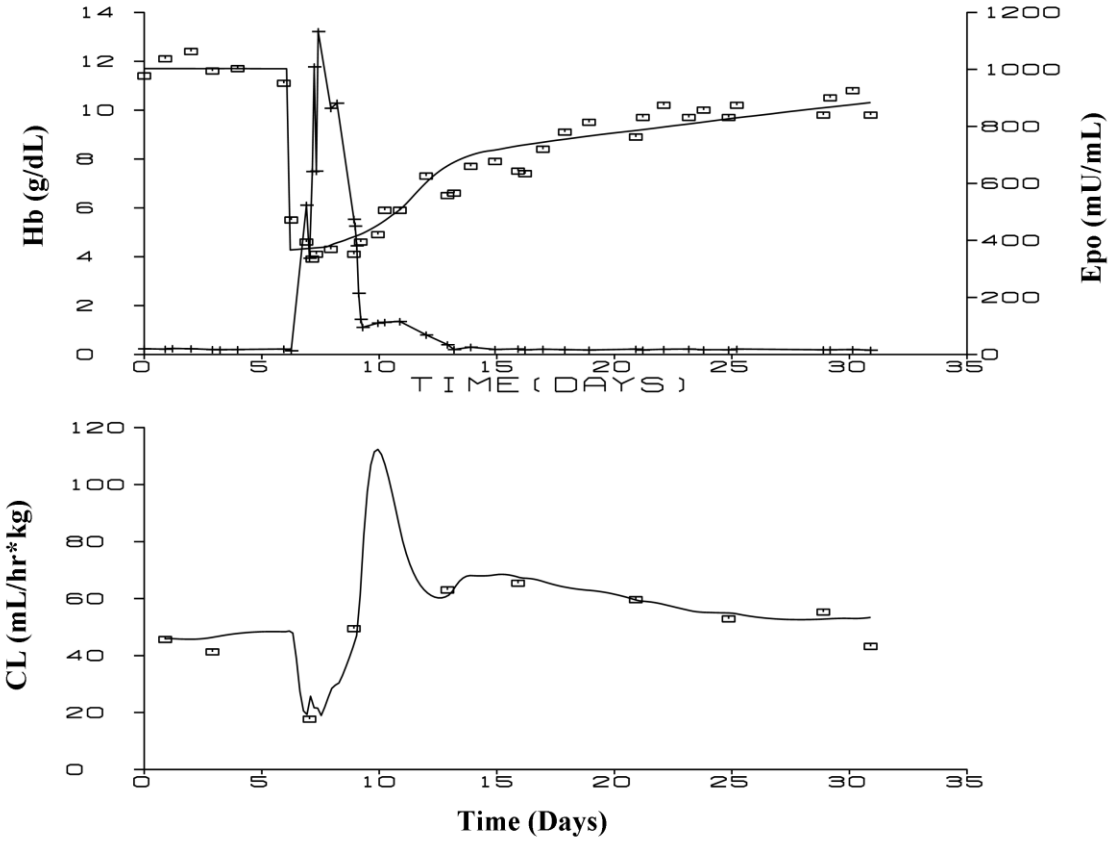


Figure 3.3 Representative estimated Hb profile (top) for model with a simulated IV bolus Epo dosing (broken curve) and without any Epo dosing (continuous curve). Representative Epo profiles (bottom) with an IV bolus Epo dosing simulation

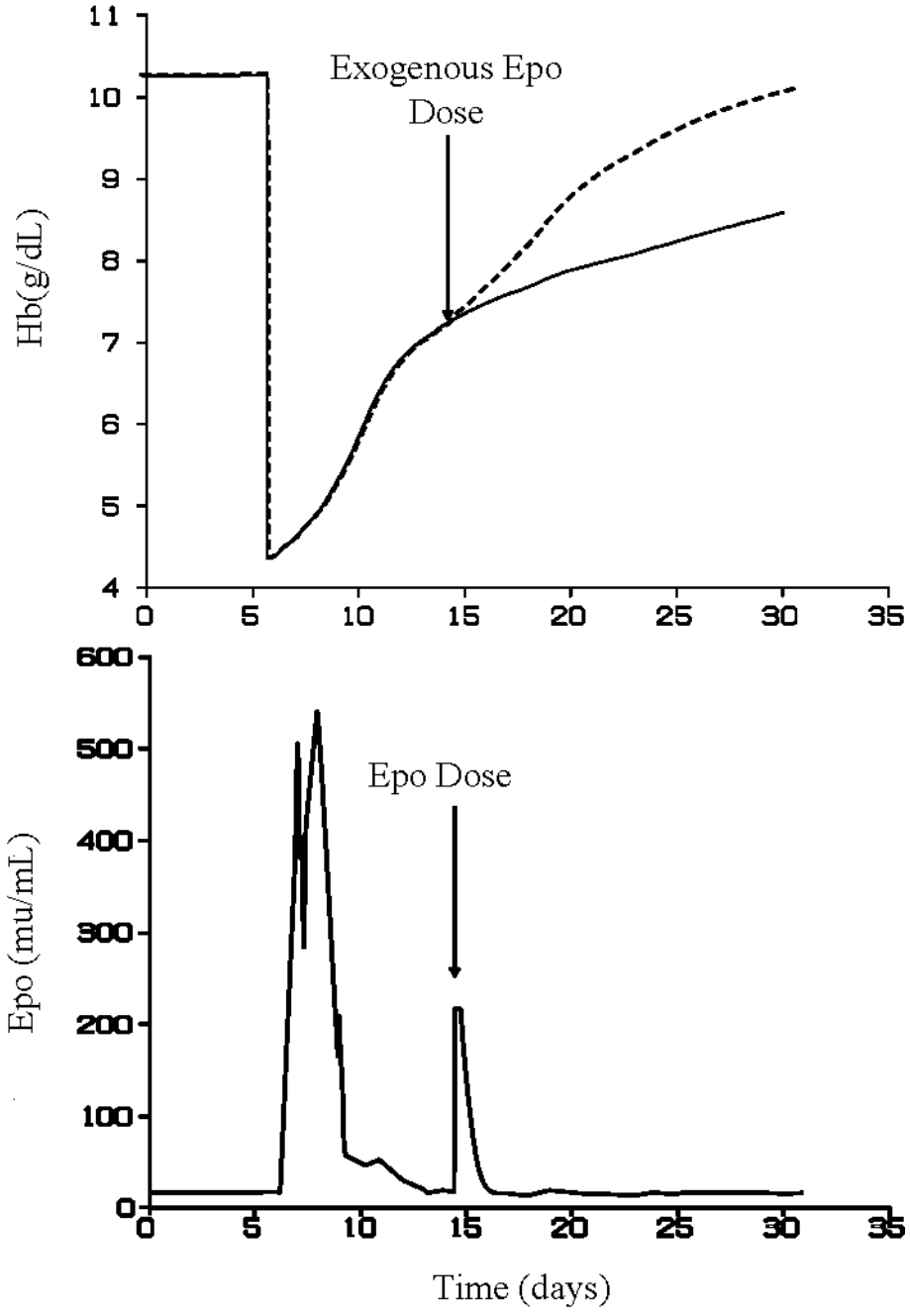
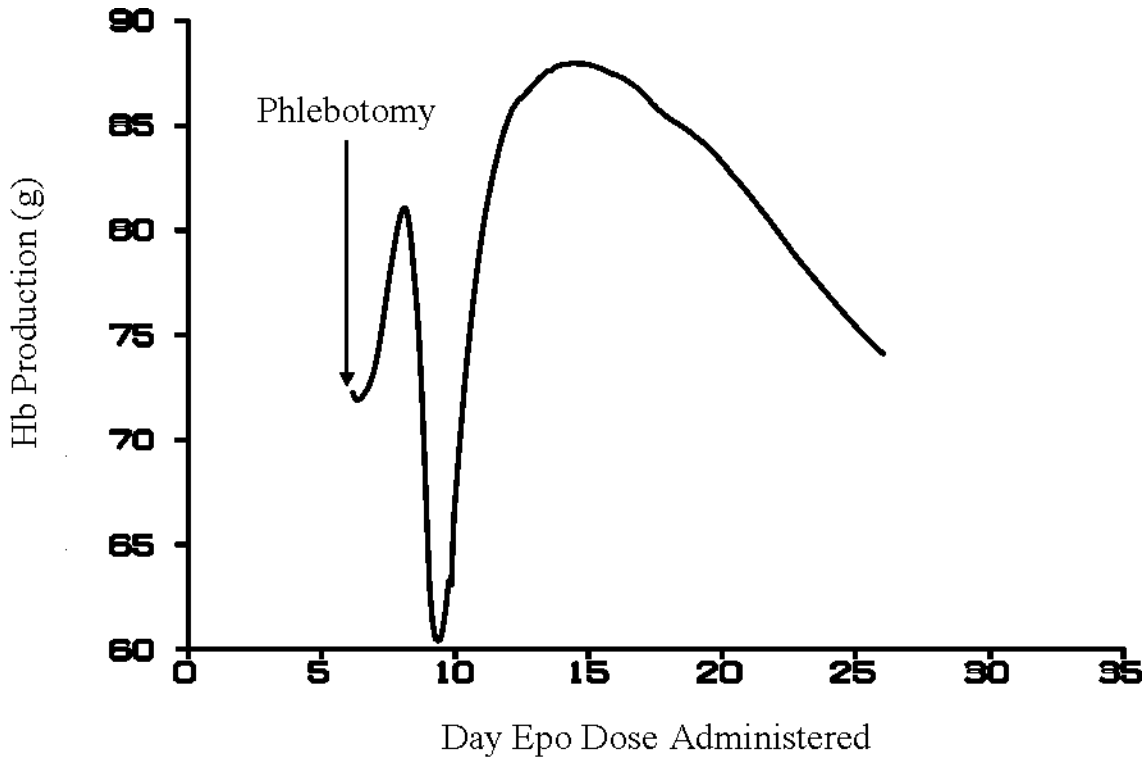


Figure 3.4 Representative plot of amount of Hb produced as a function of simulated Epo dosing time for one animal



CHAPTER 4. MULTI-DOSE OPTIMIZATION SIMULATION OF ERYTHROPOIETIN IN PRETERM INFANTS

4.1 Abstract

Preterm infants commonly develop anemia requiring red blood cell transfusions (RBCTx). Although an alternative therapy is recombinant human erythropoietin (Epo), it is not widely employed. To provide a rigorous scientific basis supporting the latter approach, a model-based analysis of endogenous erythropoiesis was developed to derive Epo pharmacodynamic (PD) hemoglobin production parameters in 27 preterm infants studied intensively during the first 28 days of life. Model-derived Epo PD parameters were combined with pharmacokinetic (PK) parameters derived from the literature to simulate an optimized IV Epo bolus dosing schedule. The goal of this simulated optimized schedule was to eliminate RBCTx, as prescribed per current guidelines, in as many preterm infants as possible. The PK/PD model identified an optimal Epo dosing algorithm in preterm infants that demonstrated maximal efficacy when Epo was dosed frequently during the early weeks of life (when phlebotomy loss is greatest). Model-based simulations, employing optimized Epo dosing, predicted that 13 of the 27 (46%) infants would avoid RBCTx ("good responders"). Importantly, simulation results identified five subject-specific covariate factors predictive of good Epo response. This simulation study provides a basis for possibly eliminating RBCTx in infants, who can be selected for optimized Epo therapy.

4.2 Introduction

All preterm infants develop anemia in the first few weeks of life as a result of multiple physiologic, pathophysiologic and iatrogenic events (79). Among neonates most critically ill, anemia is greatly exacerbated by frequent physician-ordered laboratory tests (74, 92, 93). As treatment for anemia, as many as 80% of very low birth weight infants (VLBW) weighing less than 1,500 g at birth and 95% of extremely low birth weight infants (ELBW) weighing less than 1,000 g at birth receive one or more red blood cell transfusions (RBCTx) (43). The efficacy of RBCTx, measured in terms of improving tissue oxygenation, is not always apparent. Moreover, RBCTx as given to preterm infants are expensive and can be associated with complications that include microbial infections, retinopathy of prematurity and electrolyte perturbations (1).

The hormone erythropoietin (Epo) stimulates red blood cell (RBC) production and has been investigated as an alternative therapy for the treatment and prevention of neonatal anemia. In a meta-analysis of 27 Epo clinical trials in VLBW and ELBW infants in which the goal was to reduce RBCTx, it was concluded that the effect of Epo in reducing RBCTx in preterm infants was too limited to be of clinical importance (1). However, this conclusion may be challenged for several reasons. First, there are difficulties in combining many clinical trials into a meta-analysis because of the heterogeneity of studies employing different Epo doses and schedules, different routes of Epo administration, different RBCTx criteria, different definitions of success, and different enrollment criteria. Second, the empirical choice of dosing used in some Epo clinical trials is limited because the trials were not designed to recognize the individual responsiveness to Epo treatment (different PD).

An attractive alternative approach to the design of Epo clinical trials in preterm infants is a personalized medicine approach in which Epo's complex pharmacokinetics (PK) and pharmacodynamics (PD) are more fully considered. This powerful PK/PD approach is attractive because it considers how individual infants respond to Epo. Thus, this approach can be used to develop an optimized Epo dosing regimen that both (a) targets sub-sets of infants predicted to be the most responsive to treatment with Epo: and (b) identifies those unlikely to respond. Unfortunately, compared to the extensive Epo PK/PD studies in adults, only limited Epo PK/PD data exist in this difficult-to-study infant patient group.

The mechanism of action for the clearance of Epo in both adults and infants has been identified as non-linear, erythropoietin receptor (EpoR)-mediated endocytosis (33, 34, 43, 47) followed by lysosomal degradation (17). Determining preterm infants' PD response to Epo is experimentally demanding because it requires an accurate real-time evaluation of hemoglobin (Hb) production, a procedure that is greatly impacted by the numerous phlebotomies and RBCTx very premature infants receive (79). Recently, our research group overcame these problems using a mass balance approach to determine the Hb production in 14 critically ill VLBW preterm infants studied for factors impacting endogenous erythropoiesis. This approach required meticulous accounting for Hb transfused and Hb removed by phlebotomy, for RBC losses due to senescence and for expanding blood volume as a consequence of growth (9).

The sample size was not specifically determined for this present study since this study represents a secondary analysis of data from a yet-to-be published study whose aims/hypothesis required a sample size of 24 VLBW infants.

4.3 Methods

4.3.1 Subjects

The study was approved by the University of Iowa Human Subject Internal Review Board in accordance with the Declaration of Helsinki. All parents signed informed consent. Subjects eligible for enrollment included the offspring of pregnant women presenting to labor and delivery at <29 weeks gestation and infants born at <29 weeks gestation who required intubation in the first day of life. Infants excluded were those presenting with hematological disease (except for anemia of prematurity), those receiving RBCTx prior to enrollment, and those receiving erythropoiesis stimulating agents. There were a total of 162 mothers or infants who met study eligibility criteria. Of those eligible for enrollment, 119 were not approached for the following reasons: 1) they had already been approached for another clinical study with similar eligibility criteria (n=39); 2) the significant workload imposed on the clinical lab for weighing all clinical blood samples only allowed two research subjects to be studied at a time (n=62); 3) prior blood transfusion before obtaining consent (n=13); and 4) lack of staff availability (n=5).

A total of 43 families were approached, 11 before delivery and 32 after delivery. Consent was obtained from 33 families while 10 families refused. Women who were consented antenatally but who delivered at >29 weeks and became ineligible (n=6). Twenty-seven infants were enrolled in the study and studied for a period of approximately one month (31.6 ± 2.2 days).

4.3.2 Study procedures

Laboratory, phlebotomy, and RBCTx data were obtained from the subjects' electronic medical record. This permitted the determination of endogenous Hb production by mass balance principle. Accurate weights for 96.6% of all discarded blood tests were recorded (9). Leftover plasma was saved for subsequent analysis for Epo. If the plasma volume from the sample was insufficient for these determinations, samples were pooled in 8-h epochs. Endogenous plasma Epo concentrations were determined using a double antibody radioimmunoassay as previously described (183). The left over anticoagulated blood samples <3 d old were analyzed for hematological parameters using the Sysmex XE-2100 automatic hematology analyzer (Sysmex Corporation, Kobe, Japan).

The RBCs transfused to the infants likely contained some Epo because the adult donors normally have a small baseline concentration of endogenous Epo. In order to determine the contribution from each RBCTx to the infant's total endogenous Epo level, Epo concentrations were compared before and after each RBCTx using a paired t-test. Comparison of the endogenous Epo levels before and after the RBCTx excluded RBCTxs which occurred in the first week of life because infants are often born with an elevated, above baseline, Epo level.

4.3.3 Simulation and pharmacodynamics

The infant Epo dosing simulations were designed to examine if repeated 600 U/kg IV Epo doses administered at optimal times could completely eliminate the need for RBCTx. The simulated optimized Epo dosing regimen was designed to provide 12 IV. bolus doses during the first 28 days of life. This number of "unit doses" was chosen to

correspond to the approximate number and amount of Epo doses commonly administered to preterm infants in clinical trials (1).

The RBCTx triggers were defined by a modification of criteria applied to preterm VLBW infants in the largest randomized clinical RBCTx trial to date, i.e., the "Preterm Infants In Need of Transfusion" (PINT) study (96). For simplicity, the average Hb levels for infants requiring respiratory support in the restricted and liberal study arms were used. This resulted in the following postnatal age-based Hb trigger levels: week 1: 12.5 g/dL, week 2: 11 g/dL, week 3 and beyond: 9.3 g/dL. In the PK/PD simulation model applied, Epo optimization was considered successful if Epo dosing was able to maintain the infant's Hb level above the PINT study RBCTx trigger.

4.3.4 PK/PD model used in simulations

To determine the rate of Hb production from the simulated Epo dosing regimen, the following model was used to estimate the effect of Epo dosing in individual infants based on its concentration at any given point in time:

$$E(t) = \frac{E_{\max} \cdot Epo(t - t_{lag})}{EC_{50} + Epo(t - t_{lag})} \tag{4.1}$$

where E(t) is the Hb production rate at time t; t_{lag} is the lag time between the Epo stimulation of EpoR and the appearance of newly synthesized reticulocytes, i.e., Hb appearing in the circulation; EC_{50} is the plasma concentration of Epo resulting in half of the maximum Hb production rate; E_{max} is the maximum achievable Hb production rate.

The Hb level of individual infants at birth was used as the initial Hb level in the simulations. To account for RBC production, RBC senescence and Hb loss resulting

from laboratory blood testing in the simulations, each infant's Hb concentration over the study period was modeled as follows:

$$\frac{dHb(t)}{dt} = E(t) - Hb(t) \cdot Cl_P(t) - \frac{Hb_O(t)}{\tau - t}$$
$$\frac{dHb_O(t)}{dt} = -Hb_O(t) \cdot Cl_P(t) - \frac{Hb_O(t)}{\tau - t} \quad (4.2)$$

, with initial conditions where:

$$Hb(0) = Hb_O(0) = Hb_{SS} \quad (4.3)$$

, and where:

$$C_{Hb}(t) = \frac{Hb(t)}{V_{KG} \cdot W(t)} \quad (4.4)$$

The phlebotomy clearance function, $Cl_P(t)$, is the volume of blood removed/time/blood volume; $Hb_O(t)$ is the old Hb amount remaining at time *t*, i.e., the Hb that is remaining from the RBCs present at birth; $Hb(t)$ is the total amount of Hb at time *t* (remaining old plus newly produced Hb); Hb_{ss} is the Hb amount at "steady state"; τ is the lifespan of RBCs; V_{KG} is the blood volume per kg; $W(t)$ is the weight of the infant at time t and $C_{Hb}(t)$ is the concentration of Hb at time t.

The model equations above assume that newly produced neonatal RBCs do not undergo senescence during the 28-day study period because the RBC lifespan is greater than 28 days. In the model simulations, it was assumed that newly produced RBCs had a fixed lifespan, τ at 65.8 days (9). There was no need to considerer RBC lifespans other than the endogenous RBC lifespans because the ultimate aim for the simulation study was to eliminate RBCTx and therefore RBCTx were not simulated.

4.3.5 Epo pharmacokinetics

Information about the elimination of Epo (PK) in preterm infants is required in deriving the Epo dosing simulations. A previous study in preterm infants by Juul et al. reported Epo plasma clearance values of 17.3, 13.1, and 8.20 mL/h·kg with exogenous Epo doses of 500, 1000 and 2500 U/kg, respectively (47). The 500 U/kg clearance value was similar to the value determined previously by our group of 16 mL/h·kg (43). The study by Juul et al. reported a volume of distribution of 104 mL/kg that did not change with Epo dose administered. The disposition elimination of Epo in the simulation model was estimated as follows:

$$\frac{dEpo}{dt} = -\frac{P_1 \cdot Epo}{P_2 + Epo} \tag{4.5}$$

where:

$$\begin{aligned} Epo_1(0) &= 500 / V \\ Epo_2(0) &= 1000 / V \\ Epo_3(0) &= 2500 / V \end{aligned} \tag{4.6}$$

In Eq. 5 *Epo* is the plasma Epo concentration at time t; P_1 and P_2 are disposition parameters, where P_1 is the maximum rate at which the Epo concentration declines and P_2 is the plasma Epo concentration at which Epo is eliminated at half of the maximum rate; and V is the fixed volume of distribution of Epo, i.e., 104 mL/kg (47).

For each literature reported Epo dose (i.e., 500, 1000 and 2500 U/kg) and fixed P_1 and P_2 disposition values, an area under the curve (AUC) can be calculated according to the integration from time 0 to infinity for Eq. 4.5. Therefore, for each Epo dose, corresponding to the current P_1 and P_2 parameter estimates, an AUC and clearance value can be calculated according to:

$$\begin{aligned} Dose_1 / AUC_1 &= Cl_{1,C} \\ Dose_2 / AUC_2 &= Cl_{2,C} \\ Dose_3 / AUC_3 &= Cl_{3,C} \end{aligned} \tag{4.7}$$

where AUC is the area under the curve calculated for the given Epo dose and Cl_C is the plasma clearance calculated for the same dose. The values of P_1 and P_2 were estimated such that the difference between the calculated and literature derived Epo clearance values were minimized by minimizing the value of the objective function as follows:

$$\text{Objective Function} = (Cl_{1,C} - Cl_{1,R}) - (Cl_{2,C} - Cl_{2,R}) - (Cl_{3,C} - Cl_{3,R}) \tag{4.8}$$

The notation $Cl_{1,R}$ is the reported clearance for dose 1, e.g., 17.3 mL/h·kg for a 500 U/kg dose of Epo (47). The objective function value for Eq. 8 was minimized using the derivative free optimization algorithm developed by Nelder and Mead (176) referred to as the simplex method. With the combined PK information derived from Juul et al. and given in Equation 4.5 and the PD information determined from Equation 4.1, the final model applied in the Epo dosing simulations for individual study infants was as follows:

$$\begin{aligned} \frac{dHb(t)}{dt} &= \frac{E_{\max} \cdot Epo(t - t_{lag})}{EC_{50} + Epo(t - t_{lag})} - Hb(t) \cdot Cl_P(t) - \frac{Hb_O(t)}{\tau - t} \\ \frac{dHb_O(t)}{dt} &= -Hb_O(t) \cdot Cl_P(t) - \frac{Hb_O(t)}{\tau - t} \\ \frac{dEpo}{dt} &= -\frac{P_1 \cdot Epo}{P_2 + Epo} \end{aligned} \tag{4.9}$$

, with initial conditions:

$$\begin{aligned} Hb(0) &= Hb_O(0) \\ Hb_O(0) &= E_{ss} \cdot \tau \\ Epo(0) &= DOSE / V \end{aligned} \tag{4.10}$$

where E_{ss} is the Hb production rate at birth. The final model given by Equation 4.9 was used to simulate changes in hemoglobin for individual infants based on the plasma Epo concentration, phlebotomy blood loss and endogenous RBC senescence.

4.3.6 Dosing optimization algorithm

Optimal Epo dosing was defined as the dosing schedule, which results in a Hb concentration versus time curve that is as close as possible to --- but always remains above --- the Hb level defining the RBCTx trigger. Accordingly, the Hb target level used in the simulation model, *R(t),* was 3 g/dL above the Hb-level defined by a linear spline equal to the aforementioned PINT-derived RBCTx trigger Hb level (96). The 3 g/dL Hb margin was empirically chosen to reduce the likelihood of Hb concentrations falling below the PINT RBCTx trigger. This margin is required because of the natural smoothness of the simulated Hb level, which is otherwise impossible to achieve given the staircase-shaped RBCTx trigger level. The simulated Hb and Epo levels were determined according to Equation 4.9, while the objective function to be minimized was:

$$\text{Objective Function} = \sum_{i=1}^{N} \int_{0}^{E} (S_{HB,i}(t) - R_i(t))^2 dt \qquad (4.11)$$

, where *i* denotes the individual subject; *N* is the number of subjects (*N*=27 subjects); *E* is the duration of the study (i.e., 28 days); $S_{HB,i}(t)$ is the simulated Hb profile for subject *i* at time *t*; and $R_i(t)$ is the target Hb profile at time *t* for subject *i*. The value of objective function (Equation 4.11) was again minimized by the Nelder-Mead simplex method (176). Equation 4.11 permits the determination of individual Epo dosing regimens for all 27 infants that maintains simulated Hb concentrations as close as possible to the RBCTx target Hb. The double precision Fortran subroutine RETARD was used for numerical integration of the delay-type differential equations. The Cl_P parameter was represented as a linear spline fit of the infants' phlebotomy data. All graphical output was constructed using WINFUNFIT, a Windows version evolved from the general nonlinear regression

program FUNFIT (146). The sample size was not specifically determined as the present study represents a secondary analysis of a yet-to-be published study in which the sample size was 24 VLBW infants.

4.3.7 Practical dosing schedule development

Although the dosing optimization algorithm in the previous section was designed determine the best possible times to administer 12 Epo doses, the optimized timing schedule may not be practical for hospital staff to administer because of the specific timing of the doses. In order to make the optimized dosing schedule more clinically relevant and practical, the optimized simulated dosing schedule was modified so that only one Epo dose could be received each day. In addition if two Epo doses were given in consecutive days then they would be simulated to be administered 24 hours apart (i.e. age 0.0 and 1.0 for two consecutive doses on the first two days of life). The practical dosing schedule used is given in Figure 4.5. The objective function of this "practical" dosing schedule was determined according to Equation 4.11 and compared to the objective function value for the optimized dosing schedule.

4.3.8 Accuracy of continuous bleed function

The continuous bleed function used in Equations 4.2 and 4.9 is an approximation for multiple discrete events; therefore, an error analysis was completed. Since the exact amount of blood removed from each infant is know due to the meticulous weighing of the blood samples, a general cross-validated cubic spline could be fit to this data (Figure 4.6) (184). The actual equation that was used to estimate each infant's endogenous Hb concentration (Equation 4.9) included Hb production RBC senescence and RBC loss due

to phlebotomies. Therefore, the first derivative of the general cross-validated cubic spline fit in Figure 4.6 was used in the differential equations given in Equations 4.2 and 4.9. An error analysis was conducted to determine the percentage error between the discrete phlebotomy events and the continuous bleed function. The error at a given point in time was calculated according to the following equation:

$$\theta(t) = \frac{Abs(D(t) - P(t))}{D(30)} \cdot 100 \qquad (4.12)$$

where, θ is the percent error of the continuous bleed function at time t; D(t) is the value of the discrete cumulative amount of blood removed at time t and P(t) is the value of the continuous cumulative amount of blood removed at time t. All cross-validated cubic splines and cubic spline derivatives were determined using the software WINFUNFIT (146).

4.4 Results

The preterm study infants had a mean gestational age of 26.7 ± 1.29 (mean ± SD) and a corresponding birth weight of 900 ± 254 g. All of the 27 infants enrolled were ventilated, and all but three received ≥1 RBCTx during the first 4 weeks of life (mean 3.26 ± 1.93). The RBCTx volume received by the infants was 49.6 ± 27.3 mL of packed RBCs per kg body weight at the time of RBCTx. All the infants had frequent physician-ordered phlebotomies with an average of 54.6 ± 27.2 mL/kg of whole blood removed per infant during the first 4 weeks of life. A summary of all endogenous Epo concentrations shows an initial elevated Epo level followed by the Epo level dropping to baseline (Figure 4.1). The infants had a mean endogenous Epo concentration of 22.2 ± 36.5 mu/mL and a median Epo concentration of 16.2 mu/mL. In a paired t-test analysis there

was no difference in Epo concentrations before (18.5 ± 9.6 mu/mL) and after (18.8 ± 9.6 mu/mL) the RBCTxs ($p>0.05$). None of the 27 infants died and all completed the 28 day study.

4.4.1 Epo PK/PD

The Epo PD parameters, EC50 and Emax (Eq. 1), were determined for all subjects as previously described (9) (Table 4.1). The disposition parameters, P1 and P2 characterizing Epo's elimination, which have not been previously reported, were calculated to be 33.2 U Epo/mL•hr and 5.99 U Epo/mL respectively (Eq. 5). For these values of P1 and P2, the clearance values were calculated to be 17.2 mL/h·kg for the 500 U/kg Epo dose, 13.4 mL/h·kg for the 1,000 U/kg Epo dose, and 8.02 mL/h·kg for the 2,500 U/kg Epo dose. These calculated clearances are strikingly similar to the clearance values reported as 17.3, 13.1 and 8.20 mL/h·kg for Epo doses of 500, 1000 and 2500 U/kg, respectively (47).

4.4.2 Dosing optimization

The dosing optimization simulation procedure determined the best time to administer the twelve 600 U/kg IV bolus unit doses of Epo to all 27 preterm infants (Figure 4.2). The first two simulated Epo doses corresponded to a double 600 U/kg Epo dose at birth. The same closeness is observed for the 10th and 11th doses. To maintain infants' simulated Hb as close as possible, yet above, the RBCTx target Hb level, 67% of the Epo doses for all infants were administered in the first 8 days of life.

The resultant prediction of the plasma Epo concentration profile in infants was determined using the disposition model given by Eq. 5 (Figure 4.3). The optimized

plasma Epo profile determined the simulated Hb level (Eq. 9) for all 27 infants. Four representative simulated infant Hb concentration plots are presented in Figure 4.4. In each simulated Hb concentration-time plot, weight adjusted cumulative blood loss and RBCTx Hb trigger criteria derived from the PINT trial are shown. Subjects A and B are examples of good Epo responders who were predicted to avoid RBCTx, while subjects C and D were predicted to be poor Epo responders requiring RBCTx. In 13 of the 27 (46%) study subjects, RBCTx were avoided in the simulations.

Results of the simulation study were also used to identify potentially significant clinical and laboratory differences in covariates associated with either good or poor Epo response. To do this and to initiate Epo therapy when it is likely to be the most effective, only covariates which could be determined during the first day of life were compared (i.e., using a two sample t-test). Due to the small sample size, the analysis was done in a univariate manner. Five covariates were identified as significant ($p<0.05$) in distinguishing good responders from poor responders (Table 4.2). The significant covariate factors identified included the number of RBCTx, Emax and weight adjusted cumulative blood removed. Covariates analyzed in the first 24 hours of life but not found to be significantly different between good and poor responders were Hb concentration, Epo concentration, RBC count, white blood cell concentration, mean corpuscular volume, platelet count, reticulocyte count, and heart rate.

As previously mentioned the optimal Epo dosing schedule given by Figure 4.2 is not practical for nurses to implement in a clinical setting. When the dosing times were adjusted to implement in a practical manner as described in the methods section (Figure 4.5), a drop in the objective function (Equation 4.11) of only 2.7% was observed. This

indicates that although the practical dosing schedule is not optimal, there is likely little difference in clinical outcome when making the dosing schedule practical.

4.4.3 Accuracy of continuous bleed function

The accuracy of the continuous bleed function given by Equations 4.2 and 4.9 was shown to be less than 5% for the duration of the study for all subjects (Figure 4.6). Figure 4.6 demonstrates that a general cross-validated cubic spline was able to fit the discrete phlebotomy data well. Although the cubic spline shows an error as high as 3%, for the majority of the study the error is around 1%. The subject shown in Figure 4.6 was the subject with the most cumulative blood removed out of all of the subjects.

4.5 Discussion

Although Epo has been shown to be useful in consistently stimulating erythropoiesis in preterm infants and in significantly reducing the number and volume of RBCTx in some subsets of preterm infants (1), the goal for Epo administration in the present study was to completely eliminate the need for all RBCTx. Results of our PK/PD modeling in 27 VLBW critically ill infants indicated that with optimally simulated Epo dosing, 46% of the infants were predicted to avoid RBCTx --- as judged by their simulated Hb levels staying above the RBCTx trigger criteria of the PINT trial (96). This is a higher percentage than the value of 20% (CI 14-25%) included as a composite of 16 clinical trials where Epo was administered to preterm infants per standard dose and schedule without consideration of individual infant clinical and laboratory characteristics. (1). We contend that part of this difference is likely due to the fact that, in the present

study, the simulated Epo dosing was optimized per several characteristics predicting the ability of individual infants to respond, or not, to Epo.

Another source responsible for the difference may be that the total Epo dose used in current simulation (1800 U/kg/week) is relatively high compared to published clinical trials (96). In the 16 clinical trials analyzed for avoidance of RBCTx, only one used a higher dose of Epo (2100 U/kg/week) than the current simulation study (7). This trial also reported significantly fewer infants in the Epo group needed RBCTx compared to the placebo group (risk ratio = 0.65, 95% confidence interval (0.46, 0.93). It is difficult to compare these 16 Epo clinical trials to our simulation study because of heterogeneity as indicated by their inconsistency in Epo dosing, administration routes and enrollment criteria. In applying the PK/PD model, future analyses could consider alternative RBCTx trigger criteria as more evidence-based RBCTx criteria are identified. This would be easy to implement since the optimization method does not depend on specific RBCTx criteria being applied.

4.5.1 Covariate analysis predicting Epo responsiveness

Results of the covariate analysis done to identify good responders (no RBCTx predicted) and poor responders (predicted need for one or more RBCTx) suggest that several factors may explain the difference between the two groups (Table 4.2). The E_{max} parameter was significantly higher for the good responder group indicating that based on our model predictions these infants can theoretically produce more Hb in response to given doses of Epo than the lower E_{max} group. Although the EpoR state in preterm infants has not been directly analyzed, results from our sheep bone marrow ablation study suggest that infants with a higher E_{max} parameter may have a larger EpoR pool in the

bone marrow (41). An additional key parameter found to be significantly different between the good responder and poor responder group was the cumulative, weight adjusted blood removed for laboratory testing. As expected, the cumulative laboratory blood removed in the poor responder group was much higher than in the good responder group. Infants with lower bodyweights become anemic more quickly due to their lower blood volume relative to the fixed volumes of blood removed for laboratory testing. The importance of this problem is evident from data showing that anemia in neonates is exacerbated by frequent, physician ordered phlebotomies (74, 92, 93).

The current study suggests that large Epo doses should be given in the first week of life for preterm VLBW infants followed by less frequent smaller doses. This Epo dosing strategy is in contrast to previous Epo clinical trials that employ a fixed, regular dosing. Another reason why the simulated methodology may prove to be more efficacious than previous clinical trials is that many clinical trials did not start Epo dosing soon enough after birth (4, 5, 109, 114), which the present study demonstrated is particularly important.

An additional covariate analysis done in this study considered covariates that could be determined in the first day of life for the infant (Table 4.2). Determining covariates at, or very close after birth, that may predict responsiveness to Epo is especially important because of the need to begin Epo therapy very soon after birth as demonstrated by our dosing simulations. Although the enrollment criteria for this study was a gestational age $\leq$ 29 weeks, the infants who responded well to Epo in the simulations had a mean gestation age of 27.4 weeks while the infants who responded poorly in the simulations to Epo had a mean gestation age of 25.9 weeks. This is

consistent with previous research showing that a preterm infant's average Hb increases as gestation age increases, therefore making it less likely that they will need to be transfused (64). The other initial covariates significantly different between good and poor responders were birth weight, mean arterial pressure and mean cellular hemoglobin. All of these covariates are consistent with a higher gestation age suggesting larger, more mature infants are better able to respond to Epo. Because the present study's investigation of covariates predictive of Epo responsiveness did not test all potentially important factors, this remains an important area for future research.

4.5.2 Additional covariates of interest

The current study examined several covariates when analyzing which infants would respond the best to optimal Epo therapy, however, several other factors have been shown to influence Epo PK and PD. The primary mechanism of Epo elimination from the body is though to be Epo receptor mediated endocytosis followed by lysosomal degradation (17). In addition, research in sheep has shown that the Epo receptor state changes significantly during periods of anemia suggesting the a measure of the Epo receptor state is needed to more accurately predict the PK in preterm infants (48). Currently no measure of the Epo receptor state in preterm infants is available. Chapel et al. were previously able to estimate the receptor state in sheep by using radiolabeled ^{125}I-Epo in a tracer PK study. Unfortunately, using radioactively labeled Epo is not allowed in infants due to ethical guidelines. Currently our group is working to design a non-radioactive labeling method for Epo using biotin as the label. Such a successful non-radioactive label would allow the first ever measure of the Epo receptor state in infants.

In addition to the Epo receptor's influence on Epo clearance, several cytokines have been shown to influence erythropoiesis. Specifically cytokines that are associated with iron status and inflammation have been shown to cause Epo resistance. Costa et al. examined Epo dosing in renal dialysis patients concluding that higher than normal levels of IL-1, IL-2, IL-6, C-reactive protein and tumor necrosis factor are associated with increased Epo resistance. In addition IL-3 was found to significantly inhibit erythropoietin action in a mouse study due to IL-3's inhibition of endothelial cells which produce erythroid stimulating factors (26). Hepcidin has also been previously suggested to inhibit erythropoiesis due to its metabolism of iron (27). For the current study the aforementioned covariates were not available but future studies which utilize such information will be able to determine the rate of Hb production.

4.6 Conclusions

The present Epo dosing optimization simulation study, based in part on data from actual critically ill anemic premature infants, demonstrates that administration of more frequent doses of Epo in the first week of life likely would prove to be efficacious in eliminating RBCTx needs. It also suggests the likelihood for identifying as soon as possible after birth those VLBW infants who will be best Epo responders in eliminating RBCTx. Accordingly, results of the current study can serve as the basis for initiating a prospective clinical trial in preterm VLBW infants with specific enrollment criteria and precise Epo dosing. Such a clinical trial will be necessary to confirm the results of the present simulation study.

Table 4.1 Covariate comparison of good and poor Epo responders for the infant Epo dosing simulations.

	Good Responder*		**Poor Responder**		
Factor	**Mean**	**SD**	**Mean**	**SD**	**p-value**
Number of RBCTx	1.86	1.29	4.77	1.24	<0.001
E_{max} (g/day)	0.918	0.327	0.597	0.348	<0.05
EC_{50} (mU/mL)	78.9	75.0	131	236	0.44
E_{max} / EC_{50}	0.0116	0.0273	0.0046	0.0143	0.15
Cumulative whole blood removed (mL/kg)†	36.2	19.4	74.3	19.6	<0.001

*Good responders simulated Hb stayed above the RBCTx trigger level. Poor responder simulated Hb went below the RBCTx trigger level.

† expressed per kg body weight on days when phlebotomy occurred.

Table 4.2 Comparison of covariates for good and poor Epo responders in optimized Epo dosing simulations for significant covariate factors determined in the first day of life.

	Good Responder		Poor Responder		
Factor	**Mean**	**SD**	**Mean**	**SD**	**p-value**
Birth weight (kg)	1.05	0.246	0.733	0.142	<0.001
Gestational age (wk)	27.4	0.839	25.9	1.23	<0.001
Mean Corpuscular Hemoglobin (g/dL)	36.1	2.98	39.0	2.90	<0.05
Phlebotomy loss day 0-1 (mL/kg)†	6.57	2.80	12.0	4.79	<0.001
Mean arterial pressure (torr)	38.7	5.56	29.6	4.91	<0.001

† per kg body weight on day of phlebotomy.

Figure 4.1 Endogenous Epo concentrations for the 27 preterm infants enrolled in this study for the entire duration of the study (top) and for the first 5 days of life only (bottom). The Epo concentrations are initially high and then drop to a baseline level.

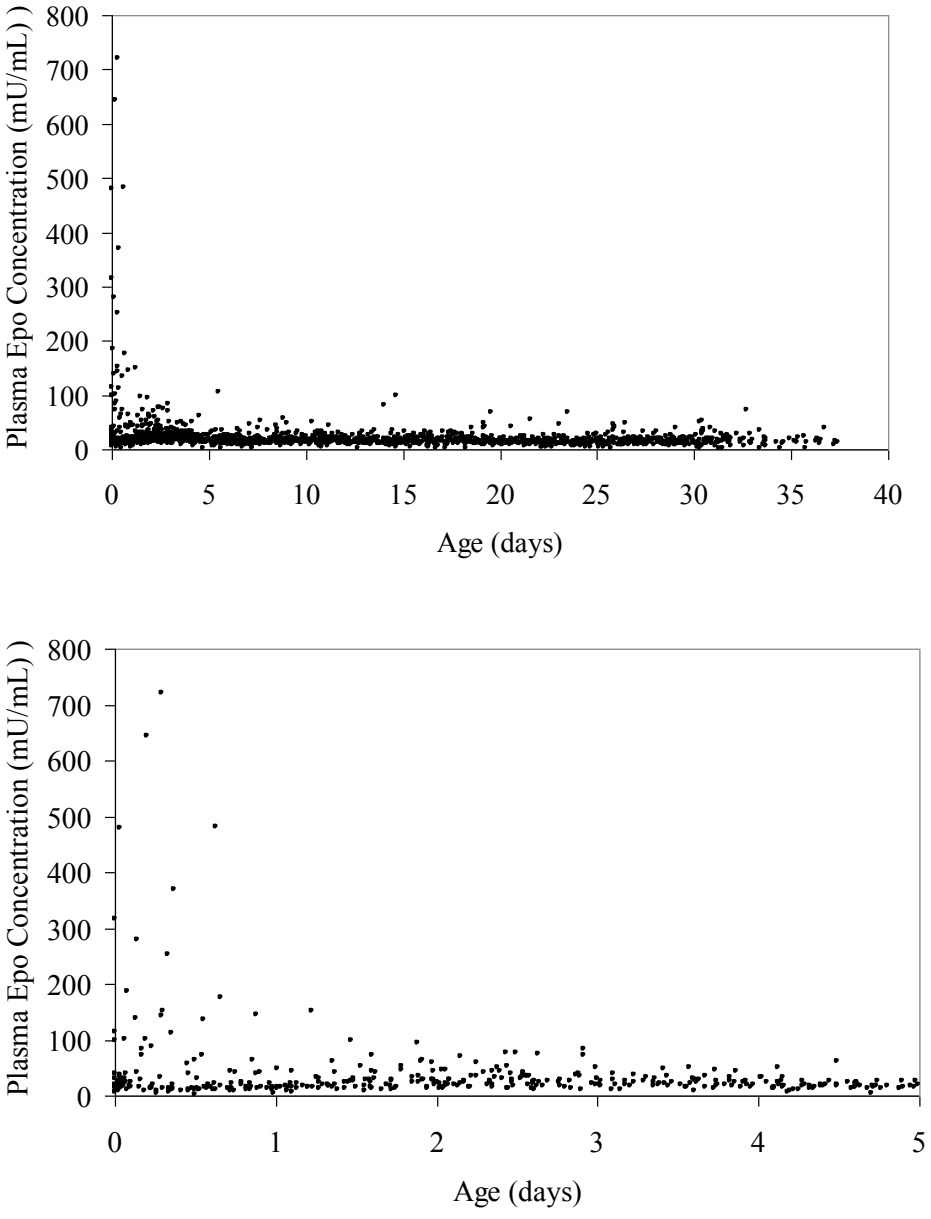


Figure 4.2 Epo Dosing Profile. Simulated Epo doses administered to 27 preterm infants optimized to eliminate RBCTx. All simulated Epo doses were 600 U/kg IV bolus doses. The dosing times for the 1st and 2nd dose were so close as to effectively represent a double dose. The same is the case for the 10th and 11th dose.

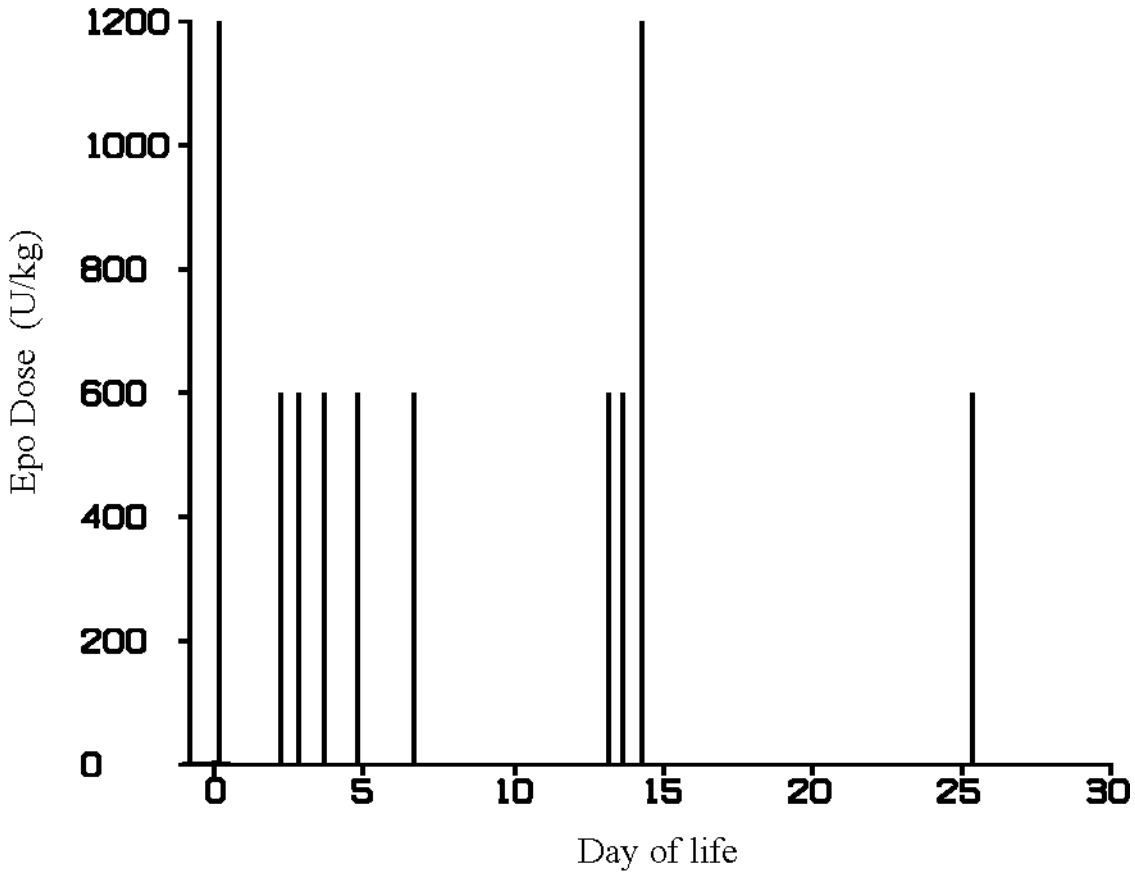


Figure 4.3 Epo Plasma Concentration Profile. Simulated plasma Epo concentration profile for an infant, corresponding to the simulated, optimized Epo dosing administered to 27 preterm infants. The Epo dosing was optimized to avoid RBCTx in the infants.

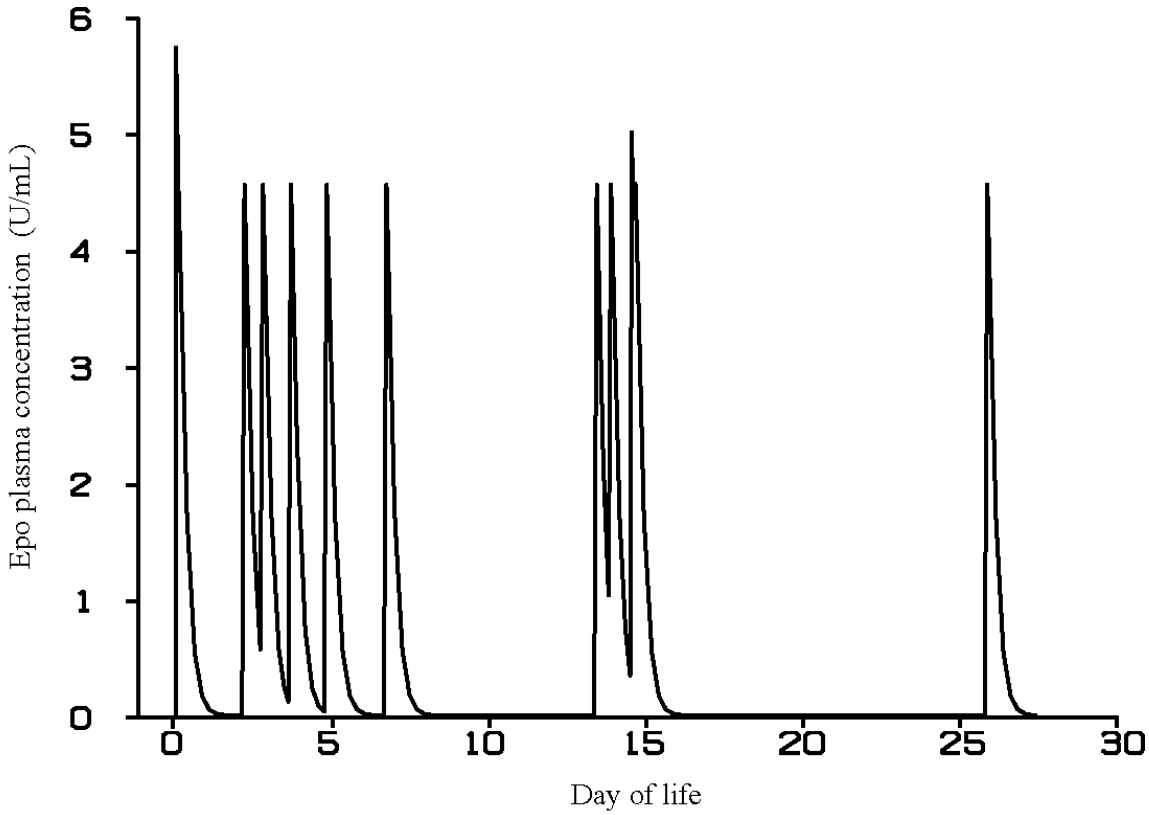


Figure 4.4 Simulated Hb Profile from Epo Dosing. Simulated infant Hb curves based on the optimized Epo dosing. The solid line is the infant's simulated Hb, the dashed staircase line is the RBCTx trigger criteria based on the PINT trial, the dashed increasing line is the cumulative amount of blood removed from the infant due to physician ordered laboratory tests. Subjects 1 and 2 were "good Epo responders" predicted to avoid transfusions, in contrast to subjects 3 and 4 were "poor Epo responders" who would have needed a RBCTx at the transfusion trigger crossing point (●).

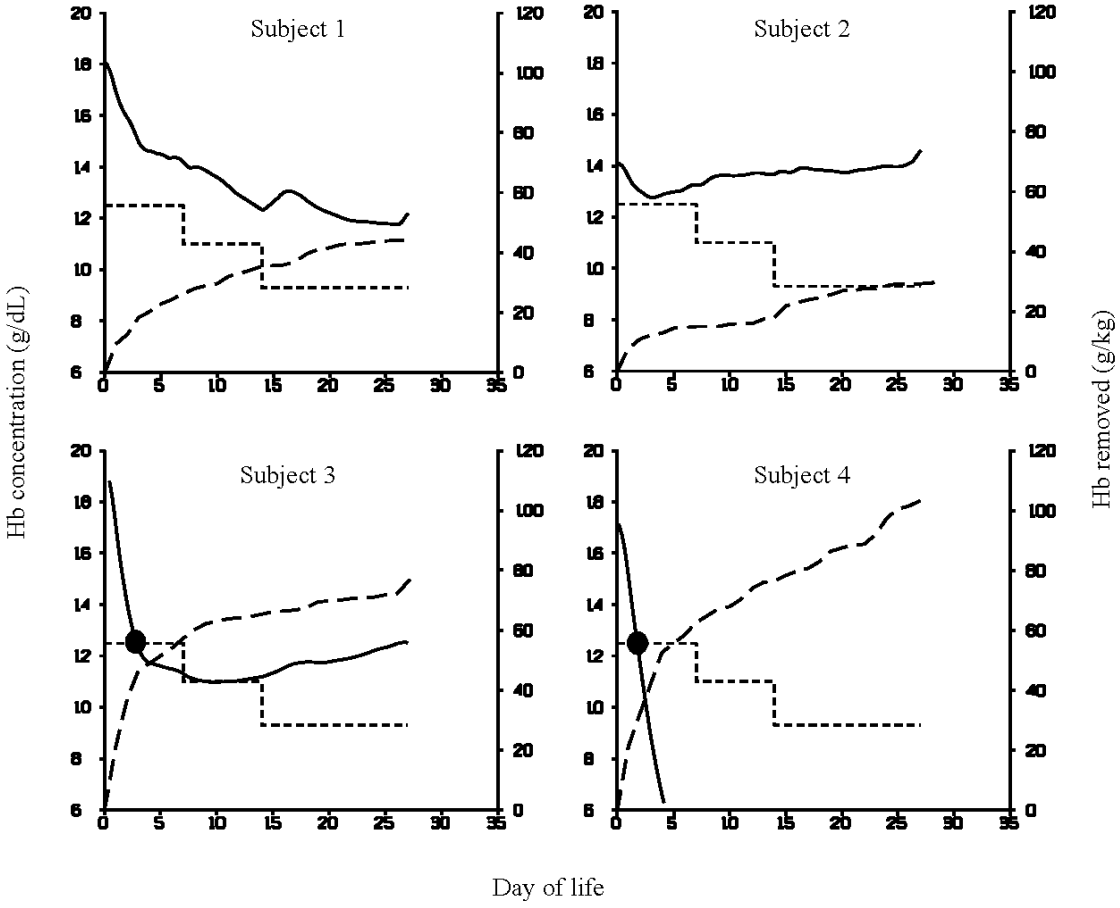


Figure 4.5 Practical Epo dosing schedule. This schedule is similar to the optimized Epo dosing schedule except doses can only be given once every 24 hours. The dosing schedule below showed a 2.7% drop in the objective function over the optimal dosing schedule.

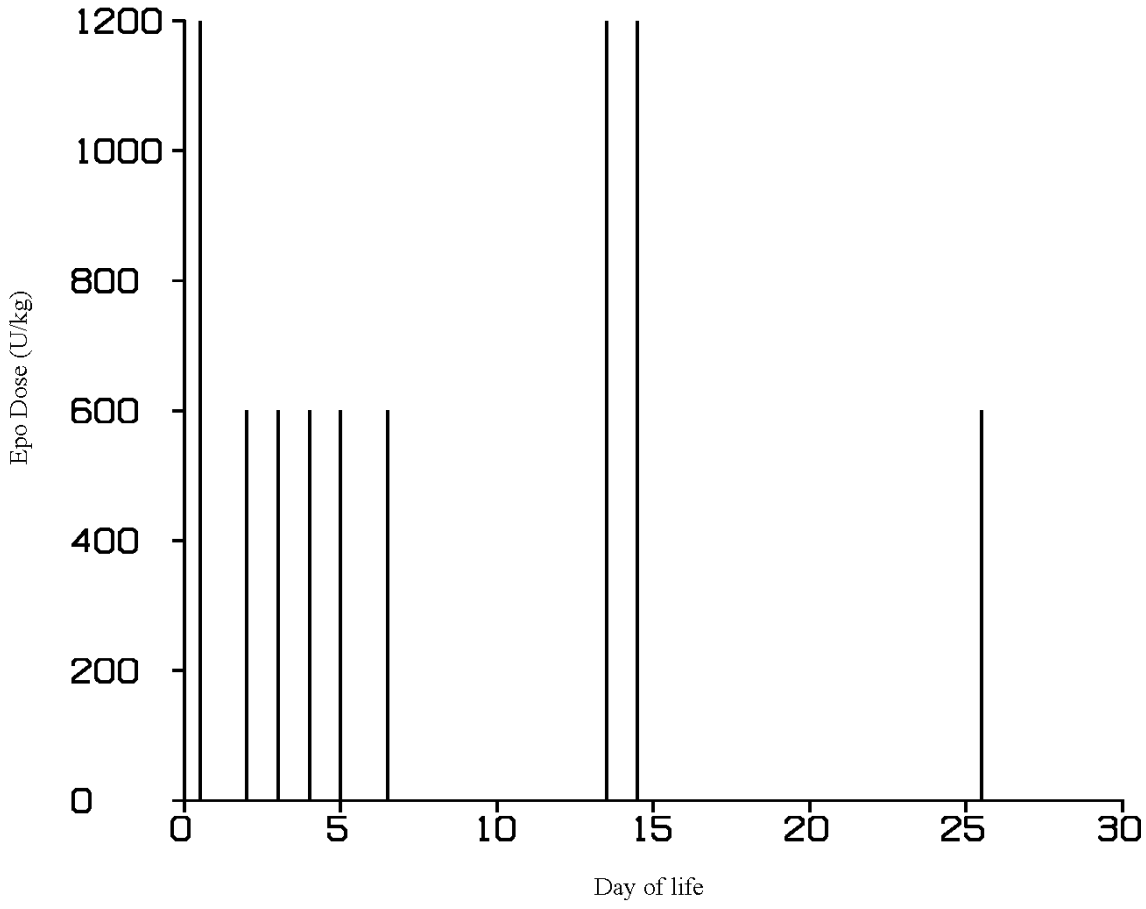
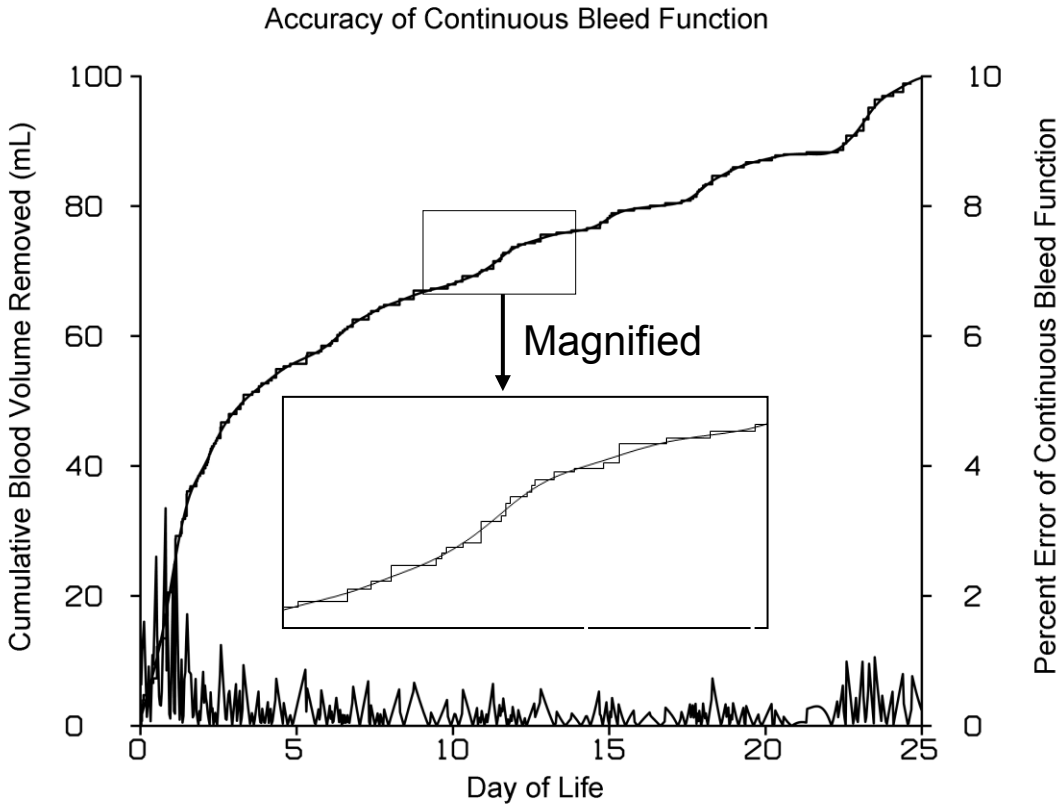


Figure 4.6 Continuous bleed function for discrete phlebotomies in infant shows very little error. The discrete infant phlebotomy data was fit using a general cross-validated cubic spline from the software WINFUNFIT.



CHAPTER 5. COMBINING EXTENSIVE PATIENT DATA WITH MATHEMATICAL MODELING TO PRECISELY QUANTITATE THE ROLE OF PHLEBOTOMY LOSSES AND NEED FOR TRANSFUSIONS IN NEONATAL ANEMIA

5.1 Abstract

Very preterm infants commonly develop anemia requiring multiple red blood cell transfusions (RBCTx). This is in part attributable to heavy laboratory phlebotomy loss. Quantification of the extent to which laboratory blood loss contributes to anemia sufficient to prompt RBCTx has not been examined. Twenty-six preterm infants weighing <1500 g at birth requiring ventilator support who received one or more RBCTx were intensively studied during the first month of life. Hemoglobin (Hb) loss via laboratory blood loss and RBC senescence, and Hb gain from RBCTx were precisely accounted for in a mathematical Hb balance simulation model developed to assess the impact of phlebotomy on RBCTx when uniformly restrictive RBCTx criteria were applied. Study subjects had a birth weight 880±240 g (mean±SD), a Hb level of 14.4±2.4 g/dL at birth and received 3.81±2.15 RBCTx during the study period. Modeling indicated that even with the total elimination of laboratory phlebotomy loss, a reduction of only a 41-48% in RBCTx was achievable. The present modeling results indicate that while phlebotomy reduction can significantly decrease the number of RBCTx administered to preterm infants, total elimination of all RBCTx will likely require other approaches, e.g., stimulation of erythropoiesis with erythropoiesis stimulating agents.

5.2 Introduction

All extremely premature infants develop anemia in the early weeks of life. This is the result of heavy laboratory blood loss, shortened red blood cell (RBC) lifespan, low plasma erythropoietin (Epo) levels, inadequate erythropoiesis, and perhaps other factors (79). As treatment for clinically significant anemia, approximately 80% of very low birth weight infants (VLBW) weighing less than 1,500 g at birth and 95% of extremely low birth weight infants (ELBW) weighing less than 1,000 g at birth receive one or more red blood cell transfusions (RBCTx) (185). RBCTx are important because they are expensive and associated with complications including infection, fluid overload, electrolyte imbalance, and exposure to plasticizers, lead, and other toxins (1). Thus, therapies for neonatal anemia resulting in fewer RBCTx are highly desirable.

Three therapies shown to reduce RBCTx in VLBW infants include treatment with recombinant human erythropoietin, institution of restrictive RBCTx criteria, and reduction in laboratory phlebotomy loss. While Epo administration has been shown to be effective in reducing the number of RBCTx in some preterm infants, its routine use is controversial because of its association with retinopathy of prematurity and its modest effect on decreasing RBCTx (1). Similarly, in the two largest randomized trials of liberal versus restrictive RBCTx criteria, restrictive criteria have demonstrated only a slight reduction in RBCTx when applied as intended (96, 97).

Reduction in the heavy laboratory phlebotomy losses experienced by very premature infants is the third, and the most promising clinical intervention associated with a reduction in RBCTx. Phlebotomy loss is particularly important among this patient

group because even seemingly small volumes of laboratory blood loss are large relative to their weight. Numerous studies have reported that laboratory phlebotomy loss in preterm infants is a major contributor to neonatal anemia and to RBCTx (74, 79, 88, 89). For example, drawing 6.5 mL of blood from a 1 kg infant whose total blood volume is approximately 80 mL/kg is equivalent to drawing a 450 mL donor blood unit (79). Although there have been successful efforts in reducing iatrogenic laboratory phlebotomy loss in preterm infants (92, 93), quantification of the relationship of phlebotomy loss and RBCTx has not been reported.

Thus, our objective was to quantitatively explore the relationship of phlebotomy loss and RBCTx by applying mathematical modeling when uniformly restrictive RBCTx criteria were applied (96). In doing so, we relied on accurate phlebotomy and RBCTx data gathered previously (186), on modeled blood volume, and on RBC lifespan data in the literature (9). The mathematical modeling was used to conduct simulations based on reductions in phlebotomy loss.

5.3 Methods

5.3.1 Subjects

Subjects eligible for enrollment included the offspring of pregnant women presenting to labor and delivery at <29 weeks gestation and infants born at <29 weeks gestation who required intubation in the first day of life. Infants excluded were those presenting with hematological disease (except for anemia of prematurity), those receiving RBCTx prior to enrollment, and those receiving erythropoiesis stimulating agents. There were a total of 162 mothers or infants who met study eligibility criteria. Of those eligible for enrollment, 119 were not approached for the following reasons: 1) they had already

been approached for another clinical study with similar eligibility criteria (n=39); 2) the significant workload imposed on the clinical lab for weighing all clinical blood samples only allowed two research subjects to be studied at a time (n=62); 3) prior blood transfusion before obtaining consent (n=13); and 4) lack of staff availability (n=5).

A total of 43 families were approached, 11 before delivery and 32 after delivery. Consent was obtained from 33 families while 10 families refused. Women who were consented antenatally but who delivered at >29 weeks and became ineligible (n=6). Twenty-seven infants were enrolled in the study and studied for a period of approximately one month (31.6 ± 2.2 days). One of the 27 infants enrolled was omitted because the infant did not receive any RBCTx during the study period.

5.3.2 Study procedures

Laboratory phlebotomy and RBCTx data were obtained from the subjects' electronic medical record. Accurate weights for 97% of all 2,656 laboratory blood samples drawn in the first month of life were recorded to 0.1 mg (9). Following clinically ordered laboratory testing, leftover anticoagulated blood less than 3 days old was analyzed for hematological parameters using the Sysmex XE-2100 automatic hematology analyzer (Sysmex Corporation, Kobe, Japan). Additionally, leftover plasma was saved for subsequent analysis for Epo. If the plasma volume from the sample was insufficient for these determinations, samples were pooled in 8-h epochs. Endogenous plasma Epo concentrations were determined using a double antibody radioimmunoassay as previously described (183).

5.3.3 Intravascular blood volume determination

The blood volume for each infant was calculated from the RBCTx volume, the Hb concentration of the transfused donor blood, and the increase in Hb concentration as previously described (187):

$$BV_{kg} = \frac{TRX_V \cdot HCT_{trx} / 3}{\Delta Hb \cdot W} \qquad (5.1)$$

where BV_{kg} is the total intravascular blood volume per kg body weight; TRX_V is the volume of packed RBCs transfused; HCT_{trx} is the hematocrit of the transfused donor RBCs and $HCT_{trx}/3$ is the Hb concentration equivalent (9); ΔHb is the change in Hb concentration after the RBCTx; W is the weight of the infant at the time of transfusion. Infant blood volume was used to convert Hb concentrations to Hb amounts. A linear regression statistical analysis was used to determine if a relationship exists between blood volume per kg and weight, age and postnatal age.

In 40 of 101 of the RBCTx, the HCT aliquots of the packed donor RBCTx were measured. For packed RBCTx in which HCT was not determined, the average HCT for all RBCTx was applied, i.e., 83.1%.

5.3.4 Modeling phlebotomy reduction

The number of RBCTx each individual infant received following simulated reductions in phlebotomy was determined. The amount of Hb remaining from the transfused blood was subtracted from each infant's total Hb to determine the quantity of Hb present in the endogenous blood. The amount of Hb each RBCTx contributed to the infant's total body Hb at different time points was determined as follows:

$$Hb(t)_{trx} = Hb(t_0)_{trx} \cdot \left(1 - \frac{t - t_0}{\tau_A}\right) \cdot \prod_{j=1}^{k} (1 - F_j) \qquad t_k < t < t_{k+1} \qquad (5.2)$$

where $Hb(t)_{trx}$ is the amount of Hb remaining from a donor RBCTx at time t; $Hb(t_0)_{trx}$ is the Hb amount in the RBCTx occurring at time t_0; t_0 is the time when the most recent RBCTx occurred; τ_A is the lifespan of transfused adult donor RBCs, i.e., 120 days (188); k is the number of phlebotomies after the RBCTx time t_0 but before time t; j is the given phlebotomy number; F_j is the fraction of the total blood volume removed in phlebotomy j.

To determine the amount of Hb administered in each RBCTx, $Hb(t_0)_{trx,}$ the following equation was used:

$$Hb(t_0)_{trx} = TRX_v \cdot \frac{HCT_{trx}}{3} \qquad (5.3)$$

where TRX_v is the volume of blood transfused and HCT_{trx} is the HCT of a given RBCTx.

Phlebotomy reduction simulation analysis was performed by adding a fraction of the amount of the Hb removed by phlebotomy to the total amount of Hb in the body of each infant. The amount of Hb added to achieve specified percent phlebotomy reductions was determined as follows:

$$Hb(T)_{phle} = PHLE_V \cdot Hb_{phle} \cdot P_R \qquad (5.4)$$

where $PHLE_V$ is the volume of a single phlebotomy; Hb_{phle} is the Hb concentration immediately before the phlebotomy; P_R is the percentage by which the phlebotomy removal was reduced (i.e., P_R=0.0 indicates no change from what was observed and P_R=1.0 is the simulation value if no phlebotomies occurred); $Hb(T)_{phle}$ is the amount of Hb added for a given P_R occurring at time T.

To determine the endogenous Hb contribution of phlebotomy reduction at different time points following birth, Equation 5.2 is modified such that:

$$Hb(t)_{phle} = Hb(T)_{phle} \cdot \left(1 - \frac{t-t}{\tau_I}\right) \cdot \prod_{j=1}^{m}(1-F_j) \qquad t_m < t < t_{m+1} \qquad (5.5)$$

where $Hb(t)_{phle}$ is the Hb amount remaining in the body at time t after the phlebotomy; τ_I is the lifespan of preterm infant RBCs fixed at 65.8 days (9); and m is the number of phlebotomies that occurred after the phlebotomy at time T but before t. Equation 5.5 determines the Hb contribution from the infant's own blood while Equation 5.2 determines the Hb contribution of transfused donor blood.

5.3.5 Transfusion simulations

Prediction of the extent to which the number of each infant's RBCTx were hypothetically reduced as a result of phlebotomy reduction was determined in two steps. It was first determined what the intravascular Hb concentration versus postnatal age profile would be for each infant in the absence of both RBCTx and phlebotomies. Second, it was determined what the "no intervention profile" would be if each infant was phlebotomized as was done clinically, but with the volume of each phlebotomy reduced by a specified percentage. This resulted in a Hb versus time profile that could be evaluated according to the PINT RBCTx trigger criteria to determine if one or more RBCTx were indicated. The effect of the phlebotomy reduction was expressed as the percent RBCTx reduction calculated from predicted RBCTx relative to the actual number of RBCTx administered. The three RBCTx criteria applied were: 1) the PINT low Hb threshold criteria for infants receiving respiratory support with capillary Hb correction (96) (Figure 1); 2) the PINT restrictive threshold criteria for infants receiving respiratory

support with no capillary Hb correction; and 3) the Hb level and day of life when infants were transfused clinically. For the PINT RBCTx criteria with Hb correction, a 10% reduction was made for capillary blood sampling (106).

To determine whole blood Hb concentration for the above analysis, the Hb amounts derived from Equations 5.2 and 5.5 were converted to Hb concentrations as follows:

$$Hb_C = \frac{Hb_A}{BV} \qquad (5.6)$$

where Hb_C is the Hb concentration and Hb_A is the Hb amount. The blood volume was determined in Equation 5.1.

When an individual infant's Hb concentration reached the specific RBCTx criteria, a simulated RBCTx was administered. All simulated RBCTx were 15 mL/kg of packed RBCs with an assumed hematocrit of 83.1%. The resulting Hb amount was estimated for each simulated RBCTx according to Equations 5.2 and 5.3. In order to determine the contribution of each RBCTx to endogenous Epo concentrations, a paired t-test statistical analysis was performed on endogenous Epo concentrations in infants before and after each RBCTx was received. The paired t-test analysis excluded Epo concentrations in the first week of life because infants often have elevated endogenous Epo concentrations in the first few days following birth before their Epo concentration reaches a baseline level (9). The Epo concentration following each RBCTx was determined 5 hours after the start of each RBCTx. The RBCTx simulations were programmed in FORTRAN and graphical output was done using WINFUNFIT (146) or Microsoft Excel.

5.4 Results

5.4.1 Study subjects

The 26 ventilated study infants had a gestational age of 26.6±1.3 weeks (mean±SD) and a mean birth weight of 880±240 g. Infants received an average of 3.81±2.15 RBCTx and underwent 138±21.2 clinical phlebotomies for laboratory blood testing during the month long study period. Pre-transfusion and post-transfusion Epo concentrations were 18.5±9.6 and 18.8±9.6 mu/mL, respectively and no statistically significant difference was found between the two values ($p>0.05$). Ninety-seven percent of all laboratory blood samples drawn were weighed. The average volume of transfused donor packed RBCs was 14.4±1.8 mL/kg with 85% of the transfusions being between 13 and 17 mL/kg of packed RBCs. At birth infants had a mean amount of total Hb of 12.1±3.29 g. During the one-month study period, 14.4±5.27 g of Hb was transfused and 6.28±2.07 g of Hb was drawn for laboratory tests. When expressed relative to birth weight, this corresponded to a mean 14.1±3.05 g/kg of Hb at birth, 18.6±10.3 g/kg of Hb transfused, and 8.03±4.06 g/kg of Hb phlebotomized. Of the 2,656 laboratory samples in which Hb concentration was determined, 36.2% of the samples were drawn centrally (arterial or venous) and 63.8% were drawn as a capillary sample. The Hb concentration was determined in 40 of the 101 RBCTx administered; the mean Hb concentration of transfused blood was 27.7±1.4 g/dL. The mean estimated infant blood volume based on Equation 5.1 was 93.2±24.9 mL/kg. There was no significant correlation between blood volume per kg and weight, age and postnatal age (Figures 5.8, 5.9 and 5.10).

5.4.2 Hb levels at time of clinical transfusion versus PINT RBCTx criteria

The PINT RBCTx criteria become increasingly restrictive with increasing postnatal age (Figure 5.1). Comparison of the Hb levels at which infants actually received clinical transfusions relative to PINT RBCTx criteria indicate that infants were liberally transfused (97). Of the 101 RBCTx administered, 88 (87%) were administered at Hb levels greater than that required had PINT RBCTx criteria been applied (Figure 5.2).

5.4.3 Fate of phlebotomized blood

The cumulative amount of laboratory blood drawn for the 26 individual study infants over the first 30 days of life was determined based on weighed blood samples (Figure 5.3). Notably, only 33% of the blood withdrawn was required by the laboratory instruments, while 59% was discarded as waste and 8% was attributable to hidden blood loss in syringes or on to gauze pads, bandages, etc. (9).

5.4.4 Blood removed and blood transfused

Based on precisely determined phlebotomy and RBCTx data, it was possible to determine the cumulative amounts of blood transfused and removed for individual infants. The amount of Hb present at birth and the cumulative amount of blood removed and transfused is illustrated for two representative infants in Figure 5.4. The infant weighing 548 g at birth (Figure 5.4A) received more RBCTx and underwent more phlebotomies than the infant weighing 1320 g (Figure 5.4B). In addition, the lighter infant received approximate 3 times more Hb from RBCTx relative to that removed for laboratory testing. The heavier infant had approximately equal amounts of Hb transfused and phlebotomized. When all infants were combined, there was a significant association

of phlebotomy loss (per kg birth weight) and birth weight (r^2=0.6329, $p<0.05$). When adjusted for birth weight, Hb removed and Hb transfused during the first month of life demonstrated that study subjects had 2.3 times as much Hb transfused as was removed (Figure 5.5). ELBW infants had more Hb transfused compared to VLBW infants.

5.4.5 Modeling of phlebotomy reduction

In modeling simulated reductions of laboratory phlebotomy loss in the 26 study subjects according to Equation 5.5, resultant reductions in RBCTx (Figure 5.6) could be determined for the three separate transfusion criteria applied. Although the average number of RBCTx per infant demonstrated a progressive decline as the phlebotomy reduction increased, the mean modeled RBCTx could not be eliminated, even with elimination of all phlebotomy loss. When applying the actual RBCTx practices and laboratory blood loss was totally eliminated in the model, mean RBCTx were reduced by only 41%, i.e., from 3.81 to 2.26 RBCTx per infant. For the PINT low Hb threshold with and without adjustment for capillary sampling, the decline was 48% (3.22 to 1.66 RBCTx per infant) and 48% (2.85 to 1.48 RBCTx per infant), respectively. When individual infants were modeled using the PINT RBCTx criteria with adjustment for capillary blood samples, elimination of all laboratory phlebotomy loss resulted in 4 infants who were predicted to require no RBCTx.

5.4.6 Modeled decline in infant Hb level with no laboratory phlebotomy loss

An advantage of modeling developed in this study is that hypothetical changes in infant Hb levels with specified reductions or increases in laboratory phlebotomy loss can be determined. With no phlebotomy loss and no RBCTx, the average Hb concentration

for all 26 subjects at birth and day 30 fell from 14.4±2.4 g/dL to 6.3±2.7 g/dL, respectively (Figure 5.7). This observation is likely attributable to the spurious assumption for the no intervention Hb profile that low Hb levels do not result in an increase in Epo production leading to increased RBC production and Hb levels.

5.5. Discussion

In the current report we demonstrate for the first time the ability to estimate the number of RBCTx received by critically ill, preterm VLBW infants by simulating reductions in laboratory phlebotomy loss when uniform RBCTx criteria are applied. We observed that with the hypothetical elimination of all laboratory phlebotomy loss and the use of the actual RBCTx criteria, the average number of RBCTx per infant decreased from 3.8±2.2 to 2.3±1.5, a 41% reduction (Figure 5.6). While this represents a highly significant decrease in RBCTx, the more desirable goal would be to completely eliminate all RBCTx received by all infants. An additional advantage of the simulation model approach is that in the examples provided in the present study it offers insight as to which factors other than laboratory blood loss may contribute to neonatal anemia and the need for RBCTx. Based on the present data, we speculate that other factors may include the inability of premature infants—particularly the smallest and least mature—to mount an effective erythropoietic response to anemia and to produce RBCs that survive sufficiently long enough in the circulation to avoid severe anemia. The first of these two possibilities may be more responsive to therapeutic intervention, e.g., administration of erythropoiesis stimulating agent and optimize nutrition, than the latter.

5.5.1 Components of the laboratory phlebotomy model applied

Uniform clinical transfusion criteria

The application of restrictive RBCTx criteria to anemic VLBW infants enrolled in clinical trials has been shown to reduce the number of RBCTxs administered by 23 to 36% (96, 97). While recognizing the current uncertainty regarding which RBCTx criteria should be applied to achieve optimal long-term neurodevelopmental outcomes (189), in the present study we arbitrarily modeled restrictive RBCTx criteria as applied in the PINT study for infants requiring respiratory (96). This was done with and without the 10% correction needed for Hb concentration when capillary blood sampling is performed (106). As anticipated the reduction that was observed in RBCTx received by study subjects for modeled reductions in phlebotomy loss from 0 to 100% averaged less for central Hb sampling compared to capillary (see Figure 5.6). Just as adjustments in our model were made in correcting for central compared to capillary blood sampling, modifications in the model can easily be made to accommodate variations in RBCTx practice.

5.5.2 Measurement of laboratory blood removed and blood transfused

Modeling of the quantitative impact of graded reductions in laboratory phlebotomy loss on RBCTx in anemic VLBW infants as illustrated in Figure 5.6 has not been reported. Successful application of the model, it requires precise, complete data for conducting the Hb balance calculations required to quantitatively determine the extent to which laboratory blood loss contributes RBCTx. Gathering such data is no small undertaking. Accomplishment of this required precise weighing to the nearest 0.1 mg of

nearly all of the 2,656 (i.e., 97% of all) phlebotomy blood samples taken and all of the RBCTx administered during the month long study period.

5.5.3 Estimates of blood volume and survival of erythrocytes

In addition to requiring direct measurement of Hb removed for laboratory blood testing, Hb added via RBCTx, the simulation model requires estimates of circulating RBC survival. Estimates of RBC survival used in the present study taken from the literature included using 65.8 d for the infants' own RBCs (9), and 120 d for adult donor RBCs (188). Based on the present study's observed 2.3-fold greater amount of Hb transfused relative to that phlebotomized (Figure 5.5) and similar report from the literature (190), the RBC survival estimates applied in the present study are likely overestimates of the infant's actual RBC survival. Our research group anticipates soon being able to overcome this impediment with the ability to directly measure *in vivo* RBC survival in all critically ill VLBW infants using a safe, accurate method of labeling RBCs with biotin using minute quantities of blood left over from laboratory sampling (191).

5.5.4 Laboratory phlebotomy loss does not account for all RBCTx in infants

Quantitative assessment of the contribution of Hb removed as part of laboratory phlebotomy loss and the amount of Hb added in RBCTx can elucidate the contribution of phlebotomy loss to anemia of prematurity. Based on the modeled results of the present study it appears that laboratory phlebotomy loss, although important, only accounts for approximately half of the RBCTx administered to critically ill VLBW infants. The present results also indicate that the greatest relative per kg phlebotomy loss and need for RBCTx is most pronounced in the smallest, least mature infants who manifest the most

severe cardio respiratory illness in the early weeks of life (Figures 5.4 and 5.5). This could be a consequence of two other potentially important factors—but as yet unstudied—associated with increasing immaturity: inability to maximally increase erythropoiesis for reasons other than phlebotomy loss, and accelerated RBC senescence.

The reason certain preterm infants cannot produce sufficient Hb to counteract their anemia is unclear. The first month of life for the infant is a period of rapid growth, which means a sufficient amount of blood must be produced in order to maintain an adequate hemoglobin level (70). Additionally, the liver, where Epo is produced primarily in the fetus and newborn, appears relatively insensitive to hypoxia compared to the kidney (71, 72). This relative insensitivity of hepatic Epo production to hypoxia may help to explain why preterm infants are less able than term infants to compensate for anemia by increasing their Hb production. Furthermore, erythropoiesis is substantially reduced immediately following birth due to an oxygen rich postnatal environment that suppresses the transcription of hypoxia-inducible factor (73).

5.5.5 Feasibility of reducing laboratory phlebotomy loss

The present results suggest that the amount of phlebotomized blood drawn from VLBW preterm infants for laboratory testing can be reduced by relatively simple changes in practice. In the current study, 59% of blood obtained from infants was discarded (Figure 5.3). This indicates the possibility of reducing laboratory phlebotomy by sampling less volume. However, if the blood sampled is insufficient for analysis this may require addition sampling from the infant. In order to further investigate this, a clinical trial could be run where less volume is obtained from the infant for each test.

The clinical outcome for such a trial would be the total blood volume removed from the infants in the intervention vs. the control group.

An additional strategy to reduce phlebotomized blood in preterm infants is utilizing point-of-care bedside analyzers and in-line monitors (92, 93). The key difference between a blood monitor and a blood analyzer is that when using a blood monitor the blood is returned to the infant while the blood used in an analyzer cannot be returned to the infant. A clinical trial involving 93 preterm infants showed a reduction in transfusions of 33% when using a blood monitor (92). The blood monitor study also suggested that if blood monitors could analyze factors such as glucose, bilirubin, blood urea nitrogen and creatinine phlebotomy reduction of up to 80% is possible (92). In a separate clinical trial, Madan et al. found a 46% drop in RBCTx in a blood monitor trial for preterm infants (93). One hypothesis for why Madan et al. demonstrated such a large drop in RBCTx is that the average infant in their placebo group received 5.7±3.7 RBCTx, while the infants from our study received 3.8±2.2 RBCTx. In a small, single-center study, Rabe et al. demonstrated a decrease in RBCTx in preterm infants by half when using delayed cord clamping, early protein and iron administration and a restrictive RBCTx threshold demonstrating that there are many additional strategies to reduce RBCTx in preterm infants (192).

Further evidence that it may not be possible to completely eliminate RBCTx in preterm infants is suggested by Figure 5.7, where the average infant Hb reaches a level of 6.5 g/dL and drops below the PINT RBCTx criteria. The natural Hb level is likely underestimated because when an infant's Hb reaches a critically low level the

endogenous Epo level increases in response to anemia, however, the extent of such erythropoiesis remains unknown.

5.5.6 Limitations in modeling phlebotomy loss to predict reductions in RBCTx

The accuracy in modeling the number of RBCTx with reductions in laboratory phlebotomy relies on the accuracy with which all other measurements can be made, or estimated. Blood volume determinations were estimated from the pre and post-transfusion Hb level based on the dilution principle (187), along with estimates of RBC lifespan. The blood volume determined for this study, 93.2±24.9 mL/kg, is within the reported range of blood volumes for preterm infants (187, 193, 194). However, a previous study reported that the an increase in birth weight of 1.0kg results in a decrease in blood volume per kg of 12mL/kg (187). This previous study also used a larger sample size (n=164) and a larger range of birth weights than the current study (1000-5000g). The current study found no correlation between blood volume per kg and weight, age and postnatal age (Figures 5.8, 5.9 and 5.10, respectively) which is possibly due to the small number of subjects (n=26) or the limited weight range of subjects enrolled in the study (0.57-1.49 kg). Therefore, we assumed a constant blood volume per kg for the duration of the study period for all infants.

The lifespan of adult donor transfused RBCs is commonly referenced as 120 days based on healthy adults in stead-state erythropoiesis (188). It is possible that transfused RBCs have a shorter lifespan as a result of environmental factors, e.g., hyper- or hypoxic stress (195). As mentioned above, future neonatal RBC survival studies will be required to more accurately determine the lifespan of transfused and endogenously produced RBC under non-“steady state” conditions. Finally, the number of simulated RBCTx is based

on the anticipated volume of packed RBCs transfused. For our study subjects, 85% received RBCTx in which the hematocrit measured was between 13-17 mL of packed RBCs per kg.

5.6 Summary and conclusions

The present study demonstrates that when uniform, restrictive RBCTx criteria together with modeled phlebotomy reduction are applied, a predictable reduction in RBCTx administered to anemic preterm VLBW infants is observed. Under modeling conditions in which 100% of laboratory phlebotomy loss was assumed, the average number of RBCTx could be reduced by 41-48%. This finding is due in part to study infants having 2.3 times more Hb transfused as that phlebotomized. This discrepancy suggests that other factors—and not merely laboratory blood loss alone—are major contributors to neonatal anemia among critically ill VLBW premature infants. The present study demonstrated complete elimination of modeled RBCTx in four of the 26 (15%) study subjects suggesting the goal of completely eliminating RBCTx in critically ill preterm infants will likely require a multistep approach. Based on our prior model-based simulation employing optimized erythropoietin dosing in which we demonstrated that 13 (46%) of the same study infants would have avoided RBCTx entirely (186), the combination of phlebotomy reduction together with optimized Epo administration may well achieve the goal of completely eliminating RBCTx in critically ill preterm infants. An important advantage of the simulation model in the present study is its versatility in being modified to accommodate to new changes taking place in RBCTx practice, direct measurements of RBC survival or blood volume measurements, or changes in volumes of RBCTx administered.

Figure 5.1 PINT restrictive threshold Hb RBCTx criteria for ventilated VLBW infants. Age versus Hb concentration for administration of RBCTx to preterm infants in PINT trial. Hb transfusion thresholds are 10% lower for central (intravascular) compared to capillary sampling.

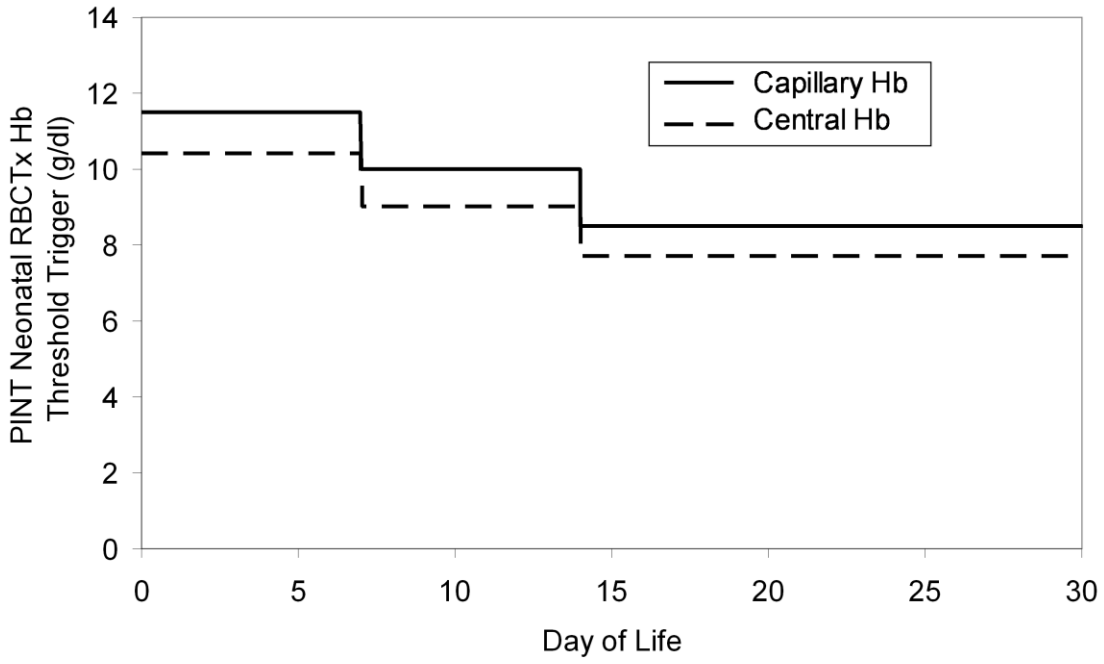


Figure 5.2 Iowa clinical RBCTx practice is more liberal than PINT restrictive threshold Hb criteria. Hb concentration when individual study infants were transfused is shown compared to the successive postnatal decline in Hb for the PINT restrictive threshold Hb RBCTx criteria for infants requiring respiratory support. The PINT transfusion criteria were reduced by 10% if a capillary blood sample was drawn. The dashed line represents the line of identity.

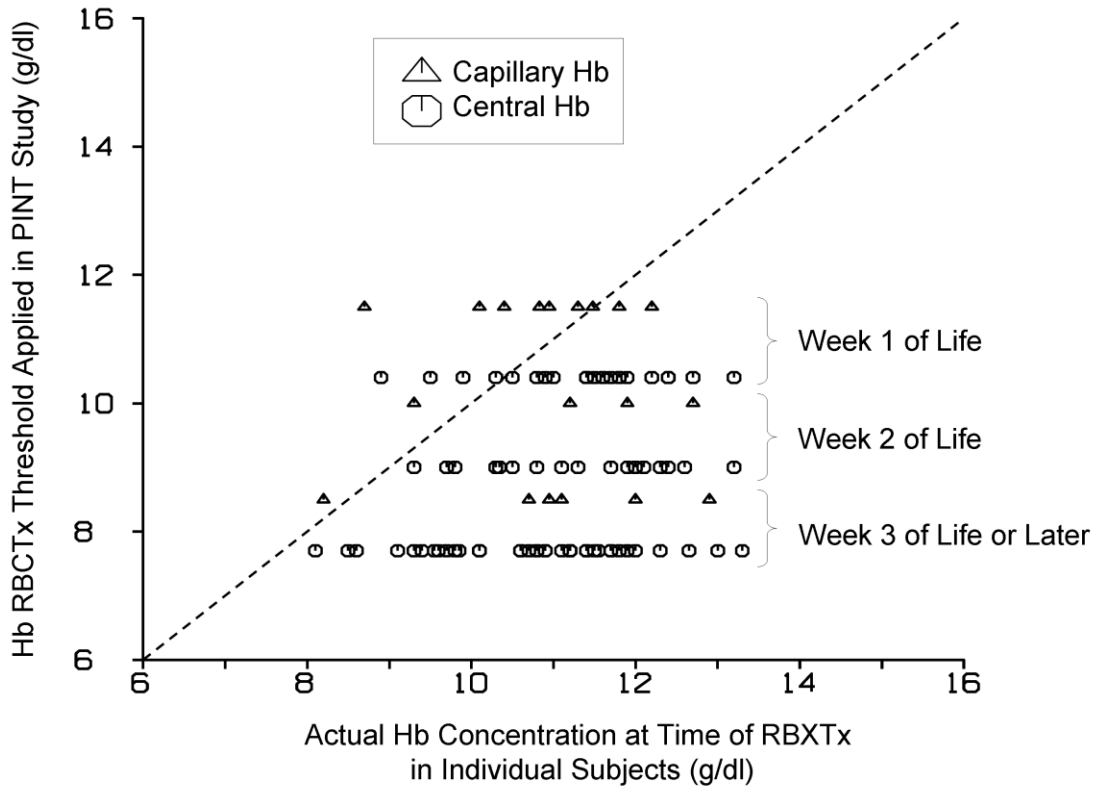


Figure 5.3 Most laboratory blood loss in VLBW preterm infants is discarded. Average cumulative infant laboratory testing blood loss during the first month. Of all blood taken from the 26 VLBW study infants, 33% is required for laboratory analysis, 59% of is discarded as waste, and 8% represents hidden blood loss (i.e., blood left in syringes or on gauze pads, bandages, etc.)

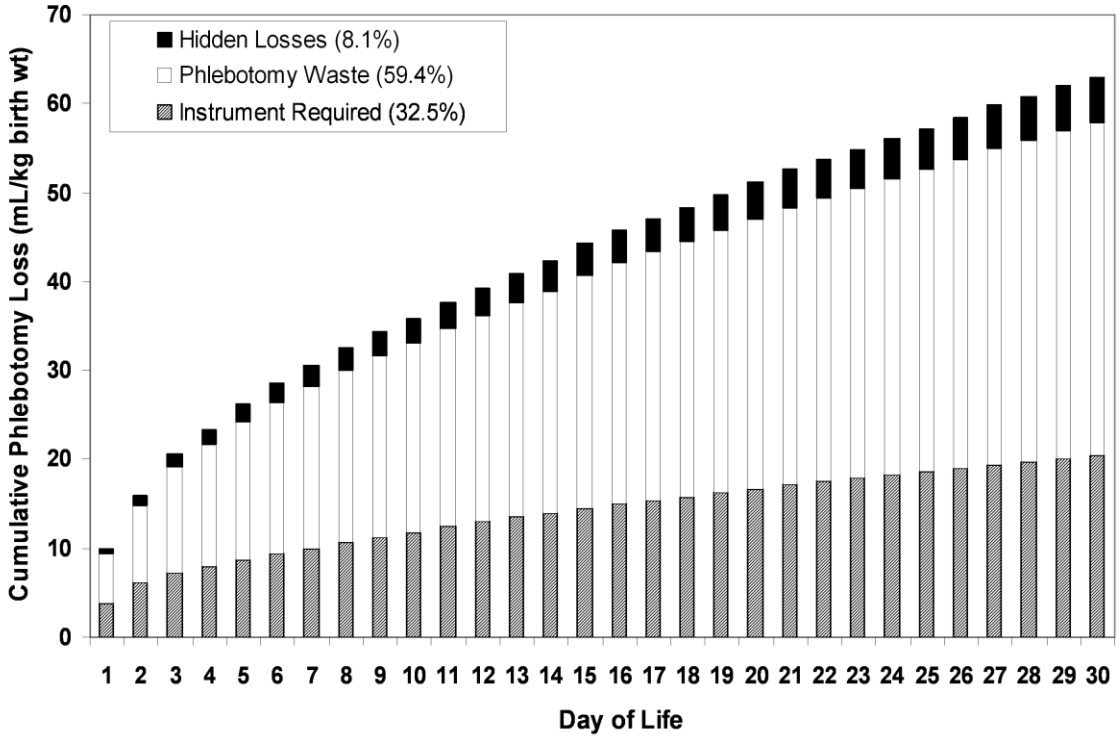


Figure 5.4 Blood removed for testing from two representative VLBW infants does not account for RBCs transfused. The cumulative amount of Hb removed per infant for laboratory testing versus the cumulative amount of Hb transfused is shown. The dashed horizontal line indicates the total amount of Hb in each infant at birth. The 548 g infant in the top panel had more Hb transfused than was phlebotomized.

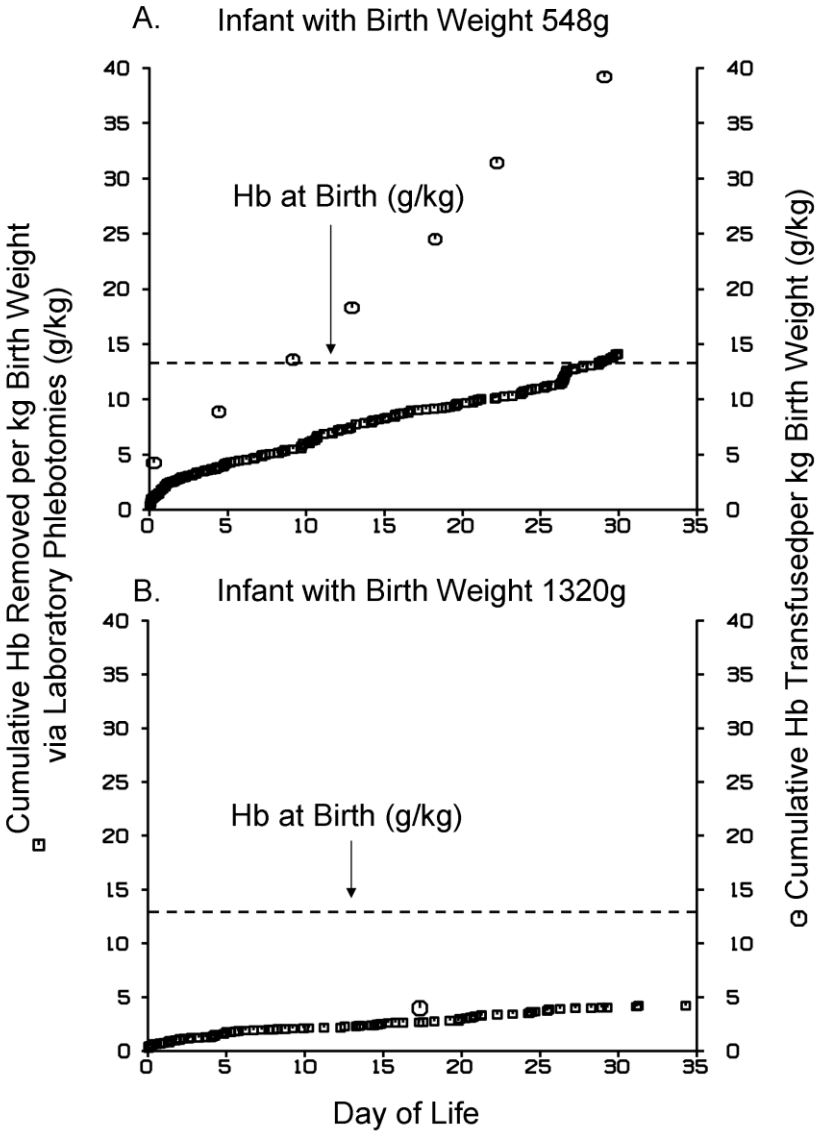


Figure 5.5 Preterm infants receive more Hb from RBCTx than is removed for laboratory testing. For 26 preterm study infants, the Hb removed and transfused is shown for ELBW (birth weight <1.0 kg) and for VLBW infants (birth weight between 1.0 and 1.5 kg). Approximately 2.3 times as much Hb was transfused as was removed. The dashed line shown is the line of identity.

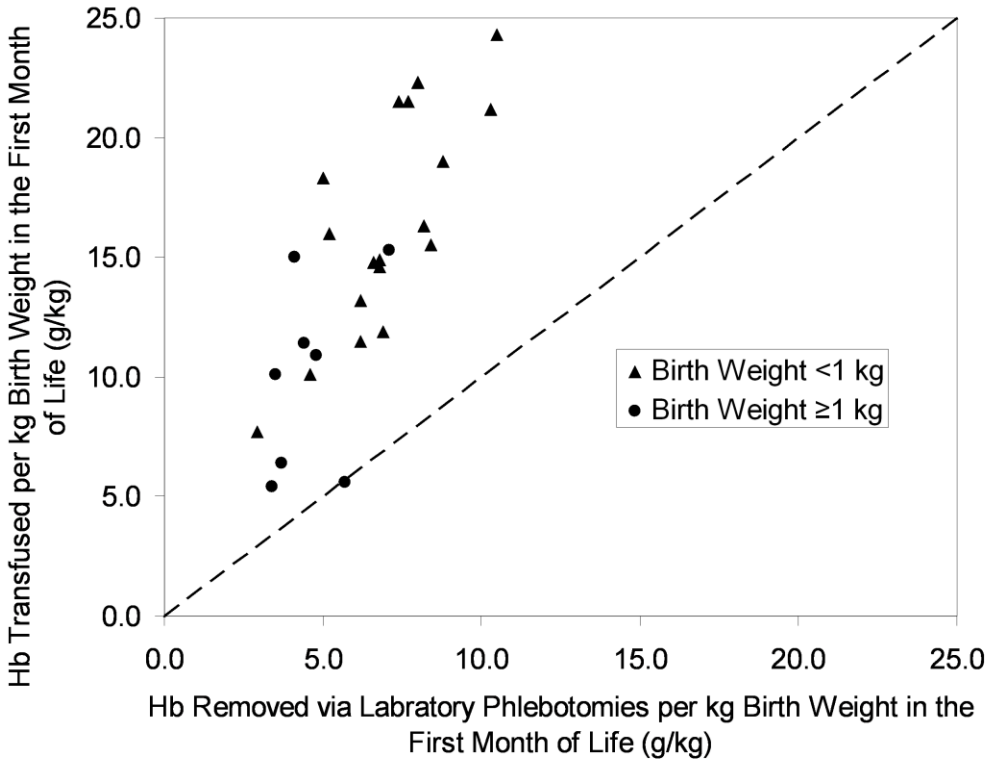


Figure 5.6 Simulation of phlebotomy reduction among VLBW preterm infants showing a reduction in the number of RBCTx. The average number of RBCTx during the first month of life determined by modeling of all 26 infant study subjects versus the percentage phlebotomy reduction. Simulations were conducted for the two PINT RBCTx criteria and for when infants were transfused clinically.

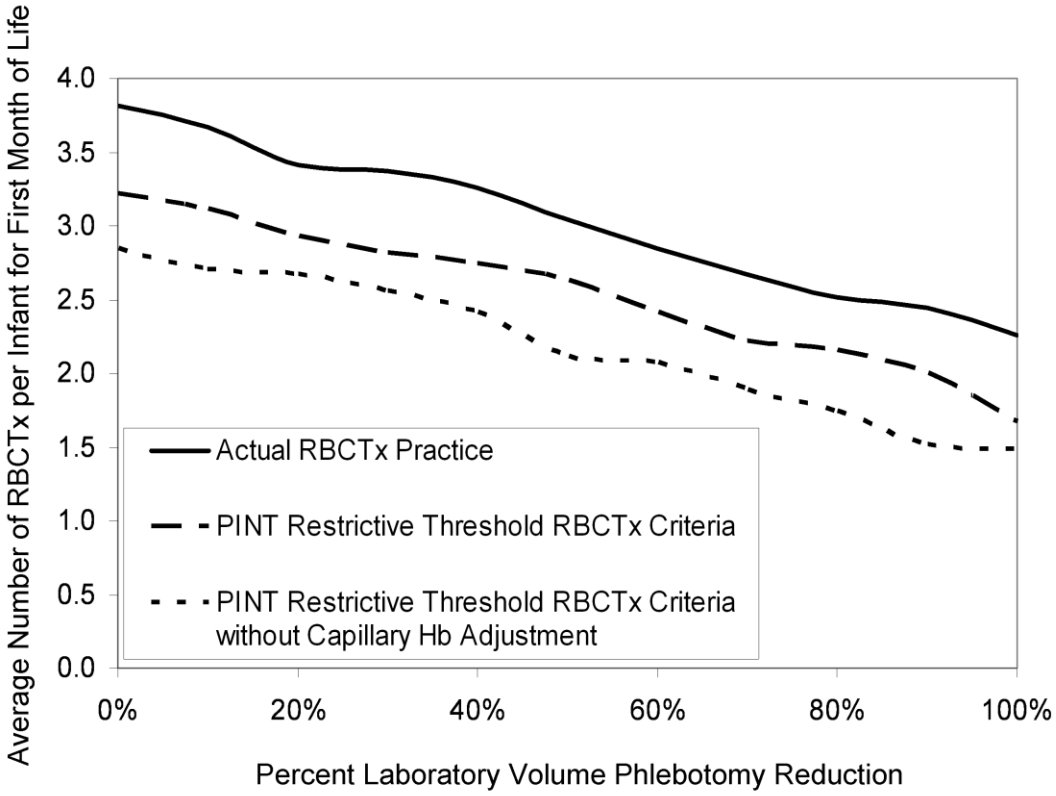


Figure 5.7 Modeled infant Hb concentration assuming no RBCTx and no phlebotomy blood loss relative to PINT Hb RBCTx criteria. The postnatal decline in modeled Hb levels is likely an under estimate because increased in endogenous Epo production would be expected to reverse the decline in Hb.

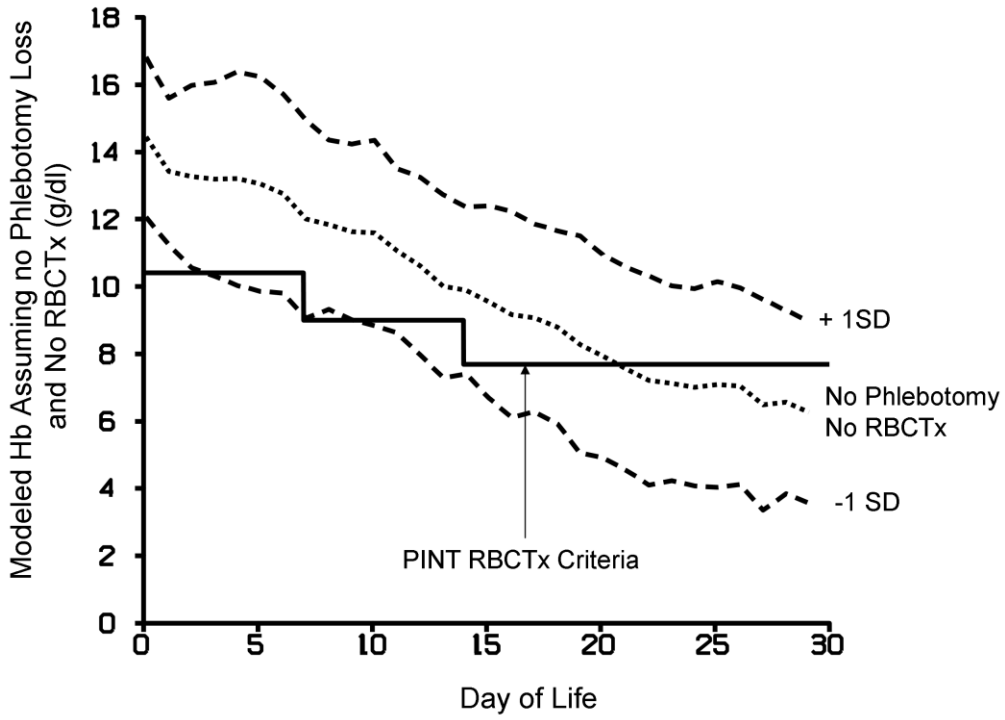


Figure 5.8 Correlation between blood volume per kg and the weight when the blood volume was determined. No significant correlation exists ($p>0.05$).

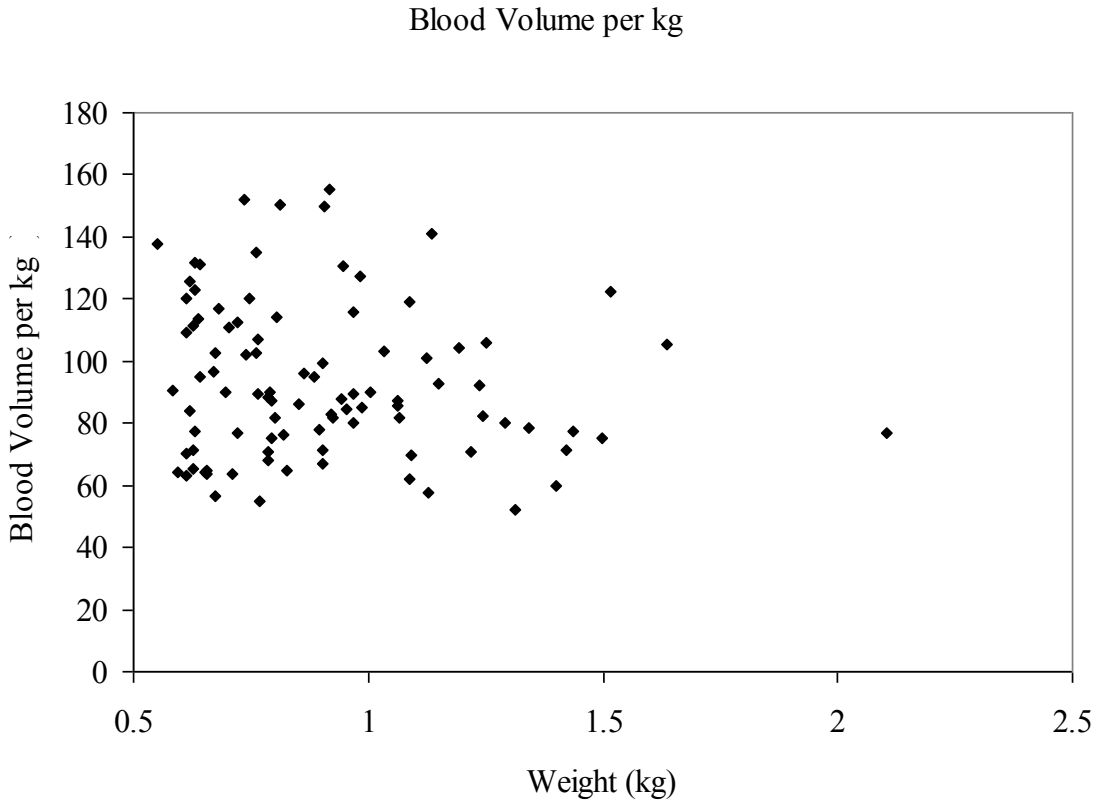


Figure 5.9 Correlation between blood volume per kg and the age when the blood volume was determined. No significant correlation exists ($p>0.05$).

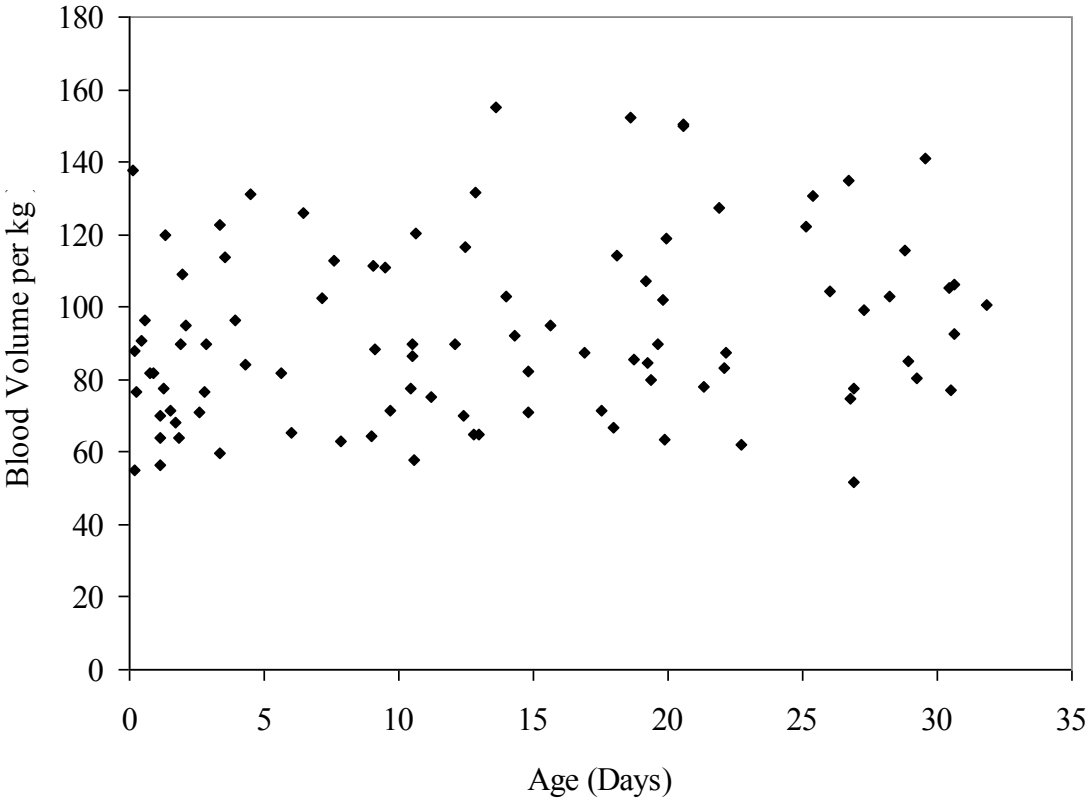
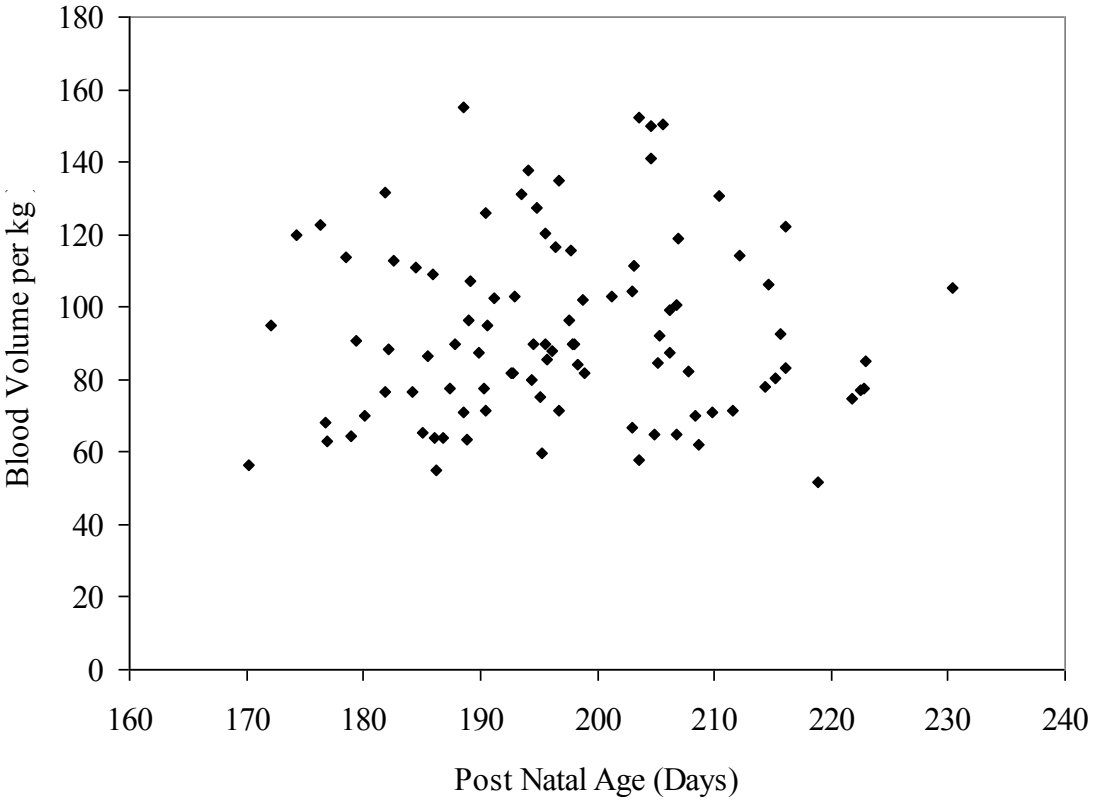


Figure 5.10 Correlation between blood volume per kg and the post natal age when the blood volume was determined. No significant correlation exists ($p>0.05$).



CHAPTER 6. MODELED ERYTHROPOIETIN DOSING OPTIMIZATION COMBINED WITH PHLEBOTOMY REDUCTION ELIMINATES BLOOD TRANSFUSIONS IN SELECTED PRETERM INFANTS

6.1 Abstract

Preterm very low birth weight (VLBW) infants with birth weights (BW) <1.5kg develop anemia requiring multiple red blood cell transfusions (RBCTx). Although individual clinical interventions have been shown to reduce RBCTx, these have rarely been used in combination. Thus, the goal of this study was to simulate the extent to which RBCTx administered to VLBW infants could be eliminated by optimizing erythropoietin (Epo) therapy and reducing laboratory blood loss. Twenty-six VLBW infants receiving ≥1 RBCTx were studied during the first month of life. RBCTx were simulated using relevant RBCTx criteria with RBCTx based on simulations utilizing optimized Epo administration derived from endogenous pharmacodynamic data and modeled reductions in phlebotomy loss, blood volume, and literature-derived RBC lifespan data. With simulated optimization of Epo administration and a ≥55% reduction in laboratory phlebotomies, RBCTx were eliminated from all infants with BW from 1.0-1.5kg during the first month. In infants with BW<1.0kg, simulated optimization of Epo administration and elimination of all laboratory phlebotomy blood loss predicted that RBCTx could be avoided in 45% of infants. The present study predicts that a combination of the two therapeutic interventions may eliminate a substantial number of RBCTx in VLBW infants, particularly for infants 1.0kg or greater at birth.

6.2 Introduction

Anemia of prematurity develops in all very low birth weight (VLBW) (BW <1.5kg) infants during the early weeks of life. It is accentuated in extremely low birth weight (ELBW) preterm infants (BW <1.0kg) and occurs as a result of several factors, including prematurity itself, laboratory blood sampling, shortened red blood cell (RBC) lifespan, inadequate erythropoiesis, hemorrhage, and other as yet unidentified factors (79). Clinically significant anemia is most commonly treated with red blood cell (RBC) transfusions (RBCTx) which themselves are associated with complications including infection, fluid overload, electrolyte imbalance, and exposure to plasticizers, lead, and other potential toxins (1). Since preterm infants are among the most highly transfused patient groups (196), the development of effective strategies to reduce RBCTxs is of high clinical importance.

Several therapeutic strategies for counteracting the most important contributors to neonatal anemia have been applied with the goal of reducing RBCTx. These include treatment with recombinant human erythropoietin (Epo) (1), adaptation of restrictive RBCTx criteria (96, 97) and reduction in laboratory phlebotomy blood loss (92, 93). Although Epo treatment has been effective in reducing the number of RBCTx infants receive (1) and has potentially beneficial neuroprotective properties (98), its administration in premature infants is controversial because of its modest efficacy and the potential risk of retinopathy of prematurity (1). The application of restrictive RBCTx criteria to decrease the administration RBCTx in VLBW preterm infants has also been demonstrated to be effective in the two largest clinical trials reported to date (96, 97). Unfortunately, results of these two trials indicate that overly restrictive RBCTx criteria

may be associated with parenchymal brain hemorrhage, periventricular leukomalacia and more frequent episodes of apnea (8).

Reducing laboratory phlebotomy loss has been proposed as a strategy for reducing RBCTx. This approach has demonstrated considerable potential for being the most effective strategy of the three. The rationale behind this strategy is that the total blood volume removed from preterm infants for laboratory testing directly correlates with the volume of RBCs transfused (94). The use of blood monitors that results in no blood loss compared to blood analyzers is a promising approach for reducing the volume of blood taken from preterm infants and the number of RBCTxs per infant (92, 93). In addition, data showing the majority of blood drawn from preterm infants is discarded indicates that future approaches for reducing laboratory blood loss are both feasible and timely (197).

Although the three aforementioned strategies for reducing RBCTxs in preterm infants show promise, application of a combination of strategies may have the greatest potential for reducing RBCTxs (192). The hypothesis of this investigation is that RBCTx can be eliminated in a significant proportion of preterm infants with optimized Epo dosing combined with a major reduction in laboratory phlebotomy. Our hypothesis is based on our previous findings in which mathematical modeling was separately used to assess RBCTx needs in VLBW preterm infants with simulated Epo dosing (186) and laboratory phlebotomy reduction (197).

6.3 Methods

6.3.1 Subjects

Subjects eligible for enrollment included pregnant women presenting to labor and delivery at <29 weeks gestation and infants born at <29 weeks gestation requiring intubation in the first day of life. Infants excluded were those presenting with hematological disease (except for anemia of prematurity), those receiving RBCTx prior to enrollment, and those receiving erythropoiesis stimulating agents. There were a total of 162 mothers or infants who met study eligibility criteria. Of those eligible for enrollment, 119 were not approached for the following reasons: 1) they had already been approached for another clinical study with similar eligibility criteria (n=39); 2) the significant workload imposed on the clinical lab for weighing all clinical blood samples only allowed two research subjects to be studied at a time (n=62); 3) prior blood transfusion before obtaining consent (n=13); and 4) lack of staff availability (n=5).

A total of 43 families were approached, 11 before delivery and 32 after delivery. Consent was obtained from 33 families while 10 families refused. Women who were consented antenatally but who delivered at >29 weeks and became ineligible (n=6). Twenty-seven infants were enrolled in the study and studied for a period of approximately one month (31.6 ± 2.2 days). One of the 27 infants enrolled was omitted because the infant did not receive any RBCTx during the study period. Because infants <1.5kg receive increasing numbers of RBCTx with decreasing BW (79), VLBW study subjects were divided into a subset of VLBW infants with BW from 1.0 to 1.5 kg, and the subset of ELBW infants, i.e., <1.0kg BW.

6.3.2 Study procedures

Demographic, laboratory, and RBCTx data were obtained from the electronic medical record. Information was also collected regarding the laboratory testing performed, the amount of blood required for individual laboratory tests, and the amount of blood requested by the laboratory staff for individual blood tests. The weights of 97% of all laboratory blood samples recorded to the nearest 0.1 mg were obtained as previously described (9). Following clinical analyses, leftover blood otherwise destined to be discarded was centrifuged and the plasma or serum frozen at -70˚C for later analysis. Hematological parameters were measured on leftover anticoagulated blood less than 3 days old using the Sysmex XE-2100 hematology automated analyzer (Sysmex Corporation, Kobe, Japan). Plasma Epo concentrations required for the Epo optimization procedure were analyzed using a double antibody radioimmunoassay technique (198).

6.3.3 Overview of the simulation modeling applied

The computer simulations developed for this study were designed to predict the number of RBCTx which would be administered to preterm infants with model optimized exogenous Epo dosing (186) combined with concurrent reduction in laboratory phlebotomy loss (197). These predictions were based on Hb levels reaching each of two different RBCTx criteria: 1) the actual Hb levels at which clinical RBCTx were administered; and 2) Hb levels when applying restrictive PINT (Preterm Infant in Need of Transfusion study) criteria for infants receiving respiratory support (96). The PINT restrictive RBCTx criteria were chosen because these criteria were used in the largest clinical randomized trial of preterm infants to date (n=451). Modeled Hb used in the simulation studies was determined from simulations utilizing optimized Epo dosing and

theoretic phlebotomy reductions as described below. Also required for the modeling applied were data regarding Epo pharmacokinetics and pharmacodynamics (PK/PD), phlebotomy volume, blood volume and RBC lifespan.

6.3.4 Pharmacokinetics and pharmacodynamics applied in Epo optimization

Simulation of optimized Epo dosing in preterm infants requires Epo PK and PD information. The elimination of Epo from the circulation occurs via a saturable, receptor-mediated pathway (17) which may be described by the following:

$$\frac{dEPO}{dt} = -\frac{P_1 \cdot Epo}{P_2 + Epo} \qquad (6.1)$$

where, Epo is the exogenous simulated plasma Epo concentration at time t; P_1 and P_2 are disposition parameters, where P_1 (U/mL/hr) is the maximum rate at which the exogenous Epo concentration declines following intravenous administration and where P_2 (U/mL) is the plasma Epo concentration at which Epo is eliminated at half of the maximum rate (U/mL). The parameters in Equation 6.1 have been estimated in the same group of preterm VLBW infants in a prior study (186). For the simulation of Epo concentrations used in this study, Epo PK parameters were used according to Equation 6.1 (186). All Epo doses were simulated as IV bolus. In the modeling, measured endogenous Epo concentrations were added to the simulated exogenous Epo concentrations determined by Equation 6.1.

In the Epo optimization the model used was the same as the E_{max} model we have previously described (9, 186):

$$E(t) = \frac{E_{\max} \cdot Epo(t - t_{lag})}{EC_{50} + Epo(t - t_{lag})} \qquad (6.2)$$

where, E(t) is the Hb production rate at time *t*; t_{lag} is the lag time between the Epo stimulation of Epo receptors and the appearance of newly synthesized reticulocytes, i.e., Hb appearing in the circulation; EC_{50} is the plasma concentration of Epo resulting in half of the maximum Hb production rate; E_{max} is the maximum achievable Hb production rate. The E_{max} parameters from individual study infants were determined in a previous study (186).

6.3.5 Phlebotomy reduction applied in model

Phlebotomies for laboratory testing lead to a decline in Hb levels as a result of the removal of both endogenously produced and transfused donor RBCs. The mathematical model applied in the present study was used to simulate endogenous infant Hb concentrations following different degrees of phlebotomy reduction and Epo simulation. To determine the Hb produced from simulated Epo doses in the presence of multiple laboratory phlebotomies the following equation was used:

$$Hb(t)_{Epo,PHLE} = Hb(t)_{Epo} \cdot \prod_{j=1}^{k}(1 - F_j) \qquad t_k < t < t_{k+1} \qquad (6.3)$$

where, $Hb(t)_{Epo}$ is the amount of Hb produced from Epo at time *t* (determined by Equation 6.2); $Hb(t)_{Epo,PHLE}$ is the amount of Hb produced from Epo in the presence of phlebotomies at time *t; k* is the number of phlebotomies which occurred before the current time; *j* is the given phlebotomy number; F_j is the fraction of the total blood volume removed in phlebotomy *j*.

Equation 6.3 contains no correction factor for RBC senescence because it was assumed that the 65.8 day lifespan of the newly produced cells is longer than the study period, i.e., approximately 30 days (9).

6.3.6 Epo dosing schedule and RBCTx simulation

The same Epo dosing schedule used in our prior Epo modeling simulation study was applied in the current study (186). In our previous model of Epo optimized dosing, we simulated the IV administration of twelve 600 U/kg doses to preterm infants and individually adjusted the time at which each Epo dose was administered. We concluded that optimized Epo dosing based on endogenous Epo PK/PD data showed significant potential for completely eliminating RBCTx in a subset of infants when using Epo doses within the range of that previously reported (47).

Following combined simulation of both Epo dosing and laboratory phlebotomy reduction, the resultant Hb profiles of individual infants were analyzed according to the two RBCTx criteria applied in the present study: 1) the RBCTx criteria that was actually applied clinically, and 2) the RBCTx criteria applied in the largest randomized clinical trial of RBCTx reported to date (96). When an individual infant's Hb concentration reached the specific RBCTx criteria, a simulated RBCTx was administered. All clinically simulated RBCTx were assumed to be 15 mL/kg of packed RBCs with an assumed hematocrit of 83.1% (197). The simulations were programmed in FORTRAN and graphical output was performed using WINFUNFIT (146) or Microsoft Excel (Microsoft Redmond, WA).

6.4 Results

6.4.1 Study subjects

Of the 26 subjects enrolled in this study meeting study eligibility criteria, 18 were ELBW infants and 8 were VLBW infants with BW between 1.0 and 1.5kg. The VLBW infants received 1.75 ± 0.71 (mean ± SD) RBCTx while the ELBW infants received 4.83±1.72 RBCTx. During the 30-day study period, 2,656 (97%) laboratory blood samples were weighed. An average of 35.2±9.4 mL of blood was removed from the group of infants weighing 1.0 to 1.5 kg for laboratory testing. For the group of infants weighing <1.0 kg, the laboratory blood volume removed was 53.2±14.0 mL. Adjusted for BW this corresponds to 30.5±9.5 mL/kg of blood removed infants from 1.0 to 1.5 kg and 74.1±25.3 mL/kg of blood removed infants <1.0 kg.

6.4.2 Analysis of usage of phlebotomized blood

An analysis of the blood removed from the infants for laboratory testing revealed that only 33 percent of the blood required was used for analysis by test instruments, thus leaving 67 percent that was unused. The unused percent included 8% that could not be recovered (i.e., dried blood spots, blood left in syringe etc.), and 59% that was discarded. In an effort to further elucidate the fate of the phlebotomized blood, the analyzed blood and blood that was leftover were placed into categories based on the laboratory analyses performed (Figure 6.1-6.3). The first 3 categories of laboratory tests included on the left of Figures 6.1-6.3 comprise 67% of all blood taken (for all infants). Although the drugs and miscellaneous chemistry determinations made up 19.6% of the total blood collected, only 4.1% (0.8% of the total) of this blood category was used by the instrument for

analysis, compared to 33.0% of the total used for all 26 subjects. With the phlebotomized blood separated into BW categories, the infants with birth weights <1.0kg had significantly more blood removed per kg of BW ($p<0.05$, Figure 6.1). Accordingly, infants in the lower BW subgroups, i.e., <1.0 kg, had a greater number of syringe samples for pH, blood gases and chemistries blood drawn than the 1.0-1.5kg infants.

6.4.3 Simulated hemoglobin with phlebotomy reduction and Epo dosing

Hemoglobin (Hb) profiles for the 26 preterm infants were simulated based on a hypothetical reduction in laboratory phlebotomy and optimized Epo dosing (Figure 6.4). The average simulated Hb profiles with different degrees of phlebotomy reduction were compared to the average actual Hb concentration observed during the month-long study period for the study subjects. The actual observed average Hb concentration profile was an erratic curve as a result of the numerous RBCTx and phlebotomies. It is apparent that optimized Epo dosing combined with a reduction of laboratory phlebotomies was successful in maintaining a Hb concentration comparable to the average simulated Hb levels for the majority of the study period.

6.4.4 Avoiding RBCTx with optimal Epo dosing and phlebotomy reduction

With individually simulated Hb concentrations it was possible to predict the number of RBCTx which would have been administered based on the two different RBCTx criteria applied (Figures 6.5-6.11), namely the one that was actually applied clinically, and the one applied in the PINT trial (96). As expected, a higher percent of the subset of VLBW infants with birth weights from 1.0 to 1.5 kg were predicted to avoid RBCTx (Figures 6.5 and 6.6) compared to ELBW infants with birth weights < 1.0 kg

(Figure 6.7). Without simulated Epo dosing and 100% reduction in phlebotomies, none of the <1.0 kg infants were predicted to avoid RBCTx completely (data not shown). However, since a decreased in the number of simulated RBCTx may have clinical implications without completely eliminating RBCTx, the number of simulated RBCTx were also shown (Figures 6.8-6.11). Although infants <1.0 kg were not predicted to completely avoid RBCTx with phlebotomy reduction alone, a predicted decrease in RBCTx of approximately 40% was observed with a 100% reduction in phlebotomies (Figure 6.10). The number of infants predicted to avoid RBCTx in both groups shows an abrupt increase at 50-80% phlebotomy reduction.

6.5 Discussion

In the current report we demonstrate the ability to model the number of RBCTx received by infants following simulated optimization of Epo dosing and reduction in laboratory phlebotomy loss. We observed that with the hypothetical elimination of all laboratory phlebotomy loss and optimized Epo dosing, RBCTx would become unnecessary in all infants with BW 1.0-1.5kg and 45% of infants with BW <1.0kg. These findings suggest that although infants with BW 1.0-1.5kg are likely to benefit from optimized Epo dosing and laboratory phlebotomy reductions, additional therapeutic interventions will be needed to eliminate all RBCTx among ELBW infants. Since the simulated therapeutic interventions in this study are hypothetical and therefore speculative, their feasibility must be tested in clinical trials to determine the clinical applications of these findings.

6.5.1 Epo administration

There have been numerous clinical trials in which Epo was administered to preterm infants with the goal of reducing or eliminating RBCTx (1). Although many of these clinical trials have successfully reduced RBCTx in preterm infants, the routine use of erythropoietin remains controversial because of its modest efficacy in reducing RBCTx and because of its association with retinopathy of prematurity (114). In contrast, since most individual clinical Epo treatment trials have reported no association between Epo and retinopathy of prematurity (1), this suggests that the benefits of avoiding RBCTx may outweigh the risks of Epo administration and that the association is not causal. Furthermore, recently it has been suggested that erythropoietin may have neuroprotective properties which would counter balance the risks of retinopathy of prematurity (98). Future clinical trials of Epo in preterm infants are needed to determine the reduction in RBCTx, the development of retinopathy of prematurity and the neurologic outcome concurrently.

6.5.2 Phlebotomy reduction

Simulations of reducing the amount of blood removed through laboratory phlebotomies were predicted to reduce the number of RBCTx in preterm infants. In simulating multiple therapeutic interventions, the current study describes the potential for reducing laboratory blood loss in preterm infants by minimizing the leftover blood obtained from the infants (Figure 6.1-6.3). The results indicate that 67% of the blood taken from the infants was discarded while only 33% was analyzed. Requesting a volume of blood for analysis that is closer to what is needed for each analysis would reduce the volume of blood discarded. However, if insufficient blood is obtained based

on the requested volume an additional blood sample could be necessary. In addition, blood samples obtained from preterm infants could be diluted before they are analyzed, however, the diluted sample may lose accuracy when analyzed (199). Future studies that can demonstrate equivalent analyte accuracy with diluted infant blood samples are needed before such protocol can be implemented.

Clinical trials utilizing point-of-care bedside analyzers and in-line monitors have also shown reduction in the RBCTx received by preterm infants (92, 93). Current blood monitors are capable of analyzing blood gases, electrolytes and Hb while returning the analyzed blood to the infant. Our group previously speculated that if blood monitors could be improved to analyze glucose, bilirubin, blood urea nitrogen and creatinine a phlebotomy reduction of 80% may be possible (92). Currently, reducing phlebotomized blood in preterm infants is theoretically possible without compromising clinical care.

6.5.3 RBCTx trigger criteria

The number of RBCTx that infants receive has been traditionally based on the infant's Hb level and clinical status at which each physician chooses to transfuse each infant (92, 96, 97). For the subjects in the current study, an average actual Hb value was calculated for all 26 subjects (Figure 6.4). From the analysis in this study, a 50% reduction in phlebotomy blood loss combined with Epo administration was simulated to maintain Hb close the actual average Hb concentration (Figure 6.4).

The optimal RBCTx trigger criteria for VLBW infants has been a long-standing subject of controversy (189). One clinical trial (n=100) which randomly assigned preterm infants to either a restrictive or liberal RBCTx group found that the liberally transfused infants had an improved clinical outcome (97). In contrast, the similar but

larger clinical trial (n=451) found no difference between the restrictive and liberal RBCTx groups (96). Due to current uncertainty regarding RBCTx criteria, two different RBCTx criteria were used for the current study: the actual RBCTx practice and a restrictive RBCTx guideline identical to a previous clinical trial (96). The RBCTx criteria used for the simulations in this study can be modified as better information becomes available regarding when it is best to transfuse preterm infants.

6.5.4 Combination therapy for avoiding RBCTx

The current study suggests that a combination of therapeutic interventions can eliminate RBCTx in certain groups of preterm infants. As expected, a larger proportion of infants with BW 1.0-1.5kg were predicted to avoid RBCTx compared to the infants with BW <1.0kg. This transfusion avoidance occurs due to increased erythropoiesis from the simulated Epo dosing compared to the natural erythropoiesis. In addition, when the simulation studies were run with a restrictive RBCTx criteria, a larger number of infants were predicted to avoid RBCTx than using the actual RBCTx practice criteria. When the phlebotomy reduction percent increases from 50-80%, there is a large increase in the number of infants predicted to avoid RBCTx for both BW groups. This 50-80% reduction in laboratory phlebotomies may serve as a clinical target for providing the greatest potential benefit to preterm infants.

From the simulation study performed, future clinical trials can be designed that test the validity of the results of the simulation study and determine the feasibility decreasing the RBCTx in preterm infants. Based on our current findings a clinical trial is recommended that attempts to reduce phlebotomies by 50%, administers optimal Epo doses and uses a restrictive but safe RBCTx criteria. The 50% phlebotomy reduction

should be feasible by using current blood monitors and by obtaining blood sample volumes close to what is needed by blood analyzers.

Additional strategies for reducing donor RBCTx in preterm infants exist beyond what was simulated in this study. These strategies include delayed cord clamping and saving cord blood for transfusions of autologous blood rather than adult donor blood. In an attempt to reduce the risks associated with blood transfusions, there have been efforts made to collect and store cord blood for future transfusions, however, procedures for collecting and storing the cord blood are technically challenging (102). Delayed cord clamping is a promising strategy which causes a higher initial Hb value in preterm infants (86). However, there are risks associated with delayed cord clamping including polycythemia and neonatal jaundice (83). Consequently, current delayed cord clamping practices are uncertain. Future clinical trials which utilized Epo administration, phlebotomy reduction, restrictive RBCTx criteria and delayed cord clamping would have the best chance for eliminating RBCTx in all infants, however, such a study would have to be closely monitored for adverse outcomes.

6.5.5 Benefits in avoiding RBCTx in preterm infants

Since the therapeutic interventions of Epo administration (1) and the utilization of restrictive RBCTx criteria (189) remain controversial in VLBW preterm infants, evidence must be developed to show that the avoidance of RBCTx leads to better, cost-effective clinical outcomes. Research has shown that RBCTx are expensive and cost between $522 and $1,183 per unit (200). In addition to the cost of administering RBCTx, the complications associated with RBCTx include infection, fluid overload, electrolyte imbalance, and exposure to plasticizers, lead and other toxins (1). Baer et al.

demonstrated a significant correlation between RBC transfusions and intraventricular hemorrhage (201). Further evidence for the correlation between intraventricular hemorrhage and transfusions is provided by the fact that delayed umbilical cord clamping was associated with lower risk of intraventricular hemorrhage (84). Additionally, it has been suggested that necrotizing enterocolitis, a potentially fatal condition among preterm infants, is temporally related to RBCTx (94). With the multiple risks associated with RBCTx in preterm infants, one could hypothesize that a decrease in RBCTx would be associated with a decrease in morbidities.

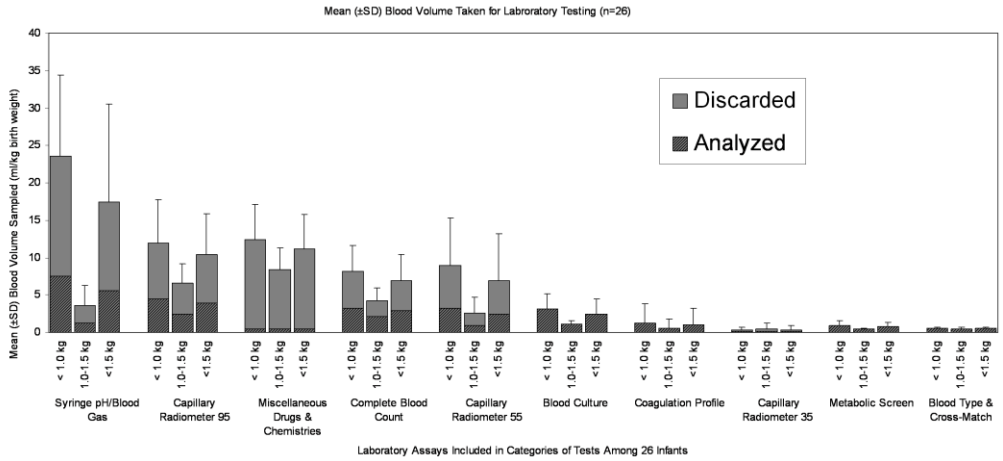
6.5.6 Limitations of modeling simulations

As with all simulation studies there are several limitations in predicting the number of RBCTx infants are anticipated to receive. The predictions made from this study were based on assumptions about the blood volume, transfused RBC lifespan and the pharmacodynamic model. Blood volume determinations were estimated from the pre and post-transfusion Hb level based on the dilution principle (187). The circulating blood volume determined for this study, 93.2±24.9 mL/kg, is within the range of that reported for preterm infants (187, 193, 194). The lifespan of adult donor transfused RBCs is commonly referenced as 120 days based on healthy adults in stead-state erythropoiesis (188). It is possible that transfused RBCs have a shorter lifespan as a result of environmental factors, e.g., hyper- or hypoxic stress (195). Finally the pharmacodynamic model does not take into account possible dynamic changes in the Epo receptor state which has been shown to influence Epo pharmacodynamics in animals (198). Although currently no measure of Epo receptors in available in preterm infants, the simulation model we applied can be modified as such information becomes available.

6.6 Summary and conclusions

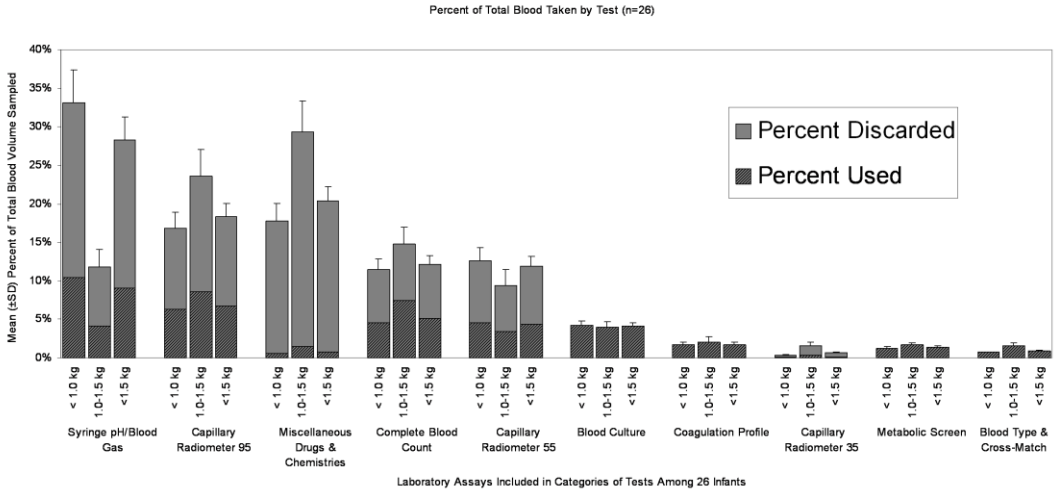
The modeled simulations included in the current report demonstrate that specific reductions in RBCTx are potentially possible in VLBW preterm infants with simulated optimized Epo administration combined with complete elimination of laboratory phlebotomy loss. Our findings predict that with optimal Epo dosing and no phlebotomy loss, total avoidance of RBCTx is possible in the subset of all VLBW infants with BW between 1.0-1.5kg, while an estimated 55% of infants with a BW of <1.0kg will continue to receive one or more RBCTx. The current study is based exclusively on simulations of RBCTx reduction in VLBW infants, and as such provides a hypothetical basis for the design of future clinical trials. The current modeled simulations strongly suggest that future clinical trials that apply both phlebotomy reduction techniques, Epo administration and restrictive RBCTx criteria may significantly reduce the number of RBCTx administered to preterm VLBW infants. If confirmed in the clinical setting, the resultant reduction in RBCTx will lower the associated complications and expense of RBCTx.

Figure 6.1 Most laboratory blood loss in VLBW preterm infants is discarded. Mean (±SD) total blood sampled by test category grouped by birth weight of study subjects. The hashed bars represent the blood required for analysis by specific instruments and the shaded bars represent the remaining leftover blood. The specific analytes obtained for each category of test are summarized in the legend of the Figure. n=26 for birth weight <1.5kg, n=8 for birth weight 1.0-1.5kg, n=18 for birth weight <1.0kg.



Test	Analytes Included in Selected Test Catagories
Syringe pH/ blood gas	Arterial or venous pH and blood gases, electrolytes, glucose, lactic acid, total billirubin, hemoglobin , methemoglobin and ionized calcium
Capillary radiometer 95	Capillary pH and blood gases, electrolytes, glucose, lactic acid, total billirubin, hemoglobin , methemoglobin and ionized calcium
Miscellaneous	Miscellaneous drug levels and chemistries, e.g., creatinine, hormone levels, direct billirubin, C-reactive protein
Capillary radiometer 55	Capillary pH and blood gases, total billirubin and methemoglobin
Capillary radiometer 35	Hemoglobin, methemoglobin, total billirubin or glucose and lactic acid

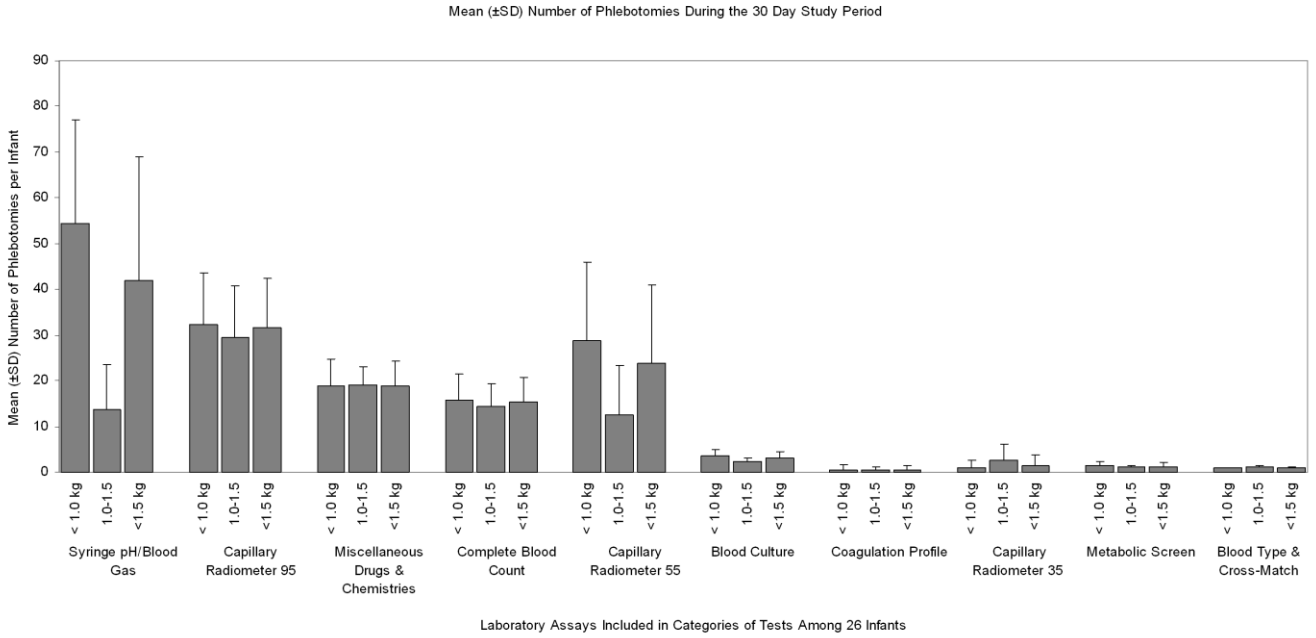
Figure 6.2 Percent of total blood taken by test. The hashed bars represent the percent of blood required for analysis by specific instruments and the shaded bars represent the remaining percentage of leftover blood. The specific analytes obtained for each category of test are summarized in the legend of the Figure. n=26 for birth weight <1.5kg, n=8 for birth weight 1.0-1.5kg, n=18 for birth weight <1.0kg.



All Percents for a given birth weight group add to 100%

Test	Analytes Included in Selected Test Catagories
Syringe pH/ blood gas	Arterial or venous pH and blood gases, electrolytes, glucose, lactic acid, total billirubin, hemoglobin , methemoglobin and ionized calcium
Capillary radiometer 95	Capillary pH and blood gases, electrolytes, glucose, lactic acid, total billirubin, hemoglobin , methemoglobin and ionized calcium
Miscellaneous	Miscellaneous drug levels and chemistries, e.g., creatinine, hormone levels, direct billirubin, C-reactive protein
Capillary radiometer 55	Capillary pH and blood gases, total billirubin and methemoglobin
Capillary radiometer 35	Hemoglobin, methemoglobin, total billirubin or glucose and lactic acid

Figure 6.3 Average number of tests ordered for all tests. The specific analytes obtained for each category of test are summarized in the legend of the Figure. n=26 for birth weight <1.5kg, n=8 for birth weight 1.0-1.5kg, n=18 for birth weight <1.0kg.



Test	Analytes Included in Selected Test Catagories
Syringe pH/ blood gas	Arterial or venous pH and blood gases, electrolytes, glucose, lactic acid, total billirubin, hemoglobin , methemoglobin and ionized calcium
Capillary radiometer 95	Capillary pH and blood gases, electrolytes, glucose, lactic acid, total billirubin, hemoglobin , methemoglobin and ionized calcium
Miscellaneous	Miscellaneous drug levels and chemistries, e.g., creatinine, hormone levels, direct billirubin, C-reactive protein
Capillary radiometer 55	Capillary pH and blood gases, total billirubin and methemoglobin
Capillary radiometer 35	Hemoglobin, methemoglobin, total billirubin or glucose and lactic acid

Figure 6.4 Simulated Epo dosing with phlebotomy reduction predicts Hb concentrations similar to actual Hb concentrations. Simulated and actual hemoglobin levels averaged over the first month of life for each time point.

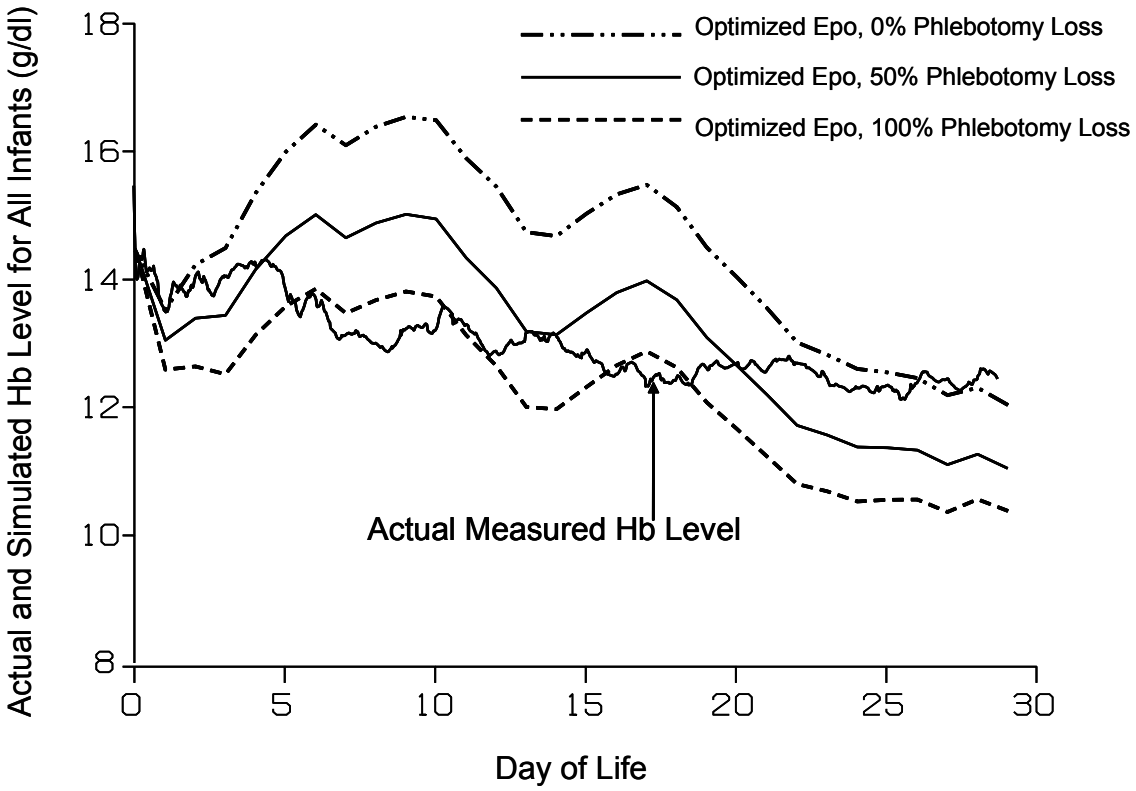


Figure 6.5 The percent of infants with birth weight from 1.0-1.5kg predicted to completely avoid RBCTx with simulated phlebotomy reduction only (n=8). The dashed line represents the PINT RBCTx criteria while the solid line represents the actual RBCTx practice applied to individual study subjects.

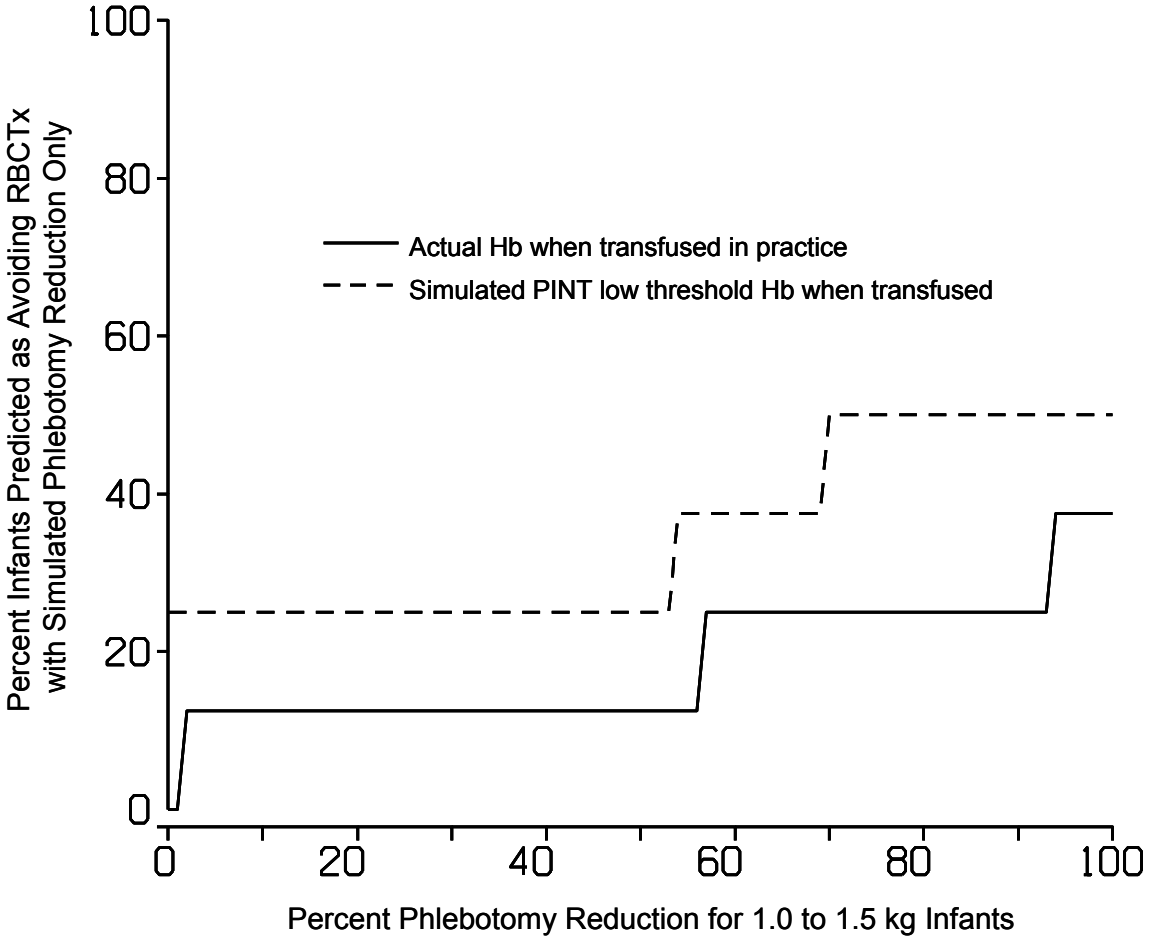


Figure 6.6 The percent of infants with birth weight 1.0-1.5kg predicted to completely avoid RBCTx with optimized Epo administration and simulated phlebotomy reduction (n=8). The dashed line represents the PINT RBCTx criteria while the solid line represents the actual RBCTx practice applied clinically.

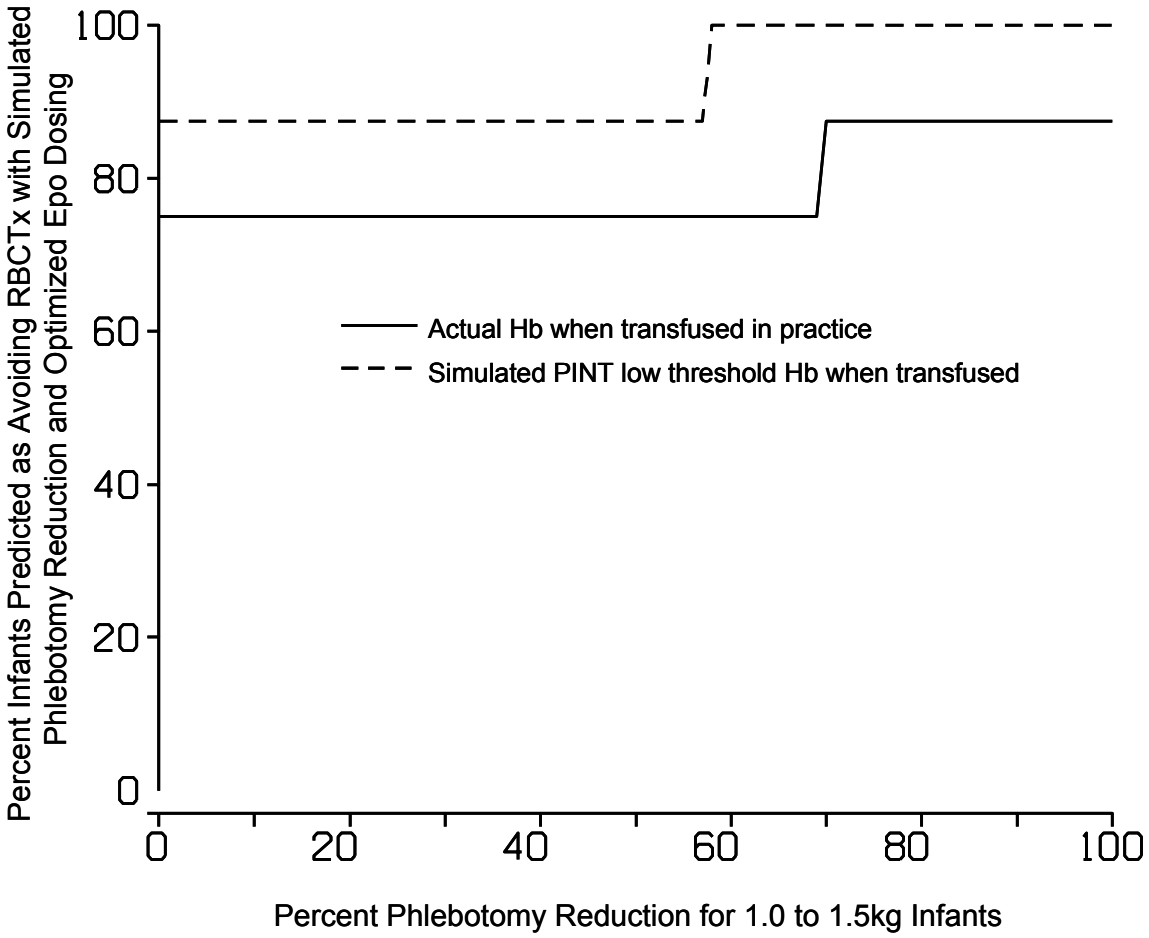


Figure 6.7 The percent of infants with birth weight <1.0kg predicted to completely avoid RBCTx with simulated phlebotomy reduction and Epo administration (n=18). The dashed line represents the PINT RBCTx criteria while the solid line represents the actual RBCTx practice.

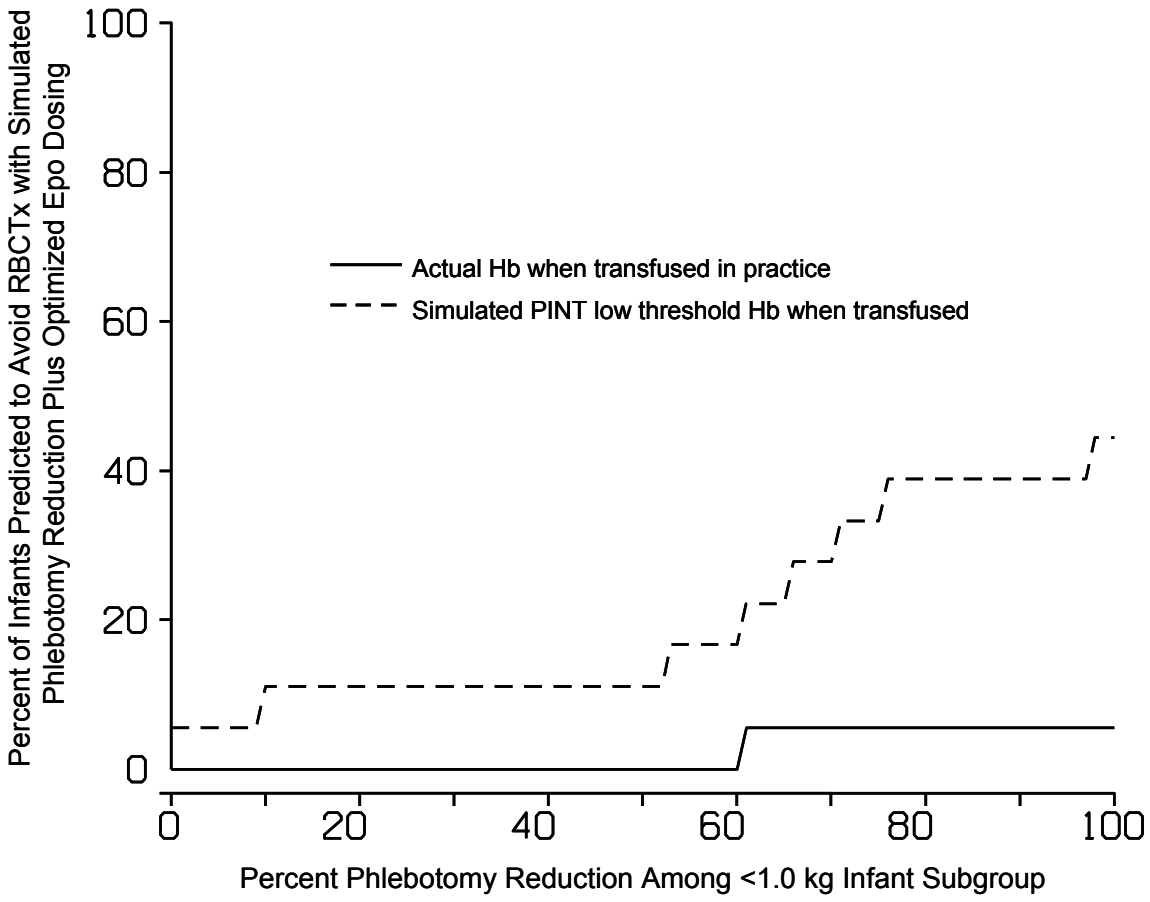


Figure 6.8 Number of RBCTx predicted for VLBW infants with simulated phlebotomy reduction only.

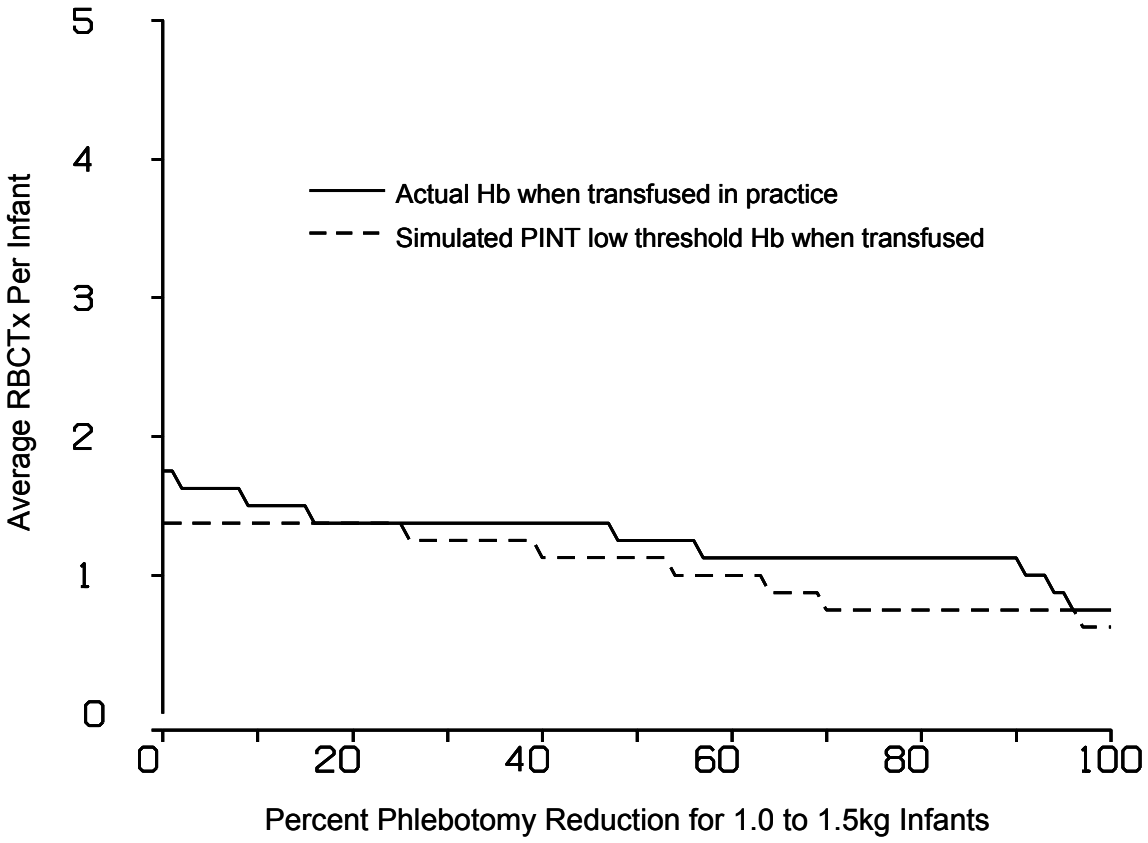


Figure 6.9 Number of RBCTx predicted for VLBW infants with simulated phlebotomy reduction and optimal Epo therapy.

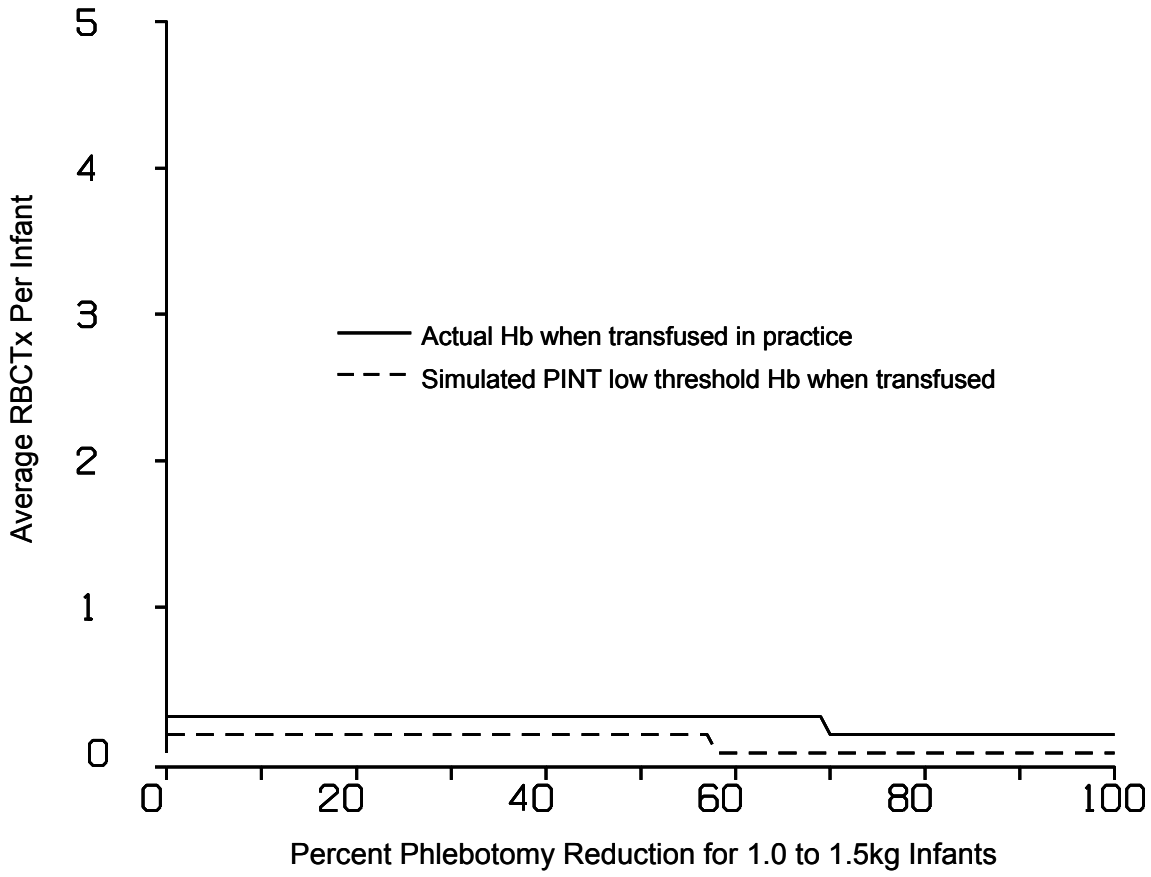


Figure 6.10 Number of RBCTx predicted for ELBW infants with simulated phlebotomy reduction only.

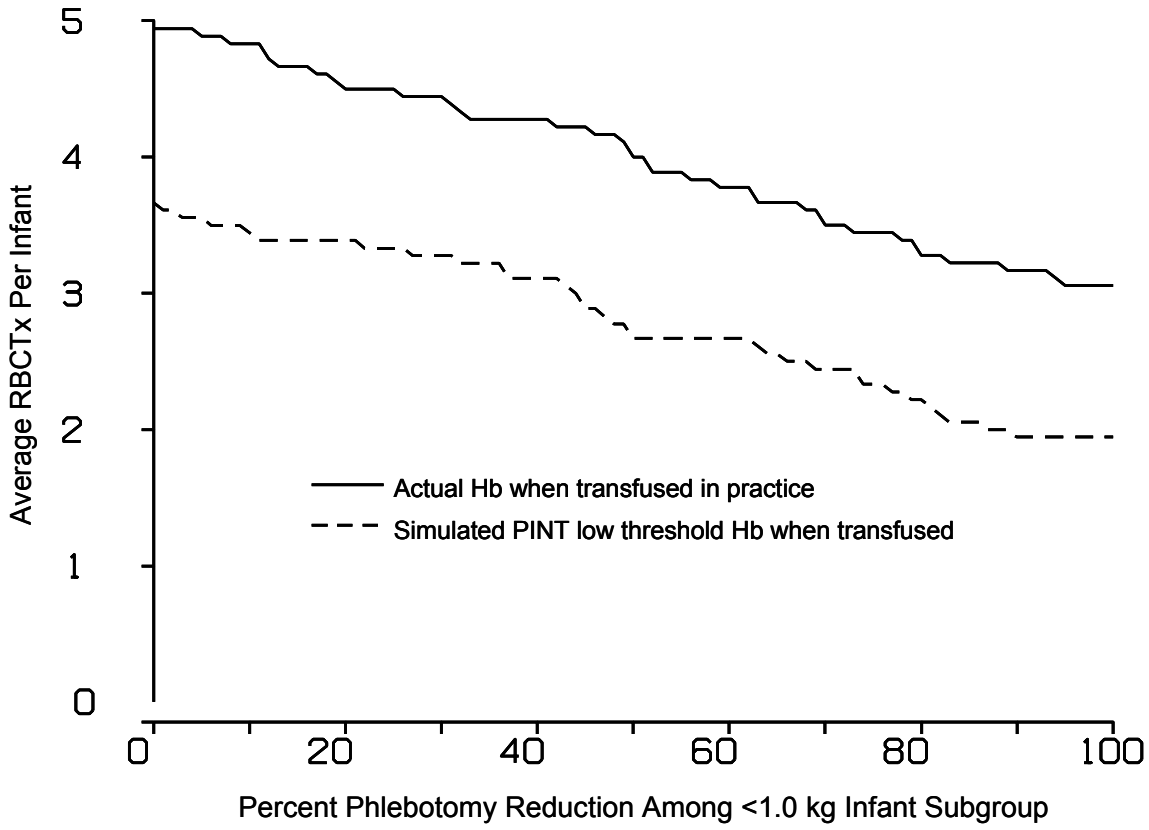
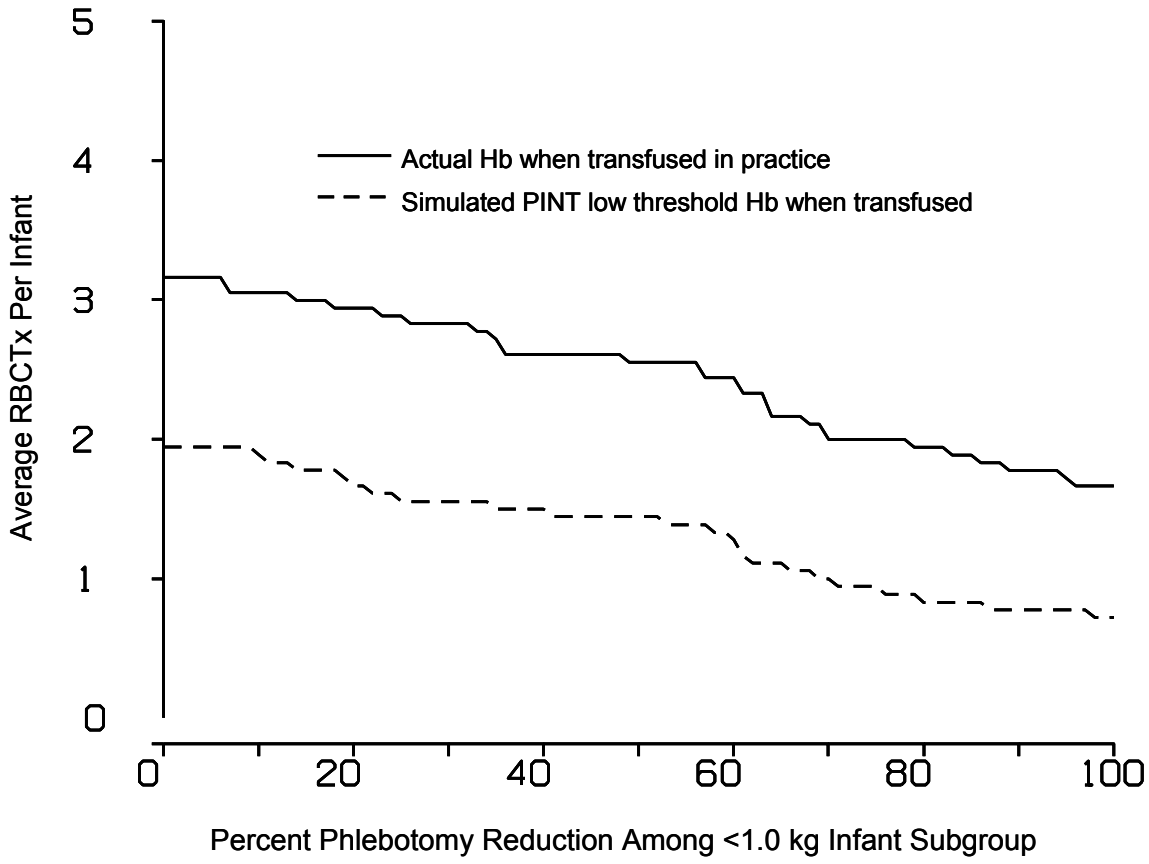


Figure 6.11 Number of RBCTx predicted for ELBW infants with simulated phlebotomy reduction and optimal Epo therapy.



CHAPTER 7. CONCLUSIONS AND FUTURE WORK

The overall aim of this thesis was to examine alternative approaches to treating anemia in preterm infants for the purpose of avoiding red blood cell transfusions. In investigating this aim, different aspects of erythropoiesis were examined in combination with simulated Epo dosing and reducing laboratory phlebotomy testing.

7.1 Main conclusions

Chapters 2 and 3 demonstrate that the Epo receptor state is important for the pharmacodynamic response of Epo in newborn sheep. This receptor state likely explains some of the large pharmacodynamic variability seen in preterm infants and elucidating the Epo receptor state could help identify candidates likely to respond well to Epo. In addition, the Epo receptor state could influence the optimal time to administer Epo doses as shown in Chapter 3. Unfortunately, no experimental data has been acquired which would give insight into the Epo receptor level in preterm infants. The methodology we used to determine the Epo receptor state in sheep cannot be used in preterm infants due to ethical issues of administering radioactive labels.

Chapter 4 examined the optimal dosing regimen of Epo for 27 preterm infants based on their pharmacodynamic parameters and pharmacokinetic information from the literature. The major finding from this chapter was that the simulations suggest more Epo than previous clinical trials should be administered frequently in the first 1-2 weeks of life when phlebotomy loss is the greatest. This chapter also suggested that infants born with a birth weight of 1-1.5 kg may be able to avoid RBCTx with optimal Epo therapy. This optimized Epo dosing regimen is quiet different from the Epo dosing in previous

clinical trials where Epo is given in a standard regimen (i.e. 3 times per week). Like all simulation studies, the dosing optimization needs to be validated by clinical studies.

In Chapter 5 the amount of blood phlebotomized from preterm infants was examined to determine if decreasing this phlebotomized blood could reduce the number of RBCTx. While the phlebotomized blood in preterm infants is significant, reducing the amount of phlebotomized blood was not simulated to completely eliminate RBCTx in most subjects. This finding is likely due to the fact that preterm infants have approximately twice as much blood transfused as removed through phlebotomies. From the data shown in Chapter 5 phlebotomy reduction was shown to be easily achievable by decreasing the wasted blood drawn from infants. Decreasing wasted blood, using blood monitors and diluting blood samples to reduce blood loss from phlebotomies will likely offer clinical benefits to infants if they are used in future clinical trials.

Chapter 6 simulated optimized Epo dosing, reduction of laboratory phlebotomy blood loss and use of a restrictive RBC transfusion criteria in order to reduce the number of transfusions each infant would receive. The simulations predicted that with optimal Epo dosing and 100% laboratory phlebotomy reduction, all infants with a birth weight of 1.0-1.5 kg and 45% of infants with a birth weight <1.0 kg would avoid RBC transfusions completely. Results from these simulation studies suggested that preterm infants could have highly significant clinical benefits from a combination of therapies. In practice, there have been clinical trials which have examined reducing RBCTx in preterm infants using Epo administration (1), phlebotomy reduction (92, 93) and applying specific restrictive RBCTx criteria (96, 97). Unfortunately, no clinical trials have examined a combination of these therapeutic interventions. Therefore, it is highly recommended that

future clinical trials use a combination of therapeutic interventions to avoid the need for RBCTx in preterm infants.

7.2 Future work

There are several areas of research that will be carried on as an extension of this thesis. Our group is currently working on a non-radioactive biotin label for Epo which could be used in preterm infants. Such a label for Epo would give a surrogate marker for the Epo receptor state in preterm infants based on the clearance. Any information on the Epo receptor pool in infants could be used to improve the pharmacokinetic and pharmacodynamic modeling and therefore improve the simulation studies from this thesis.

In addition, the lifespan assumptions used for the modeling in this thesis may not be accurate due to endogenous factors in preterm infants which could alter the lifespan. In order to address the lifespan assumption, currently lifespan studies are being conducted with biotin labeled red blood cells. These studies will give estimates of the lifespans for both the RBCs transfused to infants and the infant's own RBC lifespan. Using these new lifespan data will improve the PK/PD analysis in preterm infants as well as the corresponding simulation studies.

Finally, an Epo clinical trial will be conducted in preterm infants to confirm and improve the simulation studies completed for this thesis. The Epo clinical trial in preterm infants will improve on previous clinical trials for several reasons. (1) The Epo doses in the clinical trial will be sufficiently high to determine the saturation point such that determination of E_{max} parameters is possible, (2) There will be many additional covariates determined such as cytokines and iron status which will give further insight into the

PK/PD variability currently observed, (3) Optimized Epo dosing will be used which has been simulated to give a significant benefit over conventional Epo therapy. Furthermore, the clinical trial will enroll 100 infants which will allow for the development of a population pharmacokinetic model and elucidate which covariates determine the pharmacokinetic variability. This clinical trial will ultimately be able to determine the potential for eliminating transfusions in preterm infants with Epo therapy.

APPENDIX A. DERIVATIONS FOR CHAPTER 4

A.1 Derivation for the continuous bleed function

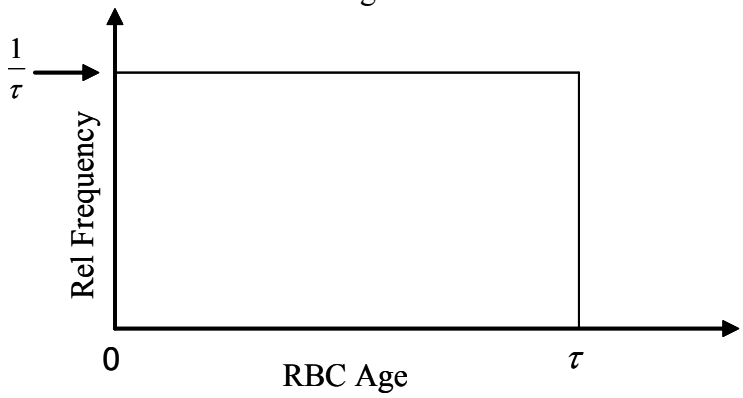
Variable Definition:

$Hb_O(0)$ = amount original of hemoglobin at t=0
$Hb_O(t)$ = amount of original hemoglobin left in blood at time t
Hb(t) = total amount of hemoglobin in blood
f(t) = production rate of Hb (amount/time)
$Cl_P(t)$ = Volume of blood removed/time/blood volume
τ = Lifespan of red blood cells

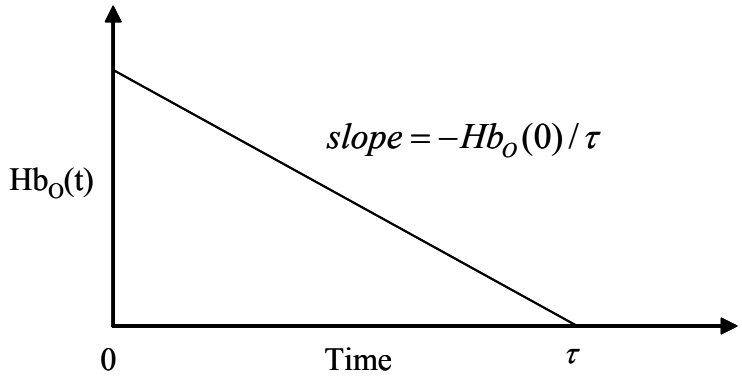
Rate of Hb removal due to continuous bleed (amount/time):

$$r_b(t) = Hb(t) \cdot CL_P(t)$$

Assumption: RBCs are under "steady state" conditions and under "steady state" conditions have a uniform age distribution.

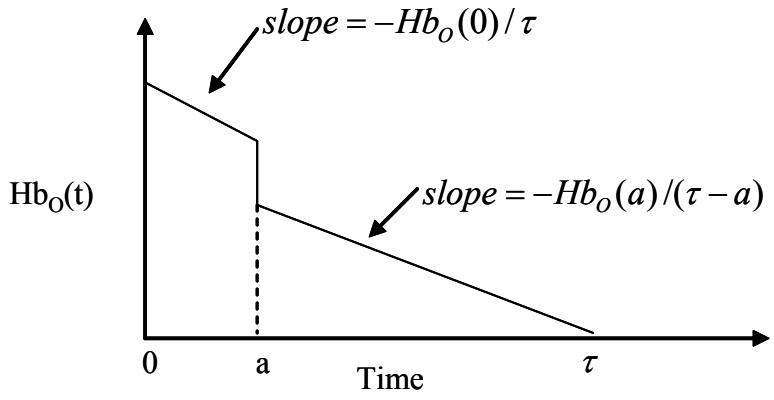


Rate of Hb removed due to aging without phlebotomy:



$$\frac{dHb_O(t)}{dt} = \frac{-Hb_O(0)}{\tau}$$

Rate of original Hb removal due to aging with phlebotomy:



$$r_a(t) = \frac{Hb_O(t)}{\tau - t} \qquad t < \tau$$

Assumption: Hemoglobin produced during the study is not removed due to aging because the study duration is much shorter than the lifespan of the red blood cells.

Change in Hb = Hb Produced – Hb Removed

$$\frac{dHb(t)}{dt} = f(t) - r_b(t) - r_a(t)$$

Final Differential Equations:

$$\frac{dHb(t)}{dt} = f(t) - Hb(t) \cdot CL_P - \frac{Hb_O(t)}{\tau - t}$$
$$\frac{dHb_O(t)}{dt} = -Hb_O(t) \cdot CL_P(t) - \frac{Hb_O(t)}{\tau - t}$$

Initial Conditions:

$$Hb(0) = Hb_O(0)$$
$$Hb_O(0) = f_{ss} \cdot \tau$$

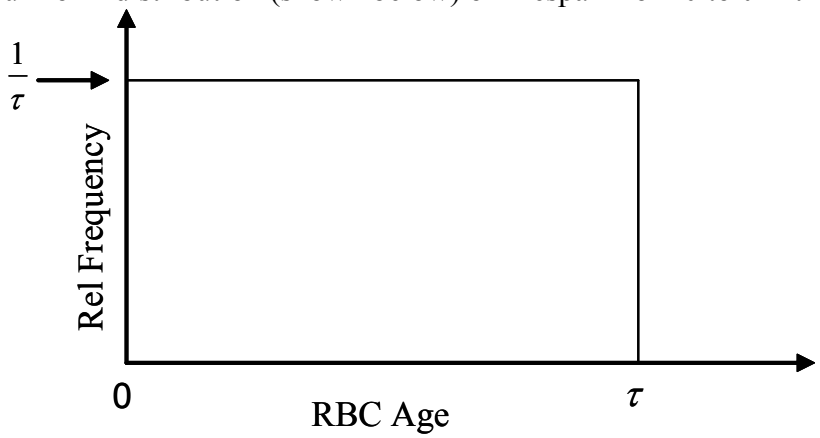
Where,

$$C_{HB}(t) = \frac{Hb(t)}{V(t)}$$

APPENDIX B. DERIVATIONS FOR CHAPTER 5

B.1 Derivation for amount of transfused blood remaining

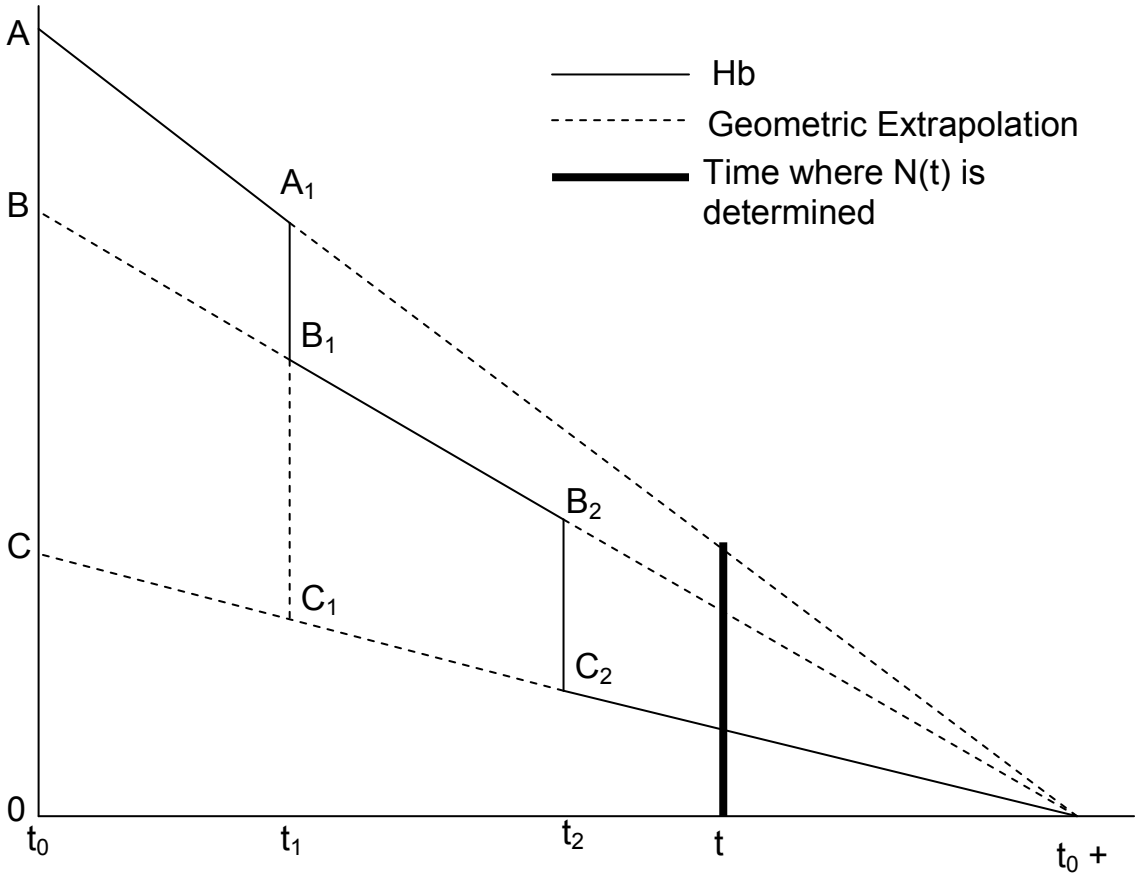
Consider a quantity, N_0, of donor cells, transfused at $t = t_0$. Let it be assumed that cells are from a subject with a SS cellular production leading to a donor cell population with a uniform distribution (shown below) of lifespan from 0 to τ in the recipient.



In the absence of any phlebotomies the number of transfused cells is given by:

$$N(t) = N_0 \cdot (1 - (t - t_0)/\tau)_+ \qquad t \geq t_0$$

The + subscript denotes the truncation function such that if the value of N(t) falls below 0 then the value of the function is equal to 0. Consider a geometric representation of a situation where multiple phlebotomies are taken following a transfusion (shown below). t_1 and t_2 are the times at which two phlebotomies occurred and t is a time after the second phlebotomy at which we need to obtain the remaining transfused cells. All points A, B and C are geometric points for deriving the amount of transfused blood remaining in the presence of multiple phlebotomies.



Fj = Fraction of blood remaining after the jth phlebotomy relative to blood present immediately before the phlebotomy

$$F_1 = B_1 / A_1 = B / A \qquad (1)$$

$$F_2 = C_2 / B_2 = C / B \qquad (2)$$

$$F_1 \cdot F_2 = C / A \qquad (3)$$

Slope of decline after 2nd phlebotomy:

$$slope = -\frac{C}{\tau} \qquad (4)$$

Value of N at time t:

$$N(t) = C + slope \cdot (t - t_0) \qquad (5)$$

$$N(t) = C - \frac{C}{\tau} \cdot (t - t_0) \qquad (6)$$

$$N(t) = C \cdot \left(1 - \frac{t - t_0}{\tau}\right) \qquad (7)$$

$$C = F_1 \cdot F_2 \cdot A = N_0 \cdot F_1 \cdot F_2 \qquad (8)$$

$$N(t) = N_0 \cdot F_1 \cdot F_2 \cdot \left(1 - \frac{t - t_0}{\tau}\right) \qquad (9)$$

Generalized:

$$N(t) = N_0 \cdot \left(1 - \frac{t - t_0}{\tau}\right) \cdot \prod_{j=0}^{k} (F_j) \qquad t_k \le t < t_{k+1} \qquad (10)$$

Where j is an indexing variable for phlebotomies and k is the number of phlebotomies which occurred after the transfusion time t_0 and before time t

Fj can also be defined as the fraction of blood removed for phlebotomy j in which cases the previous equation becomes:

$$N(t) = N_0 \cdot \left(1 - \frac{t - t_0}{\tau}\right) \cdot \prod_{j=0}^{k} (1 - F_j) \qquad t_k \le t < t_{k+1} \qquad (11)$$

Equation 11 can be found in chapter 5 of the thesis.

APPENDIX C. FORTRAN AND WINFUNFIT COMPUTER CODE

All of the computer code necessary to produce the output in the thesis is given in this appendix. Most but not all of the code is original and written by Matthew Rosebraugh. A note is put in next to each subroutine regarding the originally of the code. The program WINFUNFIT (Author Peter Veng-Pedersen) is required to run all of the code in this thesis (146). Lines with a "C" or "!" before them do not get compiled but these lines have been included to explain areas of the code.

For each chapter where Fortran code was used there is a main program and a shared program. The main file is the core program run for the given chapter and the shared file gives the common variables shared between subroutines and the main program.

C.1 Common Fortran subroutines for multiple chapters

C.1.1 FIT_LINEAR_SPLINE.F90 (Not original code)

```
subroutine spline_linear_val ( ndata, tdata, ydata, tval, yval, ypval )
!
!*******************************************************************************
!
!! SPLINE_LINEAR_VAL evaluates a linear spline at a specific point.
!
!
!  Discussion:
!
!    Because of the extremely simple form of the linear spline,
!    the raw data points ( TDATA(I), YDATA(I)) can be used directly to
!    evaluate the spline at any point.  No processing of the data
!    is required.
!
!  Modified:
!
!    06 April 1999
!
!  Author:
```

```
!
!   John Burkardt
!
!  Parameters:
!
!   Input, integer NDATA, the number of data points defining the spline.
!
!   Input, real TDATA(NDATA), YDATA(NDATA), the values of the independent
!   and dependent variables at the data points.  The values of TDATA should
!   be distinct and increasing.
!
!   Input, real TVAL, the point at which the spline is to be evaluated.
!
!   Output, real YVAL, YPVAL, the value of the spline and its first
!   derivative dYdT at TVAL.  YPVAL is not reliable if TVAL is exactly
!   equal to TDATA(I) for some I.
!
  implicit none
!
  integer ndata
!
  integer left
  integer right
  real*8 tdata(ndata)
  real*8 tval
  real*8 ydata(ndata)
  real*8 ypval
  real*8 yval
!
!  Find the interval [ TDATA(LEFT), TDATA(RIGHT) ] that contains, or is
!  nearest to, TVAL.
!
  call rvec_bracket ( ndata, tdata, tval, left, right )
!
!  Now evaluate the piecewise linear function.
!
  ypval = ( ydata(right) - ydata(left) ) / ( tdata(right) - tdata(left) )

  yval = ydata(left) +  ( tval - tdata(left) ) * ypval

  return
end

subroutine rvec_bracket ( n, x, xval, left, right )
!
!*****************************************************************************
!
!! RVEC_BRACKET searches a sorted array for successive brackets of a value.
!
!
!  Discussion:
!
!   If the values in the vector are thought of as defining intervals
!   on the real line, then this routine searches for the interval
!   nearest to or containing the given value.
!
```

```
!  Modified:
!
!    06 April 1999
!
!  Author:
!
!    John Burkardt
!
!  Parameters:
!
!    Input, integer N, length of input array.
!
!    Input, real X(N), an array sorted into ascending order.
!
!    Input, real XVAL, a value to be bracketed.
!
!    Output, integer LEFT, RIGHT, the results of the search.
!    Either:
!      XVAL < X(1), when LEFT = 1, RIGHT = 2;
!      XVAL > X(N), when LEFT = N-1, RIGHT = N;
!    or
!      X(LEFT) <= XVAL <= X(RIGHT).
!
  implicit none
!
  integer n
!
  integer i
  integer left
  integer right
  real*8 x(n)
  real*8 xval
!
  do i = 2, n - 1

    if ( xval < x(i) ) then
      left = i - 1
      right = i
      return
    end if

   end do

  left = n - 1
  right = n

  return
end
```

C.1.2 FILE_OPEN.F90 (Original code)

```
SUBROUTINE FILE_OPEN

USE SHARED
```

```
IMPLICIT NONE

INTEGER :: status, nfiles, F
REAL*8 temp1, temp2, temp3

status=0
OPEN (UNIT=10, FILE=TRX_FILE(FILENUM), STATUS='OLD', ACTION= 'READ',
IOSTAT=status)
nfiles=0
!WRITE(*,*)"OK",status
fileopen32: IF (status == 0) THEN
!WRITE(*,*) 'THE WEIGHT DATA FILE OPENED CORRECTLY'
   DO33: DO     !to identify the number of raw data files to open
      READ(10,*, IOSTAT=status) temp1,temp2,temp3


!WRITE(*,*) FILENUM, TEMP2, TEMP3
!READ*
!                    WRITE(*,*)"EVERY THING IS OK"
      IF (status/=0) EXIT
      nfiles=nfiles+1
   END DO DO33


!   WRITE(*,*)'Allocating the number of data points=',nfiles
   ALLOCATE(TRX_T(nfiles),STAT=status)
          ALLOCATE(TRX_H(nfiles),STAT=status)
          ALLOCATE(TRX_V(nfiles),STAT=status)
F=0
   ALLOCATE_OK32: IF (status==0) THEN
      REWIND (UNIT=10)
      DO34: DO
         READ(10,*, IOSTAT=status) temp1,temp2,temp3
         IF (status/=0) EXIT
         f=f+1
                              TRX_T(f)=temp1
         TRX_H(f)=temp2
                              TRX_V(f)=temp3

!       WRITE(*,*) FILENUM, EPOX(FILENUM, f), EPOY(FILENUM, f)
!                   READ*
      END DO DO34
   END IF ALLOCATE_OK32


   ELSE fileopen32
      WRITE(*,3002)status
      3002 FORMAT (1X,'Raw data trx File titles open failed -- 1 status= ',I6)

END IF fileopen32

TRX_POINTS(FILENUM)=f
!WRITE(*,*) EPO_POINTS(FILENUM)

status=0
```

```
OPEN (UNIT=11, FILE=WEIGHT_FILE(FILENUM), STATUS='OLD', ACTION= 'READ',
IOSTAT=status)
nfiles=0
!WRITE(*,*)"OK",status
fileopen33: IF (status == 0) THEN
!WRITE(*,*) 'THE EPO DATA FILE OPENED CORRECTLY'
   DO36: DO     !to identify the number of raw data files to open
      READ(11,*, IOSTAT=status) temp1,temp2
!       WRITE(*,*)"EVERY THING IS OK"
      IF (status/=0) EXIT
      nfiles=nfiles+1
   END DO DO36

!   WRITE(*,*)'Allocating the number of data points=',nfiles
   ALLOCATE(WEIGHT_X(nfiles),STAT=status)
         ALLOCATE(WEIGHT_Y(nfiles),STAT=status)
F=0
   ALLOCATE_OK33: IF (status==0) THEN
      REWIND (UNIT=11)
      DO37: DO
         READ(11,*, IOSTAT=status) temp1,temp2
                           IF (status/=0) EXIT
         f=f+1
                           WEIGHT_X(f)=temp1
         WEIGHT_Y(f)=temp2


!       WRITE(*,*) HGBX(f), HGBY(f)
      END DO DO37
!   WRITE(*,*) FILENUM, CUMLOSS_KG(f)
!          READ*
          END IF ALLOCATE_OK33

   ELSE fileopen33
      WRITE(*,3003)status
      3003 FORMAT (1X,'Raw data weight File titles open failed -- 1 status= ',I6)

END IF fileopen33

WEIGHT_POINTS(FILENUM)=f

status=0
OPEN (UNIT=12, FILE=HGB_FILE(FILENUM), STATUS='OLD', ACTION= 'READ',
IOSTAT=status)
nfiles=0
!WRITE(*,*)"OK",status
fileopen34: IF (status == 0) THEN
!WRITE(*,*) 'THE WEIGHT DATA FILE OPENED CORRECTLY'
   DO35: DO     !to identify the number of raw data files to open
      READ(12,*, IOSTAT=status) temp1,temp2,temp3

!WRITE(*,*) FILENUM, TEMP2, TEMP3
!READ*
!                    WRITE(*,*)"EVERY THING IS OK"
      IF (status/=0) EXIT
      nfiles=nfiles+1
```

```
   END DO DO35


!   WRITE(*,*)'Allocating the number of data points=',nfiles
   ALLOCATE(HGB_X(nfiles),STAT=status)
          ALLOCATE(HGB_Y(nfiles),STAT=status)
          ALLOCATE(SYRINGE(nfiles),STAT=status)
          ALLOCATE(HGB_M1(nfiles),STAT=status)
          ALLOCATE(HGB_M2(nfiles),STAT=status)
          ALLOCATE(HGB_M3(nfiles),STAT=status)
          ALLOCATE(HGB_M4(nfiles),STAT=status)
          ALLOCATE(HGB_M5(nfiles),STAT=status)
          ALLOCATE(HGB_M6(nfiles),STAT=status)
          ALLOCATE(ORIGINAL_DATA(nfiles),STAT=status)
F=0
   ALLOCATE_OK34: IF (status==0) THEN
      REWIND (UNIT=12)
      DO
         READ(12,*, IOSTAT=status) temp1,temp2,temp3
         IF (status/=0) EXIT

                              f=f+1
                              IF(temp3==1) THEN !THIS IS A SYRINGE SAMPLE
                              HGB_X(f)=temp1
         HGB_Y(f)=temp2
                              SYRINGE(f)=temp3
                              ELSE
                              HGB_X(f)=temp1
         HGB_Y(f)=temp2 !*(1D0/(-0.002404388D0*temp1+1.205176862D0)) !THIS WAS TAKEN
FROM EXCEL PLOT AND 2 REFERENCES
                              SYRINGE(f)=temp3
                              END IF

!       IF (TEMP3==0) CYCLE !PUT IN ONLY SYRINGE POINTS
!                             HGB_X(f)=temp1
!          HGB_Y(f)=temp2
!                             SYRINGE(f)=temp3

!       WRITE(*,*) FILENUM, EPOX(FILENUM, f), EPOY(FILENUM, f)
!                     READ*
      END DO
   END IF ALLOCATE_OK34


   ELSE fileopen34
      WRITE(*,3004)status
      3004 FORMAT (1X,'Raw data hgb File titles open failed -- 1 status= ',I6)

END IF fileopen34

HGB_POINTS(FILENUM)=f

status=0
OPEN (UNIT=13, FILE=PHLE_FILE(FILENUM), STATUS='OLD', ACTION= 'READ',
IOSTAT=status)
nfiles=0
```

```
!WRITE(*,*)"OK",status
fileopen35: IF (status == 0) THEN
!WRITE(*,*) 'THE EPO DATA FILE OPENED CORRECTLY'
   DO38: DO     !to identify the number of raw data files to open
      READ(13,*, IOSTAT=status) temp1,temp2
!       WRITE(*,*)"EVERY THING IS OK"
      IF (status/=0) EXIT
      nfiles=nfiles+1
   END DO DO38

!   WRITE(*,*)'Allocating the number of data points=',nfiles
   ALLOCATE(PHLE_X(nfiles),STAT=status)
         ALLOCATE(PHLE_Y(nfiles),STAT=status)
F=0
   ALLOCATE_OK39: IF (status==0) THEN
      REWIND (UNIT=13)
      DO
         READ(13,*, IOSTAT=status) temp1,temp2
                        IF (status/=0) EXIT
         f=f+1
                        PHLE_X(f)=temp1
                        PHLE_Y(f)=temp2

!       WRITE(*,*) HGBX(f), HGBY(f)
      END DO
   END IF ALLOCATE_OK39

   ELSE fileopen35
      WRITE(*,3005)status
      3005 FORMAT (1X,'Raw data PHLE File titles open failed -- 1 status= ',I6)

END IF fileopen35

PHLE_POINTS(FILENUM)=f

status=0
OPEN (UNIT=14, FILE=PHLE_FILE_KG(FILENUM), STATUS='OLD', ACTION= 'READ',
IOSTAT=status)
nfiles=0
!WRITE(*,*)"OK",status
IF (status == 0) THEN
!WRITE(*,*) 'THE EPO DATA FILE OPENED CORRECTLY'
DO     !to identify the number of raw data files to open
      READ(14,*, IOSTAT=status) temp1,temp2
!       WRITE(*,*)"EVERY THING IS OK"
IF (status/=0) EXIT
      nfiles=nfiles+1
END DO

!   WRITE(*,*)'Allocating the number of data points=',nfiles
   ALLOCATE(PHLE_X_KG(nfiles),STAT=status)
         ALLOCATE(PHLE_Y_KG(nfiles),STAT=status)
F=0
   IF (status==0) THEN
      REWIND (UNIT=14)
      DO
```

```
         READ(14,*, IOSTAT=status) temp1,temp2
                              IF (status/=0) EXIT
         f=f+1
                              PHLE_X_KG(f)=temp1
                              PHLE_Y_KG(f)=temp2
      END DO
   END IF

   ELSE
      WRITE(*,3006)status
      3006 FORMAT (1X,'Raw data PHLE_KG File titles open failed -- 1 status= ',I6)

END IF

PHLE_POINTS_KG(FILENUM)=f

status=0
OPEN (UNIT=15, FILE=THRESH_FILE(FILENUM), STATUS='OLD', ACTION= 'READ',
IOSTAT=status)
nfiles=0
!WRITE(*,*)"OK",status
IF (status == 0) THEN
!WRITE(*,*) 'THE EPO DATA FILE OPENED CORRECTLY'
DO     !to identify the number of raw data files to open
       READ(15,*, IOSTAT=status) temp1,temp2
!       WRITE(*,*)"EVERY THING IS OK"
IF (status/=0) EXIT
       nfiles=nfiles+1
END DO

!    WRITE(*,*)'Allocating the number of data points=',nfiles
   ALLOCATE(THRESH_X(nfiles),STAT=status)
          ALLOCATE(THRESH_Y(nfiles),STAT=status)
F=0
   IF (status==0) THEN
      REWIND (UNIT=15)
      DO
         READ(15,*, IOSTAT=status) temp1,temp2
                              IF (status/=0) EXIT
         f=f+1
                              THRESH_X(f)=temp1
                              THRESH_Y(f)=temp2
      END DO
   END IF

   ELSE
      WRITE(*,*) 'Raw data THRESH File titles open failed -- 1 status= ', status

END IF

THRESH_POINTS(FILENUM)=f

END SUBROUTINE FILE_OPEN
```

C.1.3 DELAY_DIFFEQ.FOR (Not original code)

```
      SUBROUTINE RETARD(N,FCN,X,Y,XEND,
     &                  RTOL,ATOL,ITOL,
     &                  SOLOUT,IOUT,
     &                  WORK,LWORK,IWORK,LIWORK,LRCONT,LICONT,IDID)
C ----------------------------------------------------------
C     NUMERICAL SOLUTION OF A SYSTEM OF FIRST 0RDER DELAY
C     ORDINARY DIFFERENTIAL EQUATIONS  Y'(X)=F(X,Y(X),Y(X-A),...).
C     THIS CODE IS BASED ON AN EXPLICIT RUNGE-KUTTA METHOD OF
C     ORDER (4)5 DUE TO DORMAND & PRINCE (WITH STEPSIZE CONTROL
C     AND DENSE OUTPUT).
C
C     AUTHORS: E. HAIRER AND G. WANNER
C              UNIVERSITE DE GENEVE, DEPT. DE MATHEMATIQUES
C              CH-1211 GENEVE 24, SWITZERLAND
C              E-MAIL:  HAIRER@UNI2A.UNIGE.CH,  WANNER@UNI2A.UNIGE.CH
C
C     THIS CODE IS DESCRIBED IN SECTION II.17 OF THE BOOK:
C        E. HAIRER, S.P. NORSETT AND G. WANNER, SOLVING ORDINARY
C        DIFFERENTIAL EQUATIONS I. NONSTIFF PROBLEMS. 2ND EDITION.
C        SPRINGER SERIES IN COMPUTATIONAL MATHEMATICS,
C        SPRINGER-VERLAG (1993)
C
C     VERSION OF March 24, 1993
C
C     INPUT PARAMETERS
C     ----------------
C     N          DIMENSION OF THE SYSTEM
C
C     FCN        NAME (EXTERNAL) OF SUBROUTINE COMPUTING THE RIGHT-
C                HAND-SIDE OF THE DELAY EQUATION, E.G.,
C                  SUBROUTINE FCN(N,X,Y,F)
C                  REAL*8 X,Y(N),F(N)
C                  EXTERNAL PHI
C                  F(1)=(1.4D0-YLAG(1,X-1.D0,PHI))*Y(1)
C                  F(2)=...   ETC.
C                FOR AN EXPLICATION OF YLAG SEE BELOW.
C                DO NOT USE YLAG(I,X-0.D0,PHI) !
C                THE INITIAL FUNCTION HAS TO BE SUPPLIED BY:
C                  FUNCTION PHI(I,X)
C                  REAL*8 PHI,X
C                WHERE I IS THE COMPONENT AND X THE ARGUMENT
C
C     X          INITIAL X-VALUE
C
C     Y(N)       INITIAL VALUES FOR Y (MAY BE DIFFERENT FROM PHI (I,X),
C                IN THIS CASE IT IS HIGHLY RECOMMENDED TO SET IWORK(6)
C                AND WORK(11),..., SEE BELOW)
C
C     XEND       FINAL X-VALUE (XEND > X)
C
C     RTOL,ATOL   RELATIVE AND ABSOLUTE ERROR TOLERANCES. THEY
C                 CAN BE BOTH SCALARS OR ELSE BOTH VECTORS OF LENGTH N.
C
C     ITOL       SWITCH FOR RTOL AND ATOL:
```

```
C              ITOL=0: BOTH RTOL AND ATOL ARE SCALARS.
C                THE CODE KEEPS, ROUGHLY, THE LOCAL ERROR OF
C                Y(I) BELOW RTOL*ABS(Y(I))+ATOL
C              ITOL=1: BOTH RTOL AND ATOL ARE VECTORS.
C                THE CODE KEEPS THE LOCAL ERROR OF Y(I) BELOW
C                RTOL(I)*ABS(Y(I))+ATOL(I).
C
C    SOLOUT      NAME (EXTERNAL) OF SUBROUTINE PROVIDING THE
C           NUMERICAL SOLUTION DURING INTEGRATION.
C           IF IOUT.GE.1, IT IS CALLED AFTER EVERY SUCCESSFUL STEP.
C           SUPPLY A DUMMY SUBROUTINE IF IOUT=0.
C           IT MUST HAVE THE FORM
C             SUBROUTINE SOLOUT (NR,XOLD,X,Y,N,IRTRN)
C             REAL*8 X,XOLD,Y(N)
C             ....
C           SOLOUT FURNISHES THE SOLUTION "Y" AT THE NR-TH
C             GRID-POINT "X" (THEREBY THE INITIAL VALUE IS
C             THE FIRST GRID-POINT).
C           "XOLD" IS THE PRECEEDING GRID-POINT.
C           "IRTRN" SERVES TO INTERRUPT THE INTEGRATION. IF IRTRN
C             IS SET <0, RETARD WILL RETURN TO THE CALLING PROGRAM.
C
C       -----  CONTINUOUS OUTPUT: -----
C           DURING CALLS TO "SOLOUT" AS WELL AS TO "FCN", A
C           CONTINUOUS SOLUTION IS AVAILABLE THROUGH HTHE FUNCTION
C                >>>   YLAG(I,S,PHI)   <<<
C           WHICH PROVIDES AN APPROXIMATION TO THE I-TH
C           COMPONENT OF THE SOLUTION AT THE POINT S. THE VALUE S
C           HAS TO LIE IN AN INTERVAL WHERE THE NUMERICAL SOLUTION
C           IS ALREADY COMPUTED. IT DEPENDS ON THE SIZE OF LRCONT
C           (SEE BELOW) HOW FAR BACK THE SOLUTION IS AVAILABLE.
C
C    IOUT       SWITCH FOR CALLING THE SUBROUTINE SOLOUT:
C             IOUT=0: SUBROUTINE IS NEVER CALLED
C             IOUT=1: SUBROUTINE IS USED FOR OUTPUT.
C
C    WORK       ARRAY OF WORKING SPACE OF LENGTH "LWORK".
C           "LWORK" MUST BE AT LEAST  8*N+11+NGRID
C           WHERE NGRID=IWORK(6)
C
C    LWORK      DECLARED LENGHT OF ARRAY "WORK".
C
C    IWORK      INTEGER WORKING SPACE OF LENGHT "LIWORK".
C           IWORK(1),...,IWORK(6) SERVE AS PARAMETERS
C           FOR THE CODE. FOR STANDARD USE, SET THEM
C           TO ZERO BEFORE CALLING.
C           "LIWORK" MUST BE AT LEAST 10 .
C
C    LIWORK     DECLARED LENGHT OF ARRAY "IWORK".
C
C    LRCONT     DECLARED LENGTH OF COMMON BLOCK
C            >>>  COMMON /CORER/RCONT(LRCONT)  <<<
C           WHICH MUST BE DECLARED IN THE CALLING PROGRAM.
C           "LRCONT" MUST BE SUFFICIENTLY LARGE. IF THE DENSE
C           OUTPUT OF MXST BACK STEPS HAS TO BE STORED, IT MUST
C           BE AT LEAST
```

```
C                   MXST * ( 5 * NRDENS + 2 )
C             WHERE NRDENS=IWORK(5) (SEE BELOW).
C
C    LICONT      DECLARED LENGTH OF COMMON BLOCK
C              >>>  COMMON /COREI/ICONT(LICONT)  <<<
C             WHICH MUST BE DECLARED IN THE CALLING PROGRAM.
C             "LICONT" MUST BE AT LEAST
C                       NRDENS + 1
C             THESE COMMON BLOCKS ARE USED FOR STORING THE COEFFICIENTS
C             OF THE CONTINUOUS SOLUTION AND MAKES THE CALLING LIST FOR
C             THE FUNCTION "CONTD5" AS SIMPLE AS POSSIBLE.
C
C
C-----------------------------------------------------------------------
C
C    SOPHISTICATED SETTING OF PARAMETERS
C    -----------------------------------
C          SEVERAL PARAMETERS (WORK(1),...,IWORK(1),...) ALLOW
C          TO ADAPT THE CODE TO THE PROBLEM AND TO THE NEEDS OF
C          THE USER. FOR ZERO INPUT, THE CODE CHOOSES DEFAULT VALUES.
C
C   WORK(1)   UROUND, THE ROUNDING UNIT, DEFAULT 2.3D-16.
C
C   WORK(2)   THE SAFETY FACTOR IN STEP SIZE PREDICTION,
C          DEFAULT 0.9D0.
C
C   WORK(3), WORK(4)   PARAMETERS FOR STEP SIZE SELECTION
C          THE NEW STEP SIZE IS CHOSEN SUBJECT TO THE RESTRICTION
C            WORK(3) <= HNEW/HOLD <= WORK(4)
C          DEFAULT VALUES: WORK(3)=0.2D0, WORK(4)=10.D0
C
C   WORK(5)   IS THE "BETA" FOR STABILIZED STEP SIZE CONTROL (SEE
C          SECTION IV.2). LARGER VALUES OF BETA (<=0.1) MAKE THE
C          STEP SIZE CONTROL MORE STABLE. NEGATIVE WORK(5) PROVOKE
C          BETA=0. DEFAULT (FOR WORK(5)=0.D0) IS WORK(5)=0.04D0.
C
C   WORK(6)   MAXIMAL STEP SIZE, DEFAULT XEND-X.
C
C   WORK(7)   INITIAL STEP SIZE, FOR WORK(7)=0.D0 AN INITIAL GUESS
C          IS COMPUTED WITH HELP OF THE FUNCTION HINIT
C
C   WORK(11),...,WORK(10+NGRID)  PRESCRIBED POINTS, WHICH THE
C          INTEGRATION METHOD HAS TO TAKE AS GRID-POINTS
C          X < WORK(11) < WORK(12) < ... < WORK(10+NGRID) <= XEND
C
C   IWORK(1)  THIS IS THE MAXIMAL NUMBER OF ALLOWED STEPS.
C          THE DEFAULT VALUE (FOR IWORK(1)=0) IS 100000.
C
C   IWORK(2)  SWITCH FOR THE CHOICE OF THE COEFFICIENTS
C          IF IWORK(2).EQ.1  METHOD OF DORMAND AND PRINCE
C          (TABLE 5.2 OF SECTION II.5).
C          AT THE MOMENT THIS IS THE ONLY POSSIBLE CHOICE.
C          THE DEFAULT VALUE (FOR IWORK(2)=0) IS IWORK(2)=1.
C
C   IWORK(3)  SWITCH FOR PRINTING ERROR MESSAGES
C          IF IWORK(3).LT.0 NO MESSAGES ARE BEING PRINTED
```

```
C            IF IWORK(3).GT.0 MESSAGES ARE PRINTED WITH
C              WRITE (IWORK(3),*) ...
C            DEFAULT VALUE (FOR IWORK(3)=0) IS IWORK(3)=6
C
C    IWORK(4)  TEST FOR STIFFNESS IS ACTIVATED AFTER STEP NUMBER
C            J*IWORK(4) (J INTEGER), PROVIDED IWORK(4).GT.0.
C            FOR NEGATIVE IWORK(4) THE STIFFNESS TEST IS
C            NEVER ACTIVATED; DEFAULT VALUE IS IWORK(4)=1000
C
C    IWORK(5)  = NRDENS = NUMBER OF COMPONENTS, FOR WHICH DENSE OUTPUT
C            IS REQUIRED (EITHER BY "SOLOUT" OR BY "FCN");
C            DEFAULT VALUE (FOR IWORK(5)=0) IS IWORK(5)=N;
C            FOR   0 < NRDENS < N   THE COMPONENTS (FOR WHICH DENSE
C            OUTPUT IS REQUIRED) HAVE TO BE SPECIFIED IN
C            ICONT(2),...,ICONT(NRDENS+1);
C            FOR  NRDENS=N  THIS IS DONE BY THE CODE.
C
C    IWORK(6)  = NGRID = NUMBER OF PRESCRIBED POINTS IN THE
C            INTEGRATION INTERVAL WHICH HAVE TO BE GRID-POINTS
C            IN THE INTEGRATION. USUALLY, AT THESE POINTS THE
C            SOLUTION OR ONE OF ITS DERIVATIVE HAS A DISCONTINUITY.
C            DEFINE THESE POINTS IN WORK(11),...,WORK(10+NGRID)
C            DEFAULT VALUE:  IWORK(6)=0
C
C-----------------------------------------------------------------------
C
C     OUTPUT PARAMETERS
C     -----------------
C     X         X-VALUE FOR WHICH THE SOLUTION HAS BEEN COMPUTED
C               (AFTER SUCCESSFUL RETURN X=XEND).
C
C     Y(N)       NUMERICAL SOLUTION AT X
C
C     H         PREDICTED STEP SIZE OF THE LAST ACCEPTED STEP
C
C     IDID       REPORTS ON SUCCESSFULNESS UPON RETURN:
C                IDID= 1  COMPUTATION SUCCESSFUL,
C                IDID= 2  COMPUT. SUCCESSFUL (INTERRUPTED BY SOLOUT)
C                IDID=-1  INPUT IS NOT CONSISTENT,
C                IDID=-2  LARGER NMAX IS NEEDED,
C                IDID=-3  STEP SIZE BECOMES TOO SMALL.
C                IDID=-4  PROBLEM IS PROBABLY STIFF (INTERRUPTED).
C                IDID=-5  COMPUT. INTERRUPTED BY YLAG
C
C-----------------------------------------------------------------------
C *** *** *** *** *** *** *** *** *** *** *** *** ***
C        DECLARATIONS
C *** *** *** *** *** *** *** *** *** *** *** *** ***
     IMPLICIT REAL*8 (A-H,O-Z)
     DIMENSION Y(N),ATOL(1),RTOL(1),WORK(LWORK),IWORK(LIWORK)
     LOGICAL ARRET
     EXTERNAL FCN,SOLOUT
     COMMON/STATRE/NFCN,NSTEP,NACCPT,NREJCT
C --- COMMON STATRE CAN BE INSPECTED FOR STATISTICAL PURPOSES:
C ---    NFCN      NUMBER OF FUNCTION EVALUATIONS
C ---    NSTEP     NUMBER OF COMPUTED STEPS
```

```
C ---    NACCPT    NUMBER OF ACCEPTED STEPS
C ---    NREJCT    NUMBER OF REJECTED STEPS (AFTER AT LEAST ONE STEP
C                  HAS BEEN ACCEPTED)
CC 50000 = LRCONT
      COMMON /CORER/RCONT(50000)
CC 4 = NRDENSE + 1 = N + 1
      COMMON /COREI/NRDS,ICONT(3)
      COMMON /POSITS/X0BEG,UROUND,HMAX,LAST,IPOS,IRTRN,IDIF,MXST,IPRINT
C *** *** *** *** *** *** ***
C       SETTING THE PARAMETERS
C *** *** *** *** *** *** ***
      NFCN=0
      NSTEP=0
      NACCPT=0
      NREJCT=0
      ARRET=.FALSE.
C -------- IPRINT FOR MONITORING THE PRINTING
      IF(IWORK(3).EQ.0)THEN
        IPRINT=6
      ELSE
        IPRINT=IWORK(3)
      END IF
C -------- NMAX , THE MAXIMAL NUMBER OF STEPS -----
      IF(IWORK(1).EQ.0)THEN
        NMAX=100000
      ELSE
        NMAX=IWORK(1)
        IF(NMAX.LE.0)THEN
          IF (IPRINT.GT.0) WRITE(IPRINT,*)
     &         ' WRONG INPUT IWORK(1)=',IWORK(1)
          ARRET=.TRUE.
        END IF
      END IF
C -------- METH   COEFFICIENTS OF THE METHOD
      IF(IWORK(2).EQ.0)THEN
        METH=1
      ELSE
        METH=IWORK(2)
        IF(METH.LE.0.OR.METH.GE.2)THEN
          IF (IPRINT.GT.0) WRITE(IPRINT,*)
     &         ' CURIOUS INPUT IWORK(2)=',IWORK(2)
          ARRET=.TRUE.
        END IF
      END IF
C -------- NSTIFF   PARAMETER FOR STIFFNESS DETECTION
      NSTIFF=IWORK(4)
      IF (NSTIFF.EQ.0) NSTIFF=1000
      IF (NSTIFF.LT.0) NSTIFF=NMAX+10
C -------- NRDENS   NUMBER OF DENSE OUTPUT COMPONENTS
      NRDENS=IWORK(5)
      IF(NRDENS.LT.0.OR.NRDENS.GT.N)THEN
        IF (IPRINT.GT.0) WRITE(IPRINT,*)
     &         ' CURIOUS INPUT IWORK(5)=',IWORK(5)
        ARRET=.TRUE.
      ELSE
        IF (NRDENS.EQ.0) NRDENS=N
```

```
C --------- CONTROL OF LENGTH OF COMMON BLOCK "CORER" -------
      IF(LRCONT.LT.(5*NRDENS+2))THEN
        IF (IPRINT.GT.0) WRITE(IPRINT,*)
     &   ' INSUFFICIENT STORAGE FOR RCONT, MIN. LRCONT=',5*NRDENS+2
        ARRET=.TRUE.
      END IF
C --------- CONTROL OF LENGTH OF COMMON BLOCK "COREI" -------
      IF(LICONT.LT.(NRDENS+1))THEN
        IF (IPRINT.GT.0) WRITE(IPRINT,*)
     &   ' INSUFFICIENT STORAGE FOR ICONT, MIN. LICONT=',NRDENS+1
        ARRET=.TRUE.
      ELSE
        NRDS=NRDENS
        IF (NRDENS.EQ.N) THEN
          DO 16 I=1,NRDENS
 16         ICONT(I)=I
        END IF
      END IF
    END IF
C -------- NGRID   NUMBER OF PRESCRIBED GRID-POINTS
    NGRID=IWORK(6)
    IF (NGRID.LT.0) NGRID=0
C -------- UROUND   SMALLEST NUMBER SATISFYING 1.D0+UROUND>1.D0
    IF(WORK(1).EQ.0.D0)THEN
      UROUND=2.3D-16
    ELSE
      UROUND=WORK(1)
      IF(UROUND.LE.1.D-35.OR.UROUND.GE.1.D0)THEN
        IF (IPRINT.GT.0) WRITE(IPRINT,*)
     &     ' WHICH MACHINE DO YOU HAVE? YOUR UROUND WAS:',WORK(1)
        ARRET=.TRUE.
      END IF
    END IF
C -------  SAFETY FACTOR -------------
    IF(WORK(2).EQ.0.D0)THEN
      SAFE=0.9D0
    ELSE
      SAFE=WORK(2)
      IF(SAFE.GE.1.D0.OR.SAFE.LE.1.D-4)THEN
        IF (IPRINT.GT.0) WRITE(IPRINT,*)
     &      ' CURIOUS INPUT FOR SAFETY FACTOR WORK(2)=',WORK(2)
        ARRET=.TRUE.
      END IF
    END IF
C -------  FAC1,FAC2     PARAMETERS FOR STEP SIZE SELECTION
    IF(WORK(3).EQ.0.D0)THEN
      FAC1=0.2D0
    ELSE
      FAC1=WORK(3)
    END IF
    IF(WORK(4).EQ.0.D0)THEN
      FAC2=10.D0
    ELSE
      FAC2=WORK(4)
    END IF
C --------- BETA FOR STEP CONTROL STABILIZATION -----------
```

```
      IF(WORK(5).EQ.0.D0)THEN
        BETA=0.04D0
      ELSE
        IF(WORK(5).LT.0.D0)THEN
          BETA=0.D0
        ELSE
          BETA=WORK(5)
          IF(BETA.GT.0.2D0)THEN
            IF (IPRINT.GT.0) WRITE(IPRINT,*)
     &        ' CURIOUS INPUT FOR BETA: WORK(5)=',WORK(5)
          ARRET=.TRUE.
        END IF
        END IF
      END IF
C -------- MAXIMAL STEP SIZE
      IF(WORK(6).EQ.0.D0)THEN
        HMAX=XEND-X
      ELSE
        HMAX=WORK(6)
      END IF
C -------- INITIAL STEP SIZE
      H=WORK(7)
C -------- GRID WITH DISCONTINUITIES
      XURO=100*UROUND*ABS(XEND)
      IF (WORK(10+NGRID)-XEND.GE.XURO) THEN
        IF(IPRINT.GT.0) WRITE(IPRINT,*)
     &        ' WORK(10+NGRID) HAS TO BE <= XEND'
        ARRET=.TRUE.
      END IF
      IF (ABS(WORK(10+NGRID)-XEND).GE.XURO) NGRID=NGRID+1
      WORK(10+NGRID)=XEND
C ------- PREPARE THE ENTRY-POINTS FOR THE ARRAYS IN WORK -----
      IEGR=11
      IEY1=11+NGRID
      IEK1=IEY1+N
      IEK2=IEK1+N
      IEK3=IEK2+N
      IEK4=IEK3+N
      IEK5=IEK4+N
      IEK6=IEK5+N
      IEYS=IEK6+N
C ------ TOTAL STORAGE REQUIREMENT -----------
      ISTORE=IEYS+N-1
      IF(ISTORE.GT.LWORK)THEN
        IF (IPRINT.GT.0) WRITE(IPRINT,*)
     &   ' INSUFFICIENT STORAGE FOR WORK, MIN. LWORK=',ISTORE
        ARRET=.TRUE.
      END IF
      ISTORE=10
      IF(ISTORE.GT.LIWORK)THEN
        IF (IPRINT.GT.0) WRITE(IPRINT,*)
     &   ' INSUFFICIENT STORAGE FOR IWORK, MIN. LIWORK=',ISTORE
        ARRET=.TRUE.
      END IF
C ------ WHEN A FAIL HAS OCCURED, WE RETURN WITH IDID=-1
      IF (ARRET) THEN
```

```
        IDID=-1
        RETURN
      END IF
C -------- CALL TO CORE INTEGRATOR ------------
      IDIF=5*NRDENS+2
      MXST=LRCONT/IDIF
      CALL RETCOR(N,FCN,X,Y,XEND,H,RTOL,ATOL,ITOL,
     &   SOLOUT,IOUT,IDID,NMAX,METH,NSTIFF,SAFE,BETA,FAC1,FAC2,NGRID,
     &   WORK(IEY1),WORK(IEK1),WORK(IEK2),WORK(IEK3),WORK(IEK4),
     &   WORK(IEK5),WORK(IEK6),WORK(IEYS),WORK(IEGR))
      WORK(7)=H
C ----------- RETURN -----------
      RETURN
      END
C
C
C
C  ----- ... AND HERE IS THE CORE INTEGRATOR  ----------
C
      SUBROUTINE RETCOR(N,FCN,X,Y,XEND,H,RTOL,ATOL,ITOL,
     &   SOLOUT,IOUT,IDID,NMAX,METH,NSTIFF,SAFE,BETA,FAC1,FAC2,NGRID,
     &   Y1,K1,K2,K3,K4,K5,K6,YSTI,GRID)
C ----------------------------------------------------------
C     CORE INTEGRATOR FOR RETARD
C     PARAMETERS SAME AS IN RETARD WITH WORKSPACE ADDED
C ----------------------------------------------------------
C        DECLARATIONS
C ----------------------------------------------------------
      IMPLICIT REAL*8 (A-H,O-Z)
      REAL*8 Y(N),Y1(N),K1(N),K2(N),K3(N),K4(N),K5(N),K6(N),YSTI(N)
      REAL*8 GRID(NGRID),ATOL(1),RTOL(1)
      LOGICAL REJECT,LAST
      EXTERNAL FCN
      COMMON/STATRE/NFCN,NSTEP,NACCPT,NREJCT
CC 50000 = LRCONT
      COMMON /CORER/CONT(50000)
CC 4 = NRDENSE + 1 = N + 1
      COMMON /COREI/NRD,ICOMP(3)
      COMMON /POSITS/X0BEG,UROUND,HMAX,IACT,IPOS,IRTRN,IDIF,MXST,IPRINT
C *** *** *** *** *** *** ***
C  INITIALISATIONS
C *** *** *** *** *** *** ***
      IF (METH.EQ.1) CALL CDOPRI(C2,C3,C4,C5,E1,E3,E4,E5,E6,E7,
     &                A21,A31,A32,A41,A42,A43,A51,A52,A53,A54,
     &                A61,A62,A63,A64,A65,A71,A73,A74,A75,A76,
     &                D1,D3,D4,D5,D6,D7)
      FACOLD=1.D-4
      EXPO1=0.2D0-BETA*0.75D0
      FACC1=1.D0/FAC1
      FACC2=1.D0/FAC2
      POSNEG=SIGN(1.D0,XEND-X)
C --- INITIAL PREPARATIONS
      IACT=1
      IPOS=1
      X0BEG=X
      XEND=GRID(1)
```

```
      IGRID=1
      UROUND=10*UROUND
      DO 3 I=0,MXST-1
    3    CONT(1+IDIF*I)=X
      ATOLI=ATOL(1)
      RTOLI=RTOL(1)
      LAST=.FALSE.
      HLAMB=0.D0
      IASTI=0
      HMAX=ABS(HMAX)
      IRTRN=2
      CALL FCN(N,X,Y,K1)
      IRTRN=1
      IORD=5
      IF (H.EQ.0.D0) H=HINIT(N,FCN,X,Y,XEND,POSNEG,K1,K2,K3,IORD,
     &                    HMAX,ATOL,RTOL,ITOL)
      NFCN=NFCN+2
      REJECT=.FALSE.
      XOLD=X
      IF (IOUT.NE.0) THEN
         CALL SOLOUT(NACCPT+1,XOLD,X,Y,N,IRTRN)
         IF (IRTRN.LT.0) GOTO 79
      END IF
C --- BASIC INTEGRATION STEP
    1 CONTINUE
      IF (NSTEP.GT.NMAX) GOTO 78
      IF (ABS(H).LE.ABS(X)*UROUND)GOTO 77
      IF ((X+1.01D0*H-XEND)*POSNEG.GT.0.D0) THEN
        H=XEND-X
        LAST=.TRUE.
      ELSE
        IF ((X+1.8D0*H-XEND)*POSNEG.GT.0.D0) H=(XEND-X)*0.55D0
      END IF
      NSTEP=NSTEP+1
C --- THE FIRST 6 STAGES
      DO 22 I=1,N
   22 Y1(I)=Y(I)+H*A21*K1(I)
      CALL FCN(N,X+C2*H,Y1,K2)
      DO 23 I=1,N
   23 Y1(I)=Y(I)+H*(A31*K1(I)+A32*K2(I))
      CALL FCN(N,X+C3*H,Y1,K3)
      DO 24 I=1,N
   24 Y1(I)=Y(I)+H*(A41*K1(I)+A42*K2(I)+A43*K3(I))
      CALL FCN(N,X+C4*H,Y1,K4)
      DO 25 I=1,N
   25 Y1(I)=Y(I)+H*(A51*K1(I)+A52*K2(I)+A53*K3(I)+A54*K4(I))
      CALL FCN(N,X+C5*H,Y1,K5)
      DO 26 I=1,N
   26 YSTI(I)=Y(I)+H*(A61*K1(I)+A62*K2(I)+A63*K3(I)+A64*K4(I)+A65*K5(I))
      XPH=X+H
      CALL FCN(N,XPH,YSTI,K6)
      DO 27 I=1,N
   27 Y1(I)=Y(I)+H*(A71*K1(I)+A73*K3(I)+A74*K4(I)+A75*K5(I)+A76*K6(I))
      IRTRN=1
      CALL FCN(N,XPH,Y1,K2)
C ------  PREPARE DENSE OUTPUT
```

```
          NRDL=4*NRD+IACT
          DO 40 J=1,NRD
          I=ICOMP(J)
          CONT(NRDL+J)=H*(D1*K1(I)+D3*K3(I)+D4*K4(I)+D5*K5(I)
     &                +D6*K6(I)+D7*K2(I))
 40       CONTINUE
C -------
      DO 28 I=1,N
 28   K4(I)=(E1*K1(I)+E3*K3(I)+E4*K4(I)+E5*K5(I)+E6*K6(I)+E7*K2(I))*H
      NFCN=NFCN+6
C ------ ERROR ESTIMATION
      ERR=0.D0
      IF (ITOL.EQ.0) THEN
        DO 41 I=1,N
        SK=ATOLI+RTOLI*MAX(ABS(Y(I)),ABS(Y1(I)))
 41     ERR=ERR+(K4(I)/SK)**2
      ELSE
        DO 42 I=1,N
        SK=ATOL(I)+RTOL(I)*MAX(ABS(Y(I)),ABS(Y1(I)))
 42     ERR=ERR+(K4(I)/SK)**2
      END IF
      ERR=SQRT(ERR/N)
C --- COMPUTATION OF HNEW
      FAC11=ERR**EXPO1
C --- LUND-STABILIZATION
      FAC=FAC11/FACOLD**BETA
C --- WE REQUIRE  FAC1 <= HNEW/H <= FAC2
      FAC=MAX(FACC2,MIN(FACC1,FAC/SAFE))
      HNEW=H/FAC
      IF(ERR.LE.1.D0)THEN
C --- STEP IS ACCEPTED
        FACOLD=MAX(ERR,1.0D-4)
        NACCPT=NACCPT+1
C ------- STIFFNESS DETECTION
        IF (MOD(NACCPT,NSTIFF).EQ.0.OR.IASTI.GT.0) THEN
          STNUM=0.D0
          STDEN=0.D0
          DO 64 I=1,N
            STNUM=STNUM+(K2(I)-K6(I))**2
            STDEN=STDEN+(Y1(I)-YSTI(I))**2
 64       CONTINUE
          IF (STDEN.GT.0.D0) HLAMB=H*SQRT(STNUM/STDEN)
          IF (HLAMB.GT.3.25D0) THEN
            NONSTI=0
            IASTI=IASTI+1
            IF (IASTI.EQ.15) THEN
              IF (IPRINT.GT.0) WRITE (IPRINT,*)
     &            ' THE PROBLEM SEEMS TO BECOME STIFF AT X = ',X
              IF (IPRINT.LE.0) GOTO 76
            END IF
          ELSE
            NONSTI=NONSTI+1
            IF (NONSTI.EQ.6) IASTI=0
          END IF
        END IF
C -------   COMPUTE DENSE OUTPUT
```

```
          DO 43 J=1,NRD
          I=ICOMP(J)
          YDIFF=Y1(I)-Y(I)
          BSPL=H*K1(I)-YDIFF
          CONT(IACT+J)=Y(I)
          CONT(IACT+NRD+J)=YDIFF
          CONT(IACT+2*NRD+J)=BSPL
          CONT(IACT+3*NRD+J)=-H*K2(I)+YDIFF-BSPL
 43       CONTINUE
          CONT(IACT)=X
          IACT=IACT+IDIF
          CONT(IACT-1)=H
          IF (IACT+IDIF-1.GT.MXST*IDIF) IACT=1
C ------
        DO 44 I=1,N
        K1(I)=K2(I)
 44     Y(I)=Y1(I)
        XOLD=X
        X=XPH
        IF (IRTRN.EQ.3) THEN
          IRTRN=4
          CALL FCN(N,X,Y,K1)
          NFCN=NFCN+1
          IRTRN=1
        END IF
        IF (IOUT.NE.0) THEN
          CALL SOLOUT(NACCPT+1,XOLD,X,Y,N,IRTRN)
          IF (IRTRN.LT.0) GOTO 79
        END IF
C ------- NORMAL EXIT
        IF (LAST) THEN
          IF (IGRID.EQ.NGRID) THEN
            H=HNEW
            IDID=1
            RETURN
          ELSE
            IGRID=IGRID+1
            LAST=.FALSE.
            XEND=GRID(IGRID)
            HNEW=0.9D0*HNEW
          END IF
        END IF
        IF(ABS(HNEW).GT.HMAX)HNEW=POSNEG*HMAX
        IF(REJECT)HNEW=POSNEG*MIN(ABS(HNEW),ABS(H))
        REJECT=.FALSE.
      ELSE
C --- STEP IS REJECTED
        IF (IRTRN.LT.0) GOTO 79
        HNEW=H/MIN(FACC1,FAC11/SAFE)
        REJECT=.TRUE.
        IF(NACCPT.GE.1)NREJCT=NREJCT+1
        LAST=.FALSE.
      END IF
      H=HNEW
      IF (IRTRN.LT.0) GOTO 75
      GOTO 1
```

```
C --- FAIL EXIT
  75  CONTINUE
      IDID=-5
      RETURN
  76  CONTINUE
      IDID=-4
      RETURN
  77  CONTINUE
      IF (IPRINT.GT.0) WRITE(IPRINT,979)X
      IF (IPRINT.GT.0) WRITE(IPRINT,*)' STEP SIZE T0O SMALL, H=',H
      IDID=-3
      RETURN
  78  CONTINUE
      IF (IPRINT.GT.0) WRITE(IPRINT,979)X
      IF (IPRINT.GT.0) WRITE(IPRINT,*)
     &    ' MORE THAN NMAX =',NMAX,'STEPS ARE NEEDED'
      IDID=-2
      RETURN
  79  CONTINUE
      IF (IPRINT.GT.0) IDID=2
C          WRITE(IPRINT,979)X
 979  FORMAT(' EXIT OF RETARD AT X=',E18.4)

      RETURN
      END
C
      FUNCTION HINIT(N,FCN,X,Y,XEND,POSNEG,F0,F1,Y1,IORD,
     &                    HMAX,ATOL,RTOL,ITOL)
C ---------------------------------------------------------
C ----  COMPUTATION OF AN INITIAL STEP SIZE GUESS
C ---------------------------------------------------------
      IMPLICIT REAL*8 (A-H,O-Z)
      DIMENSION Y(N),Y1(N),F0(N),F1(N),ATOL(1),RTOL(1)
C ---- COMPUTE A FIRST GUESS FOR EXPLICIT EULER AS
C ----   H = 0.01 * NORM (Y0) / NORM (F0)
C ---- THE INCREMENT FOR EXPLICIT EULER IS SMALL
C ---- COMPARED TO THE SOLUTION
      DNF=0.0D0
      DNY=0.0D0
      ATOLI=ATOL(1)
      RTOLI=RTOL(1)
      IF (ITOL.EQ.0) THEN
        DO 10 I=1,N
        SK=ATOLI+RTOLI*ABS(Y(I))
        DNF=DNF+(F0(I)/SK)**2
  10    DNY=DNY+(Y(I)/SK)**2
      ELSE
        DO 11 I=1,N
        SK=ATOL(I)+RTOL(I)*ABS(Y(I))
        DNF=DNF+(F0(I)/SK)**2
  11    DNY=DNY+(Y(I)/SK)**2
      END IF
      IF (DNF.LE.1.D-10.OR.DNY.LE.1.D-10) THEN
        H=1.0D-6
      ELSE
        H=SQRT(DNY/DNF)*0.01D0
```

```
      END IF
      H=MIN(H,HMAX)
      H=SIGN(H,POSNEG)
C ---- PERFORM AN EXPLICIT EULER STEP
      DO 12 I=1,N
 12   Y1(I)=Y(I)+H*F0(I)
      CALL FCN(N,X+H,Y1,F1)
C ---- ESTIMATE THE SECOND DERIVATIVE OF THE SOLUTION
      DER2=0.0D0
      IF (ITOL.EQ.0) THEN
        DO 15 I=1,N
        SK=ATOLI+RTOLI*ABS(Y(I))
 15     DER2=DER2+((F1(I)-F0(I))/SK)**2
      ELSE
        DO 16 I=1,N
        SK=ATOL(I)+RTOL(I)*ABS(Y(I))
 16     DER2=DER2+((F1(I)-F0(I))/SK)**2
      END IF
      DER2=SQRT(DER2)/H
C ---- STEP SIZE IS COMPUTED SUCH THAT
C ----  H**IORD * MAX ( NORM (F0), NORM (DER2)) = 0.01
      DER12=MAX(ABS(DER2),SQRT(DNF))
      IF (DER12.LE.1.D-15) THEN
        H1=MAX(1.0D-6,ABS(H)*1.0D-3)
      ELSE
        H1=(0.01D0/DER12)**(1.D0/IORD)
      END IF
      H=MIN(100*H,H1,HMAX)
      HINIT=SIGN(H,POSNEG)
      RETURN
      END
C
      FUNCTION YLAG(II,X,PHI)
C -------------------------------------------------------------
C     THIS FUNCTION CAN BE USED FOR CONINUOUS OUTPUT IN CONECTION
C     WITH THE OUTPUT-SUBROUTINE FOR RETARD. IT PROVIDES AN
C     APPROXIMATION TO THE I-TH COMPONENT OF THE SOLUTION AT X.
C -------------------------------------------------------------
      IMPLICIT REAL*8 (A-H,O-Z)
           INTEGER :: PROBLEM=0
CC 50000 = LRCONT
      COMMON /CORER/CON(50000)
CC 4 = NRDENSE + 1 = N + 1
      COMMON /COREI/ND,ICOMP(3)
      COMMON /POSITS/X0,UR4,HMAX,IACT,IPOS,IRTRN,IDIF,MXST,IPRINT
C ----- INITIAL PHASE
      COMPAR=UR4*MAX(ABS(X),ABS(X0))
        IF (X-X0.LE.COMPAR) THEN
          IF (IRTRN.LE.3) THEN
            YLAG=PHI(II,X)
            IF (IRTRN.EQ.2) HMAX=MIN(HMAX,X0-X)
            IF (X0-X.LE.COMPAR) IRTRN=3
            RETURN
          ELSE
            IF (X0-X.GT.COMPAR) THEN
              YLAG=PHI(II,X)
```

```
            RETURN
          END IF
        END IF
      END IF
C ----- COMPUTE PLACE OF II-TH COMPONENT
      I=0
      DO 5 J=1,ND
      IF (ICOMP(J).EQ.II) I=J
    5 CONTINUE
      IF (I.EQ.0) THEN
        IF (IPRINT.GT.0) WRITE (IPRINT,*)
     &         ' NO DENSE OUTPUT AVAILABLE FOR COMP.',II
        RETURN
      END IF
C ----- COMPUTE THE POSITION OF X
      IF (X-CON(IACT).LT.-COMPAR) THEN
        IF (IPRINT.GT.0) WRITE (IPRINT,*)
     &         ' MEMORY FULL, MXST = ',MXST
        IRTRN=-1
        RETURN
      END IF
      INEXT=IACT-IDIF
      IF (INEXT.LT.1) INEXT=(MXST-1)*IDIF+1
      XRIGHT=CON(INEXT)+CON(INEXT+IDIF-1)
      IF (X-XRIGHT.GT.UR4*MAX(ABS(X),ABS(XRIGHT))) THEN
        IF (IPRINT.GT.0) IRTRN=-1
C            WRITE (IPRINT,*)
C     &         ' DONT USE ADVANCED ARGUMENTS '
C          PROBLEM = PROBLEM + 1
C         WRITE(*,*) PROBLEM

        RETURN
      END IF
    1 CONTINUE
      IF (X-CON(IPOS).LT.-COMPAR) THEN
        IPOS=IPOS-IDIF
        IF (IPOS.LT.1) IPOS=(MXST-1)*IDIF+1
        GOTO 1
      END IF
    2 CONTINUE
      INEXT=IPOS+IDIF
      IF (INEXT.GT.(MXST-1)*IDIF+1) INEXT=1
      IF (X.GT.CON(INEXT).AND.INEXT.NE.IACT) THEN
        IPOS=INEXT
        GOTO 2
      END IF
C ----- COMPUTE DESIRED APPROXIMATION
      THETA=(X-CON(IPOS))/CON(IPOS+IDIF-1)
      THETA1=1.D0-THETA
      I=I+IPOS
      YLAG=CON(I)+THETA*(CON(ND+I)+THETA1*(CON(2*ND+I)+THETA*
     &         (CON(3*ND+I)+THETA1*CON(4*ND+I))))
      RETURN
      END
C
      SUBROUTINE CDOPRI(C2,C3,C4,C5,E1,E3,E4,E5,E6,E7,
```

```
     &                A21,A31,A32,A41,A42,A43,A51,A52,A53,A54,
     &                A61,A62,A63,A64,A65,A71,A73,A74,A75,A76,
     &                D1,D3,D4,D5,D6,D7)
C -------------------------------------------------------------
C     RUNGE-KUTTA COEFFICIENTS OF DORMAND AND PRINCE (1980)
C -------------------------------------------------------------
      IMPLICIT REAL*8 (A-H,O-Z)
      C2=0.2D0
      C3=0.3D0
      C4=0.8D0
      C5=8.D0/9.D0
      A21=0.2D0
      A31=3.D0/40.D0
      A32=9.D0/40.D0
      A41=44.D0/45.D0
      A42=-56.D0/15.D0
      A43=32.D0/9.D0
      A51=19372.D0/6561.D0
      A52=-25360.D0/2187.D0
      A53=64448.D0/6561.D0
      A54=-212.D0/729.D0
      A61=9017.D0/3168.D0
      A62=-355.D0/33.D0
      A63=46732.D0/5247.D0
      A64=49.D0/176.D0
      A65=-5103.D0/18656.D0
      A71=35.D0/384.D0
      A73=500.D0/1113.D0
      A74=125.D0/192.D0
      A75=-2187.D0/6784.D0
      A76=11.D0/84.D0
      E1=71.D0/57600.D0
      E3=-71.D0/16695.D0
      E4=71.D0/1920.D0
      E5=-17253.D0/339200.D0
      E6=22.D0/525.D0
      E7=-1.D0/40.D0
C ---- DENSE OUTPUT OF SHAMPINE (1986)
      D1=-12715105075.D0/11282082432.D0
      D3=87487479700.D0/32700410799.D0
      D4=-10690763975.D0/1880347072.D0
      D5=701980252875.D0/199316789632.D0
      D6=-1453857185.D0/822651844.D0
      D7=69997945.D0/29380423.D0
      RETURN
      END
```

C.1.4 TRAP_RULE.F90 (Original code)

```
SUBROUTINE TRAP_RULE (X, Y, NPOINTS, START, AREA)

IMPLICIT NONE

REAL*8, INTENT(IN) :: X(*), Y(*), START
INTEGER, INTENT(IN) :: NPOINTS
```

```
REAL*8, INTENT(OUT) :: AREA
REAL*8 :: A, B, WEIGHT
INTEGER :: i
EXTERNAL :: WEIGHT

AREA=0D0
DO i=1, NPOINTS-1
IF (X(i)>=START) THEN
A=WEIGHT(X(i))
B=WEIGHT(X(i+1))
AREA=AREA+(((Y(i)+Y(i+1))/2D0)*(X(i+1)-X(i)))
END IF
END DO

END SUBROUTINE TRAP_RULE
```

C.2 Fortran subroutines for Chapter 3

C.2.1 MAIN.FOR (Original code)

```
!In driver program:
!
!       parameter (N=3)
!
!in S/R RETARD:
!
!    ( 4 = N + 1)
!
!     COMMON /COREI/NRDS,ICONT(4)
!
!In S/R  RETCOR:
!
!  (4 = N + 1)
!
!     COMMON /COREI/NRD,ICOMP(4)
!
!In S/R  YLAG:
!
!   (4 = N + 1)
!
!     COMMON /COREI/ND,ICOMP(4)
!
compile retard
cfeh dr_retard retard
            USE SHARED
            IMPLICIT REAL*8 (A-H,O-Z)
            REAL :: DURATION(2) !THIS IS SINGLE PERCISION BECAUASE OF INTINSIC
FUNCTION
CC N = NUMBER OF ODEs
       PARAMETER (N=4)
       PARAMETER (NDGL=n,NGRID=11,LWORK=8*NDGL+11+NGRID,LIWORK=10)
       PARAMETER (NRDENS=NDGL,LRCONT=50000,LICONT=NRDENS+1)
       INTEGER :: J, G, POINTSH
            DIMENSION Y(NDGL),WORK(LWORK),IWORK(LIWORK)
```

```
      COMMON/STATRE/NFCN,NSTEP,NACCPT,NREJCT
      COMMON /CORER/RCONT(LRCONT)
      COMMON /COREI/NRDS,ICONT(LICONT)
      EXTERNAL FCN,SOLOUT
C --- OUTPUT ROUTINE IS USED DURING INTEGRATION
      CALL FILE_OPEN !WEIGHT AND EPON FILES SHOULD ONLY OPEN ONCE
           AREAH=0D0     !OPTIMAL AREA AND DOSETIME
           DOSETIMEH=0D0
           K0=EPON(0.1D0)*K1*CLSS
          SEN=((EMAX*(WEIGHT(0.1D0)**0.75D0)*EPON(0.1D0))/(EC50
     &     +EPON(0.1D0)))/HGBIV
           DO J=1,200 !THIS IS THE DOSING OP LOOP
           XP=0D0
           YP1=0D0
           YP2=0D0
           YP3=0D0
           YP4=0D0
           IOUT=1
C --- INITIAL VALUES AND ENDPOINT OF INTEGRATION
      X=0.0D0
      Y(1)=HGBIV
      Y(2)=CLSS
      Y(3)=(K0/K3)
           Y(4)=IVALUE4
      XEND=30.91D0
           DOSETIME=DBLE(J)/10D0+TEVENT+.01D0
           POINTS=0 !INTEGER FOR PVP PLOT
C --- REQUIRED (RELATIVE AND ABSOLUTE) TOLERANCE
      ITOL=0
      RTOL=1.0D-8 !THIS IS A HIGH ERROR TOLERANCE
      ATOL=RTOL
C --- DEFAULT VALUES FOR PARAMETERS
      DO 10 I=1,10
      IWORK(I)=0
 10   WORK(I)=0.D0
C --- SECOND COMPONENT USES RETARDED ARGUMENT
      IWORK(5)=NRDENS
      ICONT(2)=2
C ---  USE AS GRID-POINTS
      IWORK(6)=NGRID
      DO 12 I=1,NGRID-1
 12   WORK(10+I)=I
      WORK(10+NGRID)=20.D0
C --- CALL OF THE SUBROUTINE RETARD
      CALL RETARD(N,FCN,X,Y,XEND,
     &            RTOL,ATOL,ITOL,
     &            SOLOUT,IOUT,
     &            WORK,LWORK,IWORK,LIWORK,LRCONT,LICONT,IDID)
C --- PRINT FINAL SOLUTION
!       WRITE (6,99) Y(1),Y(2),Y(3)
 99     FORMAT(1X,'X = XEND     Y =',3E18.10)
C --- PRINT STATISTICS
!       WRITE (6,91) RTOL,NFCN,NSTEP,NACCPT,NREJCT
 91     FORMAT('     tol=',D8.2,'   fcn=',I5,' step=',I4,
     &           ' accpt=',I4,' rejct=',I3)
       CALL TRAP_RULE(XP, YP1, POINTS, TEVENT, AREA)
```

```
      AREAA(J)=AREA-((XEND-TEVENT)*PPHGBVALUE)
      DOSETIMEA(J)=DOSETIME
      IF(AREA>AREAH)THEN
      AREAH=AREA
      DOSETIMEH=DOSETIME
      XPH=XP
      YP1H=YP1
      YP2H=YP2
      YP4H=YP4
      POINTSH=POINTS
      DO G=1,1000
      EPOX_TOTAL(G)=XEND/1000D0*DBLE(G) !THIS STUFF ADDS SPLINES TOGETHER
      EPOY_TOTAL(G)=EPON(XEND/1000D0*DBLE(G))+EPOX(XEND/1000D0*DBLE(G))
      END DO
      END IF

    END DO

      WRITE(*,*) 'OPTIMAL DOSE TIME', DOSETIMEH
      WRITE(*,*) 'OPTIMAL DOSE AUC', AREAH

      CALL ETIME(DURATION)
      WRITE(*,*) 'PROGRAM RUN TIME', DURATION(1), 'SECONDS'
      WRITE(*,*) '**********************************'
      WRITE(*,*) 'OPTIMAL DOSE TIME AFTER PHLEBOTOMY', DOSETIMEH-TEVENT
      WRITE(*,*) '**********************************'
      CALL ADD_POINTS(XPH, YP1H, POINTSH)
      CALL ADD_CURVE(XPH, YP1H, POINTSH)
      CALL LEFT_LABEL('HGB CONCENTRATION (G/DL)')
      CALL X_LABEL('TIME(DAYS)')
      CALL TITLE (EPOFILE)
      CALL PLOT_IN_AREA(1,4)
      CALL ADD_POINTS(XPH, YP2H, POINTSH)
      CALL ADD_CURVE(XPH, YP2H, POINTSH)
      CALL LEFT_LABEL('EPO CLEARANCE')
      CALL X_LABEL('TIME(DAYS)')
      CALL PLOT_IN_AREA(2,4)
      CALL ADD_POINTS(DOSETIMEA, AREAA, J-1)
      CALL ADD_CURVE(DOSETIMEA, AREAA, J-1)
      CALL LEFT_LABEL('HGB PRODUCTION')
      CALL X_LABEL('TIME EPO DOSE GIVEN')
      CALL BEGIN_X_AT(0D0)
      CALL END_X_AT(35D0)
      CALL TITLE_WITH_VALUE_ADDED('OPTIMAL PP EPO DOSE TIME',
     &                    DOSETIMEH-TEVENT)
      CALL PLOT_IN_AREA(3,4)
      CALL ADD_POINTS(EPOX_TOTAL, EPOY_TOTAL, 1000)
      CALL ADD_CURVE(EPOX_TOTAL, EPOY_TOTAL, 1000)
      CALL LEFT_LABEL('TOTAL EPO CONC (MU/ML)')
      CALL X_LABEL('TIME(DAYS)')
      CALL PLOT_IN_AREA(4,4)
      CALL DISPLAY_PLOT
       STOP
       END

C
```

```
          SUBROUTINE SOLOUT (NR,XOLD,X,Y,N,IRTRN)
          USE SHARED
C --- PRINTS SOLUTION AT EQUIDISTANT OUTPUT-POINTS
      IMPLICIT REAL*8 (A-H,O-Z)
      DIMENSION Y(N)
      EXTERNAL PHI
      COMMON /INTERN/XOUT
      IF (NR.EQ.1) THEN
!        WRITE (6,99) X,Y(1),NR-1
      ELSE
!          WRITE (6,99) X,Y(1),NR-1
      POINTS=POINTS+1
         XP(POINTS)=X
         YP1(POINTS)=Y(1)
         YP2(POINTS)=Y(2)
         YP3(POINTS)=Y(3)
         YP4(POINTS)=Y(4)

          END IF
 99    FORMAT(1X,'X =',F6.2,'    Y =',E18.10,'    NSTEP =',I4)
      RETURN
      END
C
      SUBROUTINE FCN(N,X,Y,F)
      USE SHARED
          IMPLICIT REAL*8 (A-H,O-Z)
      DIMENSION Y(N),F(N)
      EXTERNAL PHI

          IF (X>TEVENT) THEN
          HGBPP=(HGBIV-PHLEVALUE)*EXP(-SEN*(X-TEVENT))
          ELSE
          HGBPP=HGBIV
          END IF

          IF (X>LAGTIME) THEN !SET EPO SPLINE WITH LAG
          EPONLAG=EPON(X-LAGTIME)
          ELSE
          EPONLAG=EPON(0.1D0)
          END IF
      !LAG VARIABLES
      Y2L1=YLAG(2,X-LAGTIME,PHI)
      Y4L1=YLAG(4,X-LAGTIME,PHI)

         !WRITE(*,*) Y2L1

          IF(X >= TEVENT .AND. X<=TEVENT+0.2D0) THEN
          Y(1)= PPHGBVALUE
          END IF
          IF(X >= DOSETIME .AND. X<=DOSETIME+0.3D0) THEN
          Y(4)=DOSEVALUE
          END IF

          IF (X>LAGTIME) THEN
          EMAXT=EMAX*(WEIGHT(X)**0.75D0)*(Y2L1/CLSS)
```

```
            ELSE
            EMAXT=EMAX*(WEIGHT(X)**0.75D0)
            END IF

      ET = EMAXT*(EPONLAG+Y4L1)/(EC50+(EPONLAG+Y4L1))

            F(1)=ET-SEN*Y(1)
            F(2)=K3*Y(3)-K1*(EPON(X)+Y(4))*Y(2)
            F(3)=K0+K2*MAX(CLSS-Y(2),0D0)-K3*Y(3)
            F(4)=(-(CLL+Y(2))/(V*WEIGHT(X)))*Y(4)

      RETURN
      END
C
      FUNCTION PHI(I,X)
      IMPLICIT REAL*8 (A-H,O-Z)
      IF (I.EQ.2) PHI=0.1D0
      RETURN
      END
```

C.2.2 SHARED.F90 (Original Code)

```
MODULE SHARED


REAL*8,ALLOCATABLE,DIMENSION(:):: WX, WY, EPONX, EPONY
INTEGER :: WEIGHTPOINTS, EPONPOINTS
!SET PARAMETERS FOR SUBJECT DOSING OPTIMIZATION

!CHARACTER*256 :: WEIGHTFILE='50326WEIGHT.TXT', EPOFILE='50326EPO.DAT' !70016
!
!REAL*8 :: EMAX=0.037860D0
!REAL*8 :: EC50=43.641
!REAL*8 :: K3=0.29708D0
!REAL*8 :: K1=0.0071638D0
!REAL*8 :: K2=4.4907D0
!REAL*8 :: LAGTIME=1.8573D0
!REAL*8 :: TEVENT=6.9494D0
!REAL*8 :: PHLEVALUE=2.3906D0
!REAL*8 :: SEN
!REAL*8 :: CLSS=61.2D0
!REAL*8 :: HGBIV=6.8D0
!REAL*8 :: K0
!REAL*8 :: CLL=7.4D0
!REAL*8 :: V=1D0 !THIS IS VOLUME PER KG
!REAL*8 :: PPHGBVALUE=4.4D0 !POST PHLE HGB VALUE
!REAL*8 :: DOSEVALUE=200D0 !CONC CHANGE MUST BE POSITIVE
!!REAL*8 :: DOSEVALUE=0D0
!REAL*8 :: IVALUE4=0D0
!
!CHARACTER*256 :: WEIGHTFILE='60124WEIGHT.TXT', EPOFILE='60124EPO.DAT' !70016
!
!REAL*8 :: EMAX=0.067961D0
!REAL*8 :: EC50=40.276
!REAL*8 :: K3=0.67307D0
```

```
!REAL*8 :: K1=0.013249D0
!REAL*8 :: K2=15.337D0
!REAL*8 :: LAGTIME=0.54430D0
!REAL*8 :: TEVENT=6.8919D0
!REAL*8 :: PHLEVALUE=6.6633D0
!REAL*8 :: SEN
!REAL*8 :: CLSS=42.074D0
!REAL*8 :: HGBIV=10.8D0
!REAL*8 :: K0
!REAL*8 :: CLL=7.4D0
!REAL*8 :: V=1D0 !THIS IS VOLUME PER KG
!REAL*8 :: PPHGBVALUE=4.4D0 !POST PHLE HGB VALUE
!REAL*8 :: DOSEVALUE=200D0 !CONC CHANGE MUST BE POSITIVE
!!REAL*8 :: DOSEVALUE=0D0
!REAL*8 :: IVALUE4=0D0
!
!CHARACTER*256 :: WEIGHTFILE='60210WEIGHT.TXT', EPOFILE='60210EPO.DAT' !70016
!
!REAL*8 :: EMAX=0.017490D0
!REAL*8 :: EC50=45.555
!REAL*8 :: K3=0.23465D0
!REAL*8 :: K1=0.0051273D0
!REAL*8 :: K2=24.658D0
!REAL*8 :: LAGTIME=0.27407D0
!REAL*8 :: TEVENT=5.8280D0
!REAL*8 :: PHLEVALUE=6.6786D0
!REAL*8 :: SEN
!REAL*8 :: CLSS=35.07D0
!REAL*8 :: HGBIV=11.3D0
!REAL*8 :: K0
!REAL*8 :: CLL=7.4D0
!REAL*8 :: V=1D0 !THIS IS VOLUME PER KG
!REAL*8 :: PPHGBVALUE=4.4D0 !POST PHLE HGB VALUE
!REAL*8 :: DOSEVALUE=200D0 !CONC CHANGE MUST BE POSITIVE
!!REAL*8 :: DOSEVALUE=0D0
!REAL*8 :: IVALUE4=0D0

!CHARACTER*256 :: WEIGHTFILE='70015WEIGHT.TXT', EPOFILE='70015EPO.DAT' !70015
!
!REAL*8 :: EMAX=0.057203D0
!REAL*8 :: EC50=39.417
!REAL*8 :: K3=0.72206D0
!REAL*8 :: K1=0.013827D0
!REAL*8 :: K2=23.662D0
!REAL*8 :: LAGTIME=0.19295D0
!REAL*8 :: TEVENT=6.04D0
!REAL*8 :: PHLEVALUE=5.95D0
!REAL*8 :: SEN
!REAL*8 :: CLSS=44.07D0
!REAL*8 :: HGBIV=10.3D0
!REAL*8 :: K0
!REAL*8 :: CLL=7.4D0
!REAL*8 :: V=1D0 !THIS IS VOLUME PER KG
!REAL*8 :: PPHGBVALUE=4.4D0 !POST PHLE HGB VALUE
!REAL*8 :: DOSEVALUE=200D0 !CONC CHANGE MUST BE POSITIVE
!!REAL*8 :: DOSEVALUE=0D0
```

```
!REAL*8 :: IVALUE4=0D0

CHARACTER*256 :: WEIGHTFILE='70016WEIGHT.TXT', EPOFILE='70016EPO.DAT' !70016

REAL*8 :: EMAX=0.050028D0
REAL*8 :: EC50=27.436D0
REAL*8 :: K3=0.65862D0
REAL*8 :: K1=0.011527D0
REAL*8 :: K2=21.245D0
REAL*8 :: LAGTIME=0.35610D0
REAL*8 :: TEVENT=6.0084D0
REAL*8 :: PHLEVALUE=7.3839D0
REAL*8 :: SEN
REAL*8 :: CLSS=45.67D0
REAL*8 :: HGBIV=11.7D0
REAL*8 :: K0
REAL*8 :: CLL=7.4D0
REAL*8 :: V=1D0 !THIS IS VOLUME PER KG
REAL*8 :: PPHGBVALUE=4.4D0 !POST PHLE HGB VALUE
REAL*8 :: DOSEVALUE=200D0 !CONC CHANGE MUST BE POSITIVE
!REAL*8 :: DOSEVALUE=0D0
REAL*8 :: IVALUE4=0D0

REAL*8 :: DOSETIME
INTEGER :: POINTS !FOR PVP PLOT
REAL*8 :: XP(1000), XPH(1000), YP1(1000), YP2(1000), YP3(1000), YP4(1000) !ALL OUTPUT
FOR PLOTTING
REAL*8 :: AREAA(1000), DOSETIMEA(1000), YP1H(1000), YP2H(1000), YP4H(1000)
REAL*8 :: EPOX_TOTAL(1000), EPOY_TOTAL(1000)

END MODULE SHARED

REAL*8 FUNCTION WEIGHT(T) !SHEEP WEIGHT(KG) SPLINE
USE SHARED !HAVE WEIGHTPOINTS, WX AND WY SHARED SO FILE ONLY OPENS ONCE
IMPLICIT NONE
REAL*8, INTENT(IN) :: T
REAL*8 :: WEIGHTVALUE, WEIGHTVALUEDIR !LOCAL VARIABLES
CALL spline_linear_val(WEIGHTPOINTS, WX, WY, T, WEIGHTVALUE, WEIGHTVALUEDIR)
WEIGHT=WEIGHTVALUE

END FUNCTION WEIGHT

REAL*8 FUNCTION EPON(T) !ENDOGENOUS EPO SPLINE
USE SHARED
IMPLICIT NONE
REAL*8, INTENT(IN) :: T
REAL*8 :: EPONVALUE, EPONVALUEDIR
CALL spline_linear_val(EPONPOINTS, EPONX, EPONY, T, EPONVALUE, EPONVALUEDIR)
EPON=EPONVALUE
END FUNCTION EPON

REAL*8 FUNCTION EPOX(T) !ENDOGENOUS EPO SPLINE
USE SHARED
IMPLICIT NONE
REAL*8, INTENT(IN) :: T
REAL*8 :: EPOXVALUE, EPOXVALUEDIR
```

```
CALL spline_linear_val(POINTS, XP, YP4H, T, EPOXVALUE, EPOXVALUEDIR)
EPOX=EPOXVALUE
END FUNCTION EPOX
```

C.3 Fortran subroutines for Chapter 4

C.3.1 MAIN.F90 (Original Code)

```
PROGRAM DOSING_OP_INFANTS

USE SHARED
USE rand_gen_int
USE numerical_libraries

IMPLICIT NONE

INTEGER :: J, K, M, MODE, MAXEVAL, NEVAL, STATUS
INTEGER, PARAMETER :: FUNCTION_EVAL=1
REAL*8 :: X(DOSES), XA(DOSES), XB(DOSES), FX, AVE_BELOW_STAIR_LAST,
OBJEC_VALUE(FUNCTION_EVAL), EVAL_NUM(FUNCTION_EVAL), RAND(DOSES)
REAL*8 :: EPON, DOSE(DOSES)=600D0, EPOSPLINE !U/KG for dose
REAL*8 :: P1(FUNCTION_EVAL), P2(FUNCTION_EVAL), P3(FUNCTION_EVAL),
P4(FUNCTION_EVAL), P5(FUNCTION_EVAL), P6(FUNCTION_EVAL), P7(FUNCTION_EVAL),
P8(FUNCTION_EVAL),P9(FUNCTION_EVAL), P10(FUNCTION_EVAL),
P11(FUNCTION_EVAL), P12(FUNCTION_EVAL)
EXTERNAL :: EPON, EPOSPLINE
D1=1; D2=2; D3=3

CALL FILE_NAMES

!WRITE(*,*) SUM(EMAXI(1:27))/27D0, SUM(EC50I(1:27))/27D0
!READ*

HGB_SPLINE_POINTS=0
BEST=1D8
DEBUG=0

!DO j=1,DOSES
!X(j)=DBLE(J)
!XA(j)=0.01D0+DBLE(J)/100D0
!XB(j)=26D0-0.1D0/DBLE(J)
!END DO
CALL D_RAND_GEN(RAND)
DO j=1,DOSES
X(j)=26*RAND(j)
XA(j)=0.01D0
XB(j)=26D0
END DO

!X(1)=1.5D0; X(2)=3.5D0;X(3)=5.5D0; X(4)=7D0;X(5)=9D0; X(6)=11D0;X(7)=13D0;
X(8)=15D0;X(9)=17D0; X(10)=19D0;X(11)=21D0; X(12)=23D0;
!XA(1)=0.01D0; XA(2)=2.01D0; XA(3)=4.01D0; XA(4)=6.01D0;XA(5)=8.01D0;
XA(6)=10.01D0;XA(7)=12.01D0; XA(8)=14.01D0;XA(9)=16.01D0;
XA(10)=18.01D0;XA(11)=20.01D0; XA(12)=22.01D0
!XB(1)=2D0; XB(2)=4D0; XB(3)=6D0; XB(4)=8D0;XB(5)=10D0; XB(6)=12D0;XB(7)=14D0;
XB(8)=16D0;XB(9)=18D0; XB(10)=20D0;XB(11)=22D0; XB(12)=26D0
```

```
!CALL DSET_NELMIN_FTOL(1D-4)   ! OPTIONAL DEFAULT CHANGE OF FTOL
MODE = 0
MAXEVAL = FUNCTION_EVAL
AVE_BELOW_STAIR_LAST=1D8

DO m=1,1 !LOOP FOR RANDOM INITIAL VALUES
CALL D_RAND_GEN(RAND)
DO j=1,DOSES
X(j)=26*RAND(j) !CHANGE INITIAL VALUES
IF (X(j) <= 0.01D0) THEN
X(j)=X(j)+0.01D0
END IF
IF (X(j) >= 26D0) THEN
X(j)=25.99D0
END IF
END DO
!MODE=0 !START OVER OPTIMIZATION
!FX=0D0
CALL DSVRGN (DOSES, X, X_SORT)
!WRITE(*,*) X_SORT
!         DO FILENUM=1,27
!                   CALL FILE_OPEN
!                   !YIN1=HGBIV(FILENUM); YIN2=DOSE*1000D0/V; XFIRST=0D0;
XLAST=X_SORT(1)-1D-8; CALL DIFFEQ !THIS IS GIVING INITIAL DOSE
!                   YIN1=HGBIV(FILENUM); YIN2=DOSE/V; YIN3=HGBIV(FILENUM);
XFIRST=0D0; XLAST=X_SORT(1)-1D-8; CALL DIFFEQ
!                   DO j=1,DOSES-1
!                   YIN1=YP1(HGB_SPLINE_POINTS);
YIN2=DOSE*1000D0/V+YP2(HGB_SPLINE_POINTS); YIN3=YP3(HGB_SPLINE_POINTS);
XFIRST=X_SORT(j); XLAST=X_SORT(j+1)-1D-8; CALL DIFFEQ
!                   END DO
!                   YIN1=YP1(HGB_SPLINE_POINTS);
YIN2=DOSE*1000D0/V+YP2(HGB_SPLINE_POINTS); YIN3=YP3(HGB_SPLINE_POINTS);
XFIRST=X_SORT(DOSES); XLAST=27D0; CALL DIFFEQ
!                   CALL CHECK_HGB
!                   HGB_SPLINE_POINTS=0
!                   DEALLOCATE(DOL)
!                   DEALLOCATE(WEIGHT)
!                   DEALLOCATE(CUMLOSS)
!                   DEALLOCATE(VOLLOSS)
!                   DEALLOCATE(CUMLOSS_KG)
!                   DEALLOCATE(VOLLOSS_KG)
!                   DEALLOCATE(EPOX)
!                   DEALLOCATE(EPOY)
!                   DEALLOCATE(THRESHX)
!                   DEALLOCATE(THRESHY)
!                   DEALLOCATE(IDEALX)
!                   DEALLOCATE(IDEALY)
!         END DO
!BEST=1D8

DO k=1,FUNCTION_EVAL

         P1(k)=X(1);P2(k)=X(2);P3(k)=X(3);P4(k)=X(4);P5(k)=X(5);P6(k)=X(6);P7(k)=X(7);P8(k)=
X(8);P9(k)=X(9);P10(k)=X(10);P11(k)=X(11);P12(k)=X(12)
```

```
DOSE(1)=1500D0
DOSE(2)=720D0
DOSE(3)=720D0
DOSE(4)=720D0
DOSE(5)=720D0
DOSE(6)=720D0
DOSE(7)=720D0
DOSE(8)=720D0
DOSE(9)=720D0

X_SORT(1)=2D0
X_SORT(2)=3D0
X_SORT(3)=4D0
X_SORT(4)=5D0
X_SORT(5)=6D0
X_SORT(6)=9D0
X_SORT(7)=13D0
X_SORT(8)=17D0
X_SORT(9)=21D0


        DO FILENUM=1,27
                CALL FILE_OPEN
!               OPEN (UNIT=21, FILE='ALL_EPO.txt', STATUS='OLD', ACTION= 'WRITE',
ACCESS='APPEND', IOSTAT=status) !THIS WRITES OUTPUT IN FILE
!               DO j=1,EPO_POINTS(FILENUM)
!               WRITE(21,*) EPOX(j), EPOY(j)
!               END DO
                !YIN1=HGBIV(FILENUM); YIN2=DOSE*1000D0/V; XFIRST=0D0;
XLAST=X_SORT(1)-1D-8; CALL DIFFEQ !THIS IS GIVING INITIAL DOSE
                YIN1=HGBIV(FILENUM); YIN2=DOSE(1)/V; YIN3=HGBIV(FILENUM);
XFIRST=0D0; XLAST=X_SORT(1)-1D-8; CALL DIFFEQ
                DO j=1,DOSES-1
                YIN1=YP1(HGB_SPLINE_POINTS);
YIN2=DOSE(j)*1000D0/V+YP2(HGB_SPLINE_POINTS); YIN3=YP3(HGB_SPLINE_POINTS);
XFIRST=X_SORT(j); XLAST=X_SORT(j+1)-1D-8; CALL DIFFEQ
                END DO
                YIN1=YP1(HGB_SPLINE_POINTS);
YIN2=DOSE(DOSES)*1000D0/V+YP2(HGB_SPLINE_POINTS);
YIN3=YP3(HGB_SPLINE_POINTS); XFIRST=X_SORT(DOSES); XLAST=27D0; CALL DIFFEQ
                CALL CHECK_HGB
!               WRITE(*,*) EPOSPLINE(2.99D0)
!               READ*
                HGB_SPLINE_POINTS=0
                DEALLOCATE(DOL)
                DEALLOCATE(WEIGHT)
                DEALLOCATE(CUMLOSS)
                DEALLOCATE(VOLLOSS)
                DEALLOCATE(CUMLOSS_KG)
                DEALLOCATE(VOLLOSS_KG)
                DEALLOCATE(EPOX)
                DEALLOCATE(EPOY)
                DEALLOCATE(THRESHX)
                DEALLOCATE(THRESHY)
                DEALLOCATE(IDEALX)
                DEALLOCATE(IDEALY)
```

```
          END DO
!FX = -100D0/AVE_BELOW_STAIR
FX = AVE_BELOW_STAIR
OBJEC_VALUE(k)= FX ; EVAL_NUM(K)=DBLE(K)
!WRITE(*,*) FX
!IF (MOD(m,10)==0) THEN
!WRITE(*,*) 'FX', FX , 'BEST', BEST
!END IF
!IF (MOD(k,5)==0) THEN
!WRITE(*,*) 'FX', FX , 'BEST', BEST !, K, 'INITIAL VALUES', m
!END IF
END DO
END DO !LOOP FOR RANDOM INITIAL VALUES INDEX M

!WRITE(*,*) 'MODE', MODE
!DO j=1,DOSES
!WRITE(*,*) 'TIME', X_SORT_BEST(j)
!END DO
!DO j=1,27
!WRITE(*,*) 'SUBJECT',j, AREA_BEST(j)
!END DO
!WRITE(*,*) 'AVE TIME BELOW STAIR', BEST

!OPEN (UNIT=20, FILE='SOLUTION', STATUS='NEW', ACTION= 'WRITE', IOSTAT=status)
!THIS WRITES OUTPUT IN FILE
!DO j=1,DOSES
!WRITE(20,*) X_SORT_BEST(j)
!END DO
!WRITE(20,*) 'OBJECTIVE FUNC VALUE', BEST

DO FILENUM=1,27
                CALL FILE_OPEN
                !YIN1=HGBIV(FILENUM); YIN2=DOSE*1000D0/V; XFIRST=0D0;
XLAST=X_SORT_BEST(1)-1D-8; CALL DIFFEQ
                                YIN1=HGBIV(FILENUM); YIN2=DOSE(1)/V;
YIN3=HGBIV(FILENUM); XFIRST=0D0; XLAST=X_SORT_BEST(1)-1D-8; CALL DIFFEQ
                DO j=1,DOSES-1
                YIN1=YP1(HGB_SPLINE_POINTS);
YIN2=DOSE(j)*1000D0/V+YP2(HGB_SPLINE_POINTS); YIN3=YP3(HGB_SPLINE_POINTS);
XFIRST=X_SORT_BEST(j); XLAST=X_SORT_BEST(j+1)-1D-8; CALL DIFFEQ
                END DO
                YIN1=YP1(HGB_SPLINE_POINTS);
YIN2=DOSE(DOSES)*1000D0/V+YP2(HGB_SPLINE_POINTS);
YIN3=YP3(HGB_SPLINE_POINTS); XFIRST=X_SORT_BEST(DOSES); XLAST=27D0; CALL
DIFFEQ
                IF (FILENUM==1) THEN
                YP2=YP2/1000D0 !MAKES EPO AXIS U/ML
!               CALL ADD_CURVE(XP, YP2, HGB_SPLINE_POINTS)
!               CALL BEGIN_LEFT_AT(0.0D0)
!               CALL END_LEFT_AT(10.0D0)
!               CALL LEFT_LABEL('EPO CONC(U/ML)')
!               CALL X_LABEL ('DAY OF LIFE')
!               CALL PLOT_IN_AREA(28,28)
                END IF
                !CALL ADD_CURVE (XP, YP1, HGB_SPLINE_POINTS)
                !CALL INCLUDE_CURVE_RIGHT(DOL, CUMLOSS_KG, PHLE_POINTS, 3)
```

```
                    CALL CHECK_HGB
                    HGB_SPLINE_POINTS=0
!CALL INCLUDE_CURVE(IDEALX, IDEALY, THRESH_POINTS, 2)
!CALL INCLUDE_CURVE(STAIRCASE_X, STAIRCASE_Y, 8, 2)
!CALL INCLUDE_CURVE(STAIRCASE_X, STAIRCASE_Y+3D0, 8, 4)
DO j=1, THRESH_POINTS-2 !THIS MAKES IT SO THE FIRST AND LAST POINT DONT SHOW
UP BECAUSE THEY AREN'T ACTUALLY TRANSFUSIONS
THRESHX(j)=THRESHX(j+1); THRESHY(j)=THRESHY(j+1)
END DO
!CALL TITLE_WITH_INTEGER_NUMBER_ADDED('SUBJECT', FILENUM)
!CALL TITLE_WITH_VALUE_ADDED('PROB', PROB_RES(FILENUM))
!CALL X_LABEL ('DAY OF LIFE')
!CALL LEFT_LABEL('HGB CONCENTRATION(G/DL)')
!CALL RIGHT_LABEL('HB REMOVED (G/KG)')
!CALL ADD_POINTS(THRESHX, THRESHY, THRESH_POINTS-2)
!CALL END_X_AT (27D0)
!CALL BEGIN_LEFT_AT(6.0D0)
!CALL END_LEFT_AT(19.0D0)
!CALL BEGIN_RIGHT_AT(0.0D0)
!CALL END_RIGHT_AT(110.0D0)
!CALL PLOT_IN_AREA(FILENUM,28)
                    DEALLOCATE(DOL)
                    DEALLOCATE(WEIGHT)
                    DEALLOCATE(CUMLOSS)
                    DEALLOCATE(VOLLOSS)
                    DEALLOCATE(CUMLOSS_KG)
                    DEALLOCATE(VOLLOSS_KG)
                    DEALLOCATE(EPOX)
                    DEALLOCATE(EPOY)
                    DEALLOCATE(THRESHX)
                    DEALLOCATE(THRESHY)
                    DEALLOCATE(IDEALX)
                    DEALLOCATE(IDEALY)
END DO

WRITE(*,*) 'MODE', MODE
DO j=1,DOSES
WRITE(*,*) 'TIME', X_SORT_BEST(j)
END DO
DO j=1,27
WRITE(*,*) 'SUBJECT',j, AREA_BEST(j)
END DO
WRITE(*,*) 'AVE TIME BELOW STAIR', BEST

!CALL DISPLAY_PLOT

END PROGRAM DOSING_OP_INFANTS
```

C.3.2 SHARED.F90 (Original Code)

```
MODULE SHARED

INTEGER :: FILENUM, PHLE_POINTS(27), HGB_SPLINE_POINTS, EPO_POINTS,
THRESH_POINTS
INTEGER :: D1, D2, D3, D1H, D2H, D3H, DEBUG
```

```
INTEGER, PARAMETER :: DOSES=9
REAL*8,ALLOCATABLE,DIMENSION(:)::
DOL,WEIGHT,CUMLOSS,VOLLOSS,CUMLOSS_KG,VOLLOSS_KG
REAL*8,ALLOCATABLE,DIMENSION(:):: EPOX, EPOY, THRESHX, THRESHY, IDEALX,
IDEALY
!REAL*8 ::
DOL(27,100),WEIGHT(27,100),CUMLOSS(27,100),VOLLOSS(27,100),CUMLOSS_KG(27,100),
VOLLOSS_KG(27,100)
!REAL*8 :: EPOX(27,100), EPOY(27,100)
CHARACTER*40 :: PHLE_FILE(27), EPO_FILE(27), THRESH_FILE(27)
REAL*8 :: STAIRCASE_X(8), STAIRCASE_Y(8), TIME_BELOW_STAIR_BEST(27),
AREA_BEST(27)
REAL*8 :: YIN1, YIN2, YIN3, XFIRST, XLAST, BEST, AVE_BELOW_STAIR, FACTOR
REAL*8 :: XP(10000000), YP1(10000000), YP2(10000000), YP3(10000000),
EPO_TOTAL(10000000)
REAL*8 :: X_SORT(DOSES), X_SORT_BEST(DOSES), EMAXI(27), EC50I(27), PROB_RES(27)

REAL*8 :: LAGTIME=0.9D0
REAL*8 :: LIFESPAN=42.5D0
REAL*8 :: V=104.3D0
REAL*8 :: EMAX=0.813 !g/day-kg^3/4
REAL*8 :: EC50=72.2 !mu/mL
!REAL*8 :: EMAX=0.566D0
!REAL*8 :: EC50=28.5
REAL*8 :: HGBIV(27)
REAL*8 :: CL
REAL*8 :: P1=33.1639330843279D0
REAL*8 :: P2=5.99209862385388D0

END MODULE SHARED

REAL*8 FUNCTION K(T) !PHLE LOSS SPLINE
USE SHARED
IMPLICIT NONE
REAL*8, INTENT(IN) :: T
REAL*8 :: KVALUE, KVALUEDIR
CALL spline_linear_val(PHLE_POINTS, DOL, VOLLOSS_KG, T, KVALUE, KVALUEDIR)
K=KVALUE/93D0 !THIS MAKES IT A K VALUE
END FUNCTION K

REAL*8 FUNCTION WEIGHTS(T) !WEIGHT SPLINE
USE SHARED
IMPLICIT NONE
REAL*8, INTENT(IN) :: T
REAL*8 :: WEIGHTVALUE, WEIGHTVALUEDIR
INTEGER :: i
CALL spline_linear_val(PHLE_POINTS, DOL, WEIGHT, T, WEIGHTVALUE,
WEIGHTVALUEDIR)
WEIGHTS=WEIGHTVALUE
END FUNCTION WEIGHTS

REAL*8 FUNCTION EPOSPLINE(T) !EXOGENOUS EPO SPLINE
USE SHARED
IMPLICIT NONE
REAL*8, INTENT(IN) :: T
REAL*8 :: EPOSPLINEVALUE, EPOSPLINEVALUEDIR
```

```
CALL spline_linear_val(HGB_SPLINE_POINTS, XP, YP2, T, EPOSPLINEVALUE,
EPOSPLINEVALUEDIR)
EPOSPLINE=EPOSPLINEVALUE*1000D0
END FUNCTION EPOSPLINE

REAL*8 FUNCTION EPON(T) !ENDOGENOUS EPO SPLINE
USE SHARED
IMPLICIT NONE
REAL*8, INTENT(IN) :: T
REAL*8 :: EPONVALUE, EPONVALUEDIR
CALL spline_linear_val(EPO_POINTS, EPOX, EPOY, T, EPONVALUE, EPONVALUEDIR)
EPON=EPONVALUE
END FUNCTION EPON

REAL*8 FUNCTION CUM_LOSS_SPLINE(T) !ENDOGENOUS EPO SPLINE
USE SHARED
IMPLICIT NONE
REAL*8, INTENT(IN) :: T
REAL*8 :: CUM_LOSS_SPLINEVALUE, CUM_LOSS_SPLINEVALUEDIR
CALL spline_linear_val(PHLE_POINTS, DOL, CUMLOSS, T, CUM_LOSS_SPLINEVALUE,
CUM_LOSS_SPLINEVALUEDIR)
CUM_LOSS_SPLINE=CUM_LOSS_SPLINEVALUE
END FUNCTION CUM_LOSS_SPLINE

SUBROUTINE FILE_NAMES

USE SHARED

IMPLICIT NONE

STAIRCASE_X(1)=0D0 ; STAIRCASE_Y(1)=12.5D0
STAIRCASE_X(2)=7D0 ; STAIRCASE_Y(2)=12.5D0
STAIRCASE_X(3)=7.001D0 ; STAIRCASE_Y(3)=11.0D0
STAIRCASE_X(4)=14D0 ; STAIRCASE_Y(4)=11.0D0
STAIRCASE_X(5)=14.001D0 ; STAIRCASE_Y(5)=9.3D0
STAIRCASE_X(6)=21D0 ; STAIRCASE_Y(6)=9.3D0
STAIRCASE_X(7)=21.001D0 ; STAIRCASE_Y(7)=9.3D0
STAIRCASE_X(8)=27D0 ; STAIRCASE_Y(8)=9.3D0

PHLE_FILE(1)='phle loss\phle_loss_301.txt'; HGBIV(1)=15.9D0
PHLE_FILE(2)='phle loss\phle_loss_302.txt'; HGBIV(2)=12.6D0
PHLE_FILE(3)='phle loss\phle_loss_303.txt'; HGBIV(3)=18.1D0
PHLE_FILE(4)='phle loss\phle_loss_304.txt'; HGBIV(4)=19.3D0
PHLE_FILE(5)='phle loss\phle_loss_305.txt'; HGBIV(5)=17.0D0
PHLE_FILE(6)='phle loss\phle_loss_306.txt'; HGBIV(6)=13.9D0
PHLE_FILE(7)='phle loss\phle_loss_307.txt'; HGBIV(7)=17.4D0
PHLE_FILE(8)='phle loss\phle_loss_308.txt'; HGBIV(8)=17.0D0
PHLE_FILE(9)='phle loss\phle_loss_309.txt'; HGBIV(9)=18.3D0
PHLE_FILE(10)='phle loss\phle_loss_310.txt'; HGBIV(10)=14.5D0
PHLE_FILE(11)='phle loss\phle_loss_311.txt'; HGBIV(11)=17.9D0
PHLE_FILE(12)='phle loss\phle_loss_312.txt'; HGBIV(12)=17.2D0
PHLE_FILE(13)='phle loss\phle_loss_313.txt'; HGBIV(13)=14.2D0
PHLE_FILE(14)='phle loss\phle_loss_314.txt'; HGBIV(14)=11.3D0
PHLE_FILE(15)='phle loss\phle_loss_315.txt'; HGBIV(15)=12.2D0
PHLE_FILE(16)='phle loss\phle_loss_316.txt'; HGBIV(16)=15.2D0
PHLE_FILE(17)='phle loss\phle_loss_317.txt'; HGBIV(17)=12.9D0
```

```
PHLE_FILE(18)='phle loss\phle_loss_318.txt'; HGBIV(18)=14.1D0
PHLE_FILE(19)='phle loss\phle_loss_319.txt'; HGBIV(19)=13.1D0
PHLE_FILE(20)='phle loss\phle_loss_320.txt'; HGBIV(20)=17.3D0
PHLE_FILE(21)='phle loss\phle_loss_321.txt'; HGBIV(21)=15.4D0
PHLE_FILE(22)='phle loss\phle_loss_322.txt'; HGBIV(22)=17.0D0
PHLE_FILE(23)='phle loss\phle_loss_323.txt'; HGBIV(23)=12.1D0
PHLE_FILE(24)='phle loss\phle_loss_324.txt'; HGBIV(24)=17.0D0
PHLE_FILE(25)='phle loss\phle_loss_325.txt'; HGBIV(25)=18.2D0
PHLE_FILE(26)='phle loss\phle_loss_326.txt'; HGBIV(26)=18.8D0
PHLE_FILE(27)='phle loss\phle_loss_327.txt'; HGBIV(27)=14.1D0

epo_file(1)='epo\epo_301.txt'
epo_file(2)='epo\epo_302.txt'
epo_file(3)='epo\epo_303.txt'
epo_file(4)='epo\epo_304.txt'
epo_file(5)='epo\epo_305.txt'
epo_file(6)='epo\epo_306.txt'
epo_file(7)='epo\epo_307.txt'
epo_file(8)='epo\epo_308.txt'
epo_file(9)='epo\epo_309.txt'
epo_file(10)='epo\epo_310.txt'
epo_file(11)='epo\epo_311.txt'
epo_file(12)='epo\epo_312.txt'
epo_file(13)='epo\epo_313.txt'
epo_file(14)='epo\epo_314.txt'
epo_file(15)='epo\epo_315.txt'
epo_file(16)='epo\epo_316.txt'
epo_file(17)='epo\epo_317.txt'
epo_file(18)='epo\epo_318.txt'
epo_file(19)='epo\epo_319.txt'
epo_file(20)='epo\epo_320.txt'
epo_file(21)='epo\epo_321.txt'
epo_file(22)='epo\epo_322.txt'
epo_file(23)='epo\epo_323.txt'
epo_file(24)='epo\epo_324.txt'
epo_file(25)='epo\epo_325.txt'
epo_file(26)='epo\epo_326.txt'
epo_file(27)='epo\epo_327.txt'

thresh_file(1)='thresh\thresh_301.txt'
thresh_file(2)='thresh\thresh_302.txt'
thresh_file(3)='thresh\thresh_303.txt'
thresh_file(4)='thresh\thresh_304.txt'
thresh_file(5)='thresh\thresh_305.txt'
thresh_file(6)='thresh\thresh_306.txt'
thresh_file(7)='thresh\thresh_307.txt'
thresh_file(8)='thresh\thresh_308.txt'
thresh_file(9)='thresh\thresh_309.txt'
thresh_file(10)='thresh\thresh_310.txt'
thresh_file(11)='thresh\thresh_311.txt'
thresh_file(12)='thresh\thresh_312.txt'
thresh_file(13)='thresh\thresh_313.txt'
thresh_file(14)='thresh\thresh_314.txt'
thresh_file(15)='thresh\thresh_315.txt'
thresh_file(16)='thresh\thresh_316.txt'
thresh_file(17)='thresh\thresh_317.txt'
```

thresh_file(18)='thresh\thresh_318.txt'
thresh_file(19)='thresh\thresh_319.txt'
thresh_file(20)='thresh\thresh_320.txt'
thresh_file(21)='thresh\thresh_321.txt'
thresh_file(22)='thresh\thresh_322.txt'
thresh_file(23)='thresh\thresh_323.txt'
thresh_file(24)='thresh\thresh_324.txt'
thresh_file(25)='thresh\thresh_325.txt'
thresh_file(26)='thresh\thresh_326.txt'
thresh_file(27)='thresh\thresh_327.txt'

EMAXI(1)=0.65211
EMAXI(2)=0.7187
EMAXI(3)=0.79529
EMAXI(4)=0.57204
EMAXI(5)=0.87313
EMAXI(6)=1.0587
EMAXI(7)=1.5869
EMAXI(8)=1.5901
EMAXI(9)=0.40535
EMAXI(10)=0.47913
EMAXI(11)=0.9972
EMAXI(12)=0.49248
EMAXI(13)=0.82134
EMAXI(14)=1.0126
EMAXI(15)=0.52835
EMAXI(16)=0.30943
EMAXI(17)=0.27923
EMAXI(18)=0.8056
EMAXI(19)=0.85498
EMAXI(20)=0.8156
EMAXI(21)=0.88296
EMAXI(22)=0.18854
EMAXI(23)=1.434
EMAXI(24)=0.5009
EMAXI(25)=1.0133
EMAXI(26)=0.38503
EMAXI(27)=0.5555

EC50I(1)=65.854
EC50I(2)=61.686
EC50I(3)=97.676
EC50I(4)=7.2515
EC50I(5)=39.38
EC50I(6)=38.268
EC50I(7)=15.68
EC50I(8)=310.84
EC50I(9)=13.265
EC50I(10)=11.768
EC50I(11)=50.091
EC50I(12)=34.117
EC50I(13)=99.772
EC50I(14)=110.15
EC50I(15)=19.684
EC50I(16)=15.563
EC50I(17)=7.3872

```
EC50I(18)=38.257
EC50I(19)=181.98
EC50I(20)=44.741
EC50I(21)=59.889
EC50I(22)=877.93
EC50I(23)=80.532
EC50I(24)=105.55
EC50I(25)=144.96
EC50I(26)=243.62
EC50I(27)=31.599

PROB_RES(1)=0.96180D0
PROB_RES(2)=0.94286D0
PROB_RES(3)=0.45918D0
PROB_RES(4)=0.00010D0
PROB_RES(5)=0.91266D0
PROB_RES(6)=0.99914D0
PROB_RES(7)=0.99896D0
PROB_RES(8)=0.89835D0
PROB_RES(9)=0.00031D0
PROB_RES(10)=0.01997D0
PROB_RES(11)=0.95232D0
PROB_RES(12)=0.00447D0
PROB_RES(13)=0.13489D0
PROB_RES(14)=0.04154D0
PROB_RES(15)=0.03728D0
PROB_RES(16)=0.00168D0
PROB_RES(17)=0.91848D0
PROB_RES(18)=0.98636D0
PROB_RES(19)=0.00378D0
PROB_RES(20)=0.93351D0
PROB_RES(21)=0.95315D0
PROB_RES(22)=0.01011D0
PROB_RES(23)=0.02406D0
PROB_RES(24)=0.82449D0
PROB_RES(25)=0.99984D0
PROB_RES(26)=0.00001D0
PROB_RES(27)=0.99924D0


END SUBROUTINE FILE_NAMES
```

C.3.3 CHECK_HGB.F90 (Original Code)

```
SUBROUTINE CHECK_HGB

USE SHARED

IMPLICIT NONE

REAL*8 :: HGBS, STAIR_S, k, THRESH=10.5D0
REAL*8 :: TIME_BELOW_STAIR(27), THRESH_S
REAL*8 :: A, B, A1, AREA(27)
INTEGER :: LOWPOINTS
EXTERNAL :: HGBS, STAIR_S, THRESH_S
```

```
LOWPOINTS=0

AREA(FILENUM)=0D0
DO k=0,26, 0.1
A=(STAIR_S(K)+STAIR_S(k+0.1D0))/2D0 !THRESH HAS CHANGED TO IDEAL HB
B=(HGBS(K)+HGBS(k+0.1D0))/2D0
IF (B>A) THEN
FACTOR=0.25D0
ELSE
FACTOR=1D0
END IF
AREA(FILENUM)=AREA(FILENUM)+SQRT(((A-B)*0.1)**2)*FACTOR
END DO

!TIME_BELOW_STAIR(FILENUM)=DBLE(LOWPOINTS)/100D0
!WRITE(*,*) FILENUM, TIME_BELOW_STAIR(FILENUM), HGB_SPLINE_POINTS

IF (FILENUM == 27) THEN
!WRITE(*,*) AREA
!AVE_BELOW_STAIR=SUM(TIME_BELOW_STAIR(1:27))/27D0
AVE_BELOW_STAIR=SUM(AREA(1:27))/27D0
!WRITE(*,*) X_SORT
!WRITE(*,*) AVE_BELOW_STAIR
!READ*
!AVE_BELOW_STAIR=(SUM(AREA(1:27))-AREA(1)-AREA(2))/25D0
!WRITE(*,*) 'OBJECTIVE FUNCTION', AVE_BELOW_STAIR
!          IF (AVE_BELOW_STAIR<BEST) THEN
          IF (AVE_BELOW_STAIR<BEST) THEN
!          TIME_BELOW_STAIR_BEST=TIME_BELOW_STAIR
   AREA_BEST=AREA
          BEST=AVE_BELOW_STAIR
          X_SORT_BEST=X_SORT
!          D1H=D1
!          D2H=D2
!          D3H=D3
          END IF
END IF

!WRITE(*,*) 'SUBJECT', FILENUM, 'DAYS UNDER THRESH', TIME_BELOW_STAIR(FILENUM)
!IF (FILENUM == 27) THEN
!WRITE(*,*) 'AVERAGE DAYS UNDER THRESH', SUM(TIME_BELOW_STAIR(1:27))/27D0
!END IF

END SUBROUTINE CHECK_HGB

REAL*8 FUNCTION HGBS(T) !ENDOGENOUS K VALUE
USE SHARED
IMPLICIT NONE
REAL*8, INTENT(IN) :: T
REAL*8 :: HGBVALUE, HGBVALUEDIR
CALL spline_linear_val(HGB_SPLINE_POINTS, XP, YP1, T, HGBVALUE, HGBVALUEDIR)
HGBS=HGBVALUE
END FUNCTION HGBS

REAL*8 FUNCTION STAIR_S(T) !ENDOGENOUS K VALUE
```

```
USE SHARED
IMPLICIT NONE
REAL*8, INTENT(IN) :: T
REAL*8 :: STAIRVALUE, STAIRVALUEDIR
CALL spline_linear_val(8, STAIRCASE_X, STAIRCASE_Y, T, STAIRVALUE, STAIRVALUEDIR)
STAIR_S=STAIRVALUE+3D0
END FUNCTION STAIR_S

REAL*8 FUNCTION THRESH_S(T) !ENDOGENOUS K VALUE
USE SHARED
IMPLICIT NONE
REAL*8, INTENT(IN) :: T
REAL*8 :: THRESHVALUE, THRESHVALUEDIR
CALL spline_linear_val(THRESH_POINTS, THRESHX, THRESHY, T, THRESHVALUE,
THRESHVALUEDIR)
THRESH_S=(THRESHVALUE+20D0)/2D0 !THIS IS THE IDEAL HGB OBJECTIVE FUNCTION
END FUNCTION THRESH_S
```

C.4 Fortran subroutines for Chapter 5

C.4.1 MAIN_V_3.F90 (Original code)

```
PROGRAM PHLE_RED

!THIS IS VERSION 3 WHICH CHANGES THE EQUATIONS SLIGHTLY

USE SHARED

IMPLICIT NONE

INTEGER :: i,j,k, status, VENOUS=0
REAL*8 :: WEIGHT, ALIFESPAN=120D0, ILIFESPAN=65.8D0, PHLE_RED_FACTOR=1.0D0
!THIS IS PERCENT OF REMOVED BLOOD THAT WILL STAY IN FOR SIMULATION
REAL*8 :: VOL_KG_ADDED, AMOUNT_HGB_ADDED, HGB, THRESH, PINT,
SIM_TRX_HGB=83.1D0/3D0
REAL*8 :: SIM_TRX_X(10), SIM_TRX_Y(10)=7.0D0, SIM_TRX_X_PINT(10),
TRX_LENGTH=5D0/24D0
REAL*8 :: TEMP1, TEMP2, SD1, SD2, MEAN1, MEAN2, SD, TEMP_PHLE_PROD,
DELTA_HGB, HGB_NAT(26,28), HGB_NAT_AVE_X(28), HGB_NAT_AVE_Y(28)
REAL*8 :: HGB_NAT_AVE_Y_PLUS(28), HGB_NAT_AVE_Y_MINUS(28), TEMP_SD(26)
EXTERNAL :: WEIGHT, HGB, THRESH, PINT, SD

CALL FILE_NAMES
CALL TRX_PRED !THIS WILL GIVE THE HGB CHANGE VALUES

!CALL GRAPH_PINT
!CALL GRAPH_CUM_PHLE2

DO1: DO FILENUM=1,26
CALL FILE_OPEN

!JUST MODIFY THRESH POINTS BECAUSE ALREADY ALLOCATED CORRECTLY

DO i=2,THRESH_POINTS(FILENUM)-1
```

```
THRESH_Y(i)=HGB(THRESH_X(i))
END DO

ORIGINAL_DATA=HGB_Y

DO2: DO i=1,TRX_POINTS(FILENUM)
        DO3: DO j=1,HGB_POINTS(FILENUM)
                TEMP_PHLE_PROD=1D0
                DO k=1, PHLE_POINTS_KG(FILENUM)
                        IF (PHLE_X_KG(k)>TRX_T(i) .AND. PHLE_X_KG(k)<HGB_X(j)) THEN
!THIS SHOULD MULTIPLY ALL PHLE AFTER TRX AND BEFORE CURRENT CORRECTION
                        TEMP_PHLE_PROD=TEMP_PHLE_PROD*(1D0-
PHLE_Y_KG(k)/AVE_BV(FILENUM))
                        END IF
                END DO
        IF (HGB_X(j)>TRX_T(i)) THEN !WE HAVE A HGB POINT AFTER THE TRX
                DELTA_HGB=TRX_H(i)
                IF (HGB_X(j)>TRX_T(i)+TRX_LENGTH) THEN
                HGB_Y(j)=(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*HGB_Y(j)/100D0-
DELTA_HGB*TEMP_PHLE_PROD*(1D0-(HGB_X(j)-TRX_T(i))/(ALIFESPAN-
TRX_T(i))))/(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*0.01D0)
                ELSE !POINT OCCURS DURING TRX
                HGB_Y(j)=(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*HGB_Y(j)/100D0-
DELTA_HGB*((HGB_X(j)-TRX_T(i))/TRX_LENGTH)*TEMP_PHLE_PROD*(1D0-(HGB_X(j)-
TRX_T(i))/(ALIFESPAN-TRX_T(i))))/(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*0.01D0)
                END IF
        END IF
        END DO DO3
END DO DO2
HGB_M1=HGB_Y !ONCE Y MODIFIED AGAIN MAKE HGB_M2=HGB_Y

DO6: DO i=1,PHLE_POINTS_KG(FILENUM)
        DO7: DO j=1, HGB_POINTS(FILENUM)
                        TEMP_PHLE_PROD=1D0
                        DO k=1, PHLE_POINTS_KG(FILENUM)
                        IF (PHLE_X_KG(k)>PHLE_X_KG(i) .AND. PHLE_X_KG(k)<HGB_X(j))
THEN !THIS SHOULD MULTIPLY ALL PHLE AFTER CURRENT PHLE AND BEFORE
CURRENT CORRECTION
                        TEMP_PHLE_PROD=TEMP_PHLE_PROD*(1D0-
PHLE_Y_KG(k)/AVE_BV(FILENUM))
                        END IF
                        END DO
        IF (HGB_X(j)>PHLE_X_KG(i)) THEN !WE HAVE A POINT AFTER THE PHLE
        DELTA_HGB=PHLE_RED_FACTOR*(PHLE_Y_KG(i)*WEIGHT(PHLE_X_KG(i))*HGB(P
HLE_X_KG(i))*0.01D0)
        IF (DELTA_HGB < 0D0) DELTA_HGB=0D0 !MAKE SURE NO PHLE CORRECTION
FOR NEG HGB
        HGB_Y(j)=(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*HGB_Y(j)/100D0+DELTA_HGB*TE
MP_PHLE_PROD*(1D0-(HGB_X(j)-PHLE_X_KG(i))/(ILIFESPAN-
PHLE_X_KG(i))))/(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*0.01D0)
        END IF
        END DO DO7
END DO DO6
HGB_M2=HGB_Y

DO i=1,28 !THIS LOOP SHOULD COME UP WITH NATURAL AVERAGE HB VALUES
```

```
HGB_NAT(FILENUM,i)=MAX(HGB(DBLE(i)-0.9D0),0D0)
HGB_NAT_AVE_X(i)=DBLE(i)-0.9D0
IF (FILENUM==26) THEN !LAST HB
HGB_NAT_AVE_Y(i)=SUM(HGB_NAT(1:FILENUM,i))/26D0
DO j=1,26
TEMP_SD(j)=HGB_NAT(j,i)
END DO
HGB_NAT_AVE_Y_PLUS(i)=HGB_NAT_AVE_Y(i)+SD(26,TEMP_SD)
HGB_NAT_AVE_Y_MINUS(i)=HGB_NAT_AVE_Y(i)-SD(26,TEMP_SD)
END IF
END DO


DO10: DO i=1, HGB_POINTS(FILENUM)
DELTA_HGB=15D0*WEIGHT(HGB_X(i))*SIM_TRX_HGB*0.01D0 !THIS WILL BE FIXED FOR A
GIVEN BLOOD VOLUME
        IF (HGB_Y(i)<=THRESH(HGB_X(i))) THEN !HGB GOES BELOW TRX THRESH
        SIM_TRX_NUM(FILENUM)=SIM_TRX_NUM(FILENUM)+1
        SIM_TRX_X(SIM_TRX_NUM(FILENUM))=HGB_X(i)
        DO11: DO j=i, HGB_POINTS(FILENUM)
                        TEMP_PHLE_PROD=1D0
                        DO k=1, PHLE_POINTS_KG(FILENUM) !A SIMULATED TRX HAS
OCCURED B/C INSIDE IF STATEMENT
                         IF (PHLE_X_KG(k)>HGB_X(i) .AND. PHLE_X_KG(k)<HGB_X(j)) THEN
!THIS SHOULD MULTIPLY ALL PHLE AFTER TRX AND BEFORE CURRENT CORRECTION
!                        TEMP_PHLE_PROD=TEMP_PHLE_PROD*(1D0-
PHLE_Y_KG(k)/AVE_BV(FILENUM))
                         TEMP_PHLE_PROD=TEMP_PHLE_PROD*(1D0-
PHLE_Y_KG(k)*(1D0-PHLE_RED_FACTOR)/AVE_BV(FILENUM))
                         END IF
                        END DO
        HGB_Y(j)=(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*HGB_Y(j)/100D0+DELTA_HGB*TE
MP_PHLE_PROD*(1D0-(HGB_X(j)-HGB_X(i))/(ALIFESPAN-
HGB_X(i))))/(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*0.01D0) !ADDS TRX
        END DO DO11
        END IF
END DO DO10
HGB_M3=HGB_Y
HGB_Y=HGB_M2 !RESET Y SO WE CAN APPLY A DIFFERENT TRX CRITERIA

DO12: DO i=1, HGB_POINTS(FILENUM)
DELTA_HGB=15D0*WEIGHT(HGB_X(i))*SIM_TRX_HGB*0.01D0 !THIS WILL BE FIXED FOR A
GIVEN BLOOD VOLUME
IF (SYRINGE(i)==0) VENOUS=VENOUS+1
!       IF (SYRINGE(i)==0) HGB_Y(i)=HGB_Y(i)*0.9D0
        IF (HGB_Y(i)<=PINT(HGB_X(i))) THEN !HGB GOES BELOW PINT CRITERIA
        SIM_TRX_NUM_PINT(FILENUM)=SIM_TRX_NUM_PINT(FILENUM)+1
        SIM_TRX_X_PINT(SIM_TRX_NUM_PINT(FILENUM))=HGB_X(i)
        DO13: DO j=i, HGB_POINTS(FILENUM)
                        TEMP_PHLE_PROD=1D0
                        DO k=1, PHLE_POINTS_KG(FILENUM) !A SIMULATED TRX HAS
OCCURED B/C INSIDE IF STATEMENT
                         IF (PHLE_X_KG(k)>HGB_X(i) .AND. PHLE_X_KG(k)<HGB_X(j)) THEN
!THIS SHOULD MULTIPLY ALL PHLE AFTER TRX AND BEFORE CURRENT CORRECTION
!                        TEMP_PHLE_PROD=TEMP_PHLE_PROD*(1D0-
PHLE_Y_KG(k)/AVE_BV(FILENUM))
```

```
                              TEMP_PHLE_PROD=TEMP_PHLE_PROD*(1D0-
PHLE_Y_KG(k)*(1D0-PHLE_RED_FACTOR)/AVE_BV(FILENUM))
                             END IF
                            END DO
          HGB_Y(j)=(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*HGB_Y(j)/100D0+DELTA_HGB*TE
MP_PHLE_PROD*(1D0-(HGB_X(j)-HGB_X(i))/(ALIFESPAN-
HGB_X(i))))/(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*0.01D0) !ADDS TRX
          END DO DO13
          END IF
END DO DO12
HGB_M4=HGB_Y


!CALL ADD_POINTS(HGB_X, HGB_M2, HGB_POINTS(FILENUM))
!!CALL ADD_POINTS(SIM_TRX_X, SIM_TRX_Y, SIM_TRX_NUM(FILENUM))
!!CALL ADD_POINTS(SIM_TRX_X_PINT, SIM_TRX_Y, SIM_TRX_NUM_PINT(FILENUM))
!!CALL ADD_CURVE(HGB_X, HGB_M1, HGB_POINTS(FILENUM))
!!CALL INCLUDE_CURVE(HGB_X, HGB_M2, HGB_POINTS(FILENUM),2)
!!CALL INCLUDE_CURVE(HGB_X, HGB_M3, HGB_POINTS(FILENUM),1)
!!CALL ADD_CURVE(HGB_X, HGB_M6, HGB_POINTS(FILENUM))
!!CALL ADD_POINTS(THRESH_X, THRESH_Y, THRESH_POINTS(FILENUM))
!CALL ADD_POINTS(TRX_T, SIM_TRX_Y, TRX_POINTS(FILENUM))
!CALL ADD_CURVE(HGB_X, ORIGINAL_DATA, HGB_POINTS(FILENUM))
!CALL X_LABEL ('DAY OF LIFE')
!CALL TITLE_WITH_INTEGER_NUMBER_ADDED('SUBJECT',filenum+301)
!CALL LEFT_LABEL('HGB')
!CALL PLOT_IN_AREA(FILENUM,26)

IF (FILENUM==26) THEN
!CALL TITLE('AVERAGE NATURAL HGB')
!CALL X_LABEL ('DAY OF LIFE')
!CALL LEFT_LABEL('HGB')
!CALL ADD_CURVE(HGB_NAT_AVE_X, HGB_NAT_AVE_Y, 28)
!CALL INCLUDE_CURVE(HGB_NAT_AVE_X, HGB_NAT_AVE_Y_PLUS, 28,2)
!CALL INCLUDE_CURVE(HGB_NAT_AVE_X, HGB_NAT_AVE_Y_MINUS, 28,2)
!CALL BEGIN_LEFT_AT(0D0)
!WRITE(*,*) SUM(SIM_TRX_NUM(1:26)) !TOTAL TRX NUM
!WRITE(*,*) SUM(SIM_TRX_NUM_PINT(1:26)) !TOTAL TRX NUM
!CALL DISPLAY_PLOT
!CALL GRAPH_TRX_DIST
!WRITE(*,*) SUM(PHLE_POINTS(1:26))/26D0
!WRITE(*,*) SD(26, PHLE_POINTS)
!WRITE(*,*) VENOUS
END IF

CALL UNALLOCATE

!WRITE(*,*) AVE_BV(FILENUM)

!WRITE(*,*) 'SUBJECT', 301+FILENUM, SIM_TRX_NUM(FILENUM), TRX_POINTS(FILENUM)
!WRITE(*,*) 'SUBJECT', 301+FILENUM, SIM_TRX_NUM(FILENUM)-TRX_POINTS(FILENUM)
!WRITE(*,*) 'SUBJECT', 301+FILENUM, SIM_TRX_NUM_PINT(FILENUM)-
TRX_POINTS(FILENUM)

END DO DO1
```

```
MEAN1= SUM(SIM_TRX_NUM(1:26))/27D0; MEAN2=SUM(SIM_TRX_NUM_PINT(1:26))/27D0
DO i=1,26
TEMP1=TEMP1+(SIM_TRX_NUM(i)-MEAN1)**2D0
TEMP2=TEMP2+(SIM_TRX_NUM_PINT(i)-MEAN2)**2D0
END DO
TEMP1=TEMP1+(0D0-MEAN1)**2D0
TEMP2=TEMP2+(0D0-MEAN2)**2D0
SD1=SQRT(TEMP1/26D0)
SD2=SQRT(TEMP2/26D0)

WRITE(*,*) 'IOWA',SUM(SIM_TRX_NUM(1:26))/27D0, SD1
WRITE(*,*) 'PINT',SUM(SIM_TRX_NUM_PINT(1:26))/27D0, SD2

END PROGRAM PHLE_RED
```

C.4.2 SHARED.F90(Original code)

```
MODULE SHARED

INTEGER :: FILENUM, TRX_POINTS(26), WEIGHT_POINTS(26), HGB_POINTS(26),
PHLE_POINTS(26), PHLE_POINTS_KG(26), THRESH_POINTS(26), GA(26)
INTEGER :: SIM_TRX_NUM(26)=0D0, SIM_TRX_NUM_PINT(26)=0D0
REAL*8,ALLOCATABLE,DIMENSION(:):: TRX_T, TRX_H, TRX_V, WEIGHT_X, WEIGHT_Y,
HGB_X, HGB_Y, PHLE_X, PHLE_Y
REAL*8,ALLOCATABLE,DIMENSION(:):: PHLE_X_KG, PHLE_Y_KG, THRESH_X, THRESH_Y
REAL*8,ALLOCATABLE,DIMENSION(:):: HGB_M1, HGB_M2, HGB_M3, HGB_M4, HGB_M5,
HGB_M6, ORIGINAL_DATA
INTEGER, ALLOCATABLE,DIMENSION(:):: SYRINGE
REAL*8 :: HGB_DELTA(26,8),  AVE_BV(26), PINT_X(6), PINT_Y(6) !HGB CHANGE FOR
GIVEN SUBJECT AND TRX
CHARACTER*256 :: TRX_FILE(26), WEIGHT_FILE(26), HGB_FILE(26), PHLE_FILE(26),
PHLE_FILE_KG(26), THRESH_FILE(26)
!FOR DIFFEQ
INTEGER :: HGB_SPLINE_POINTS, HGB_SPLINE_POINTS2(26)
INTEGER, PARAMETER :: DOSES=12, VLBW_NUM=8, ELBW_NUM=18, LIMIT=1000000
INTEGER, DIMENSION(VLBW_NUM) :: VLBW = (/1,4,10,17,19,20,24,26 /)
INTEGER, DIMENSION(ELBW_NUM) :: ELBW =
(/2,3,5,6,7,8,9,11,12,13,14,15,16,18,21,22,23,25 /)
REAL*8 :: DOSE_X(DOSES), DOSE_Y(DOSES)
REAL*8 :: XP(26,LIMIT), YP1(26,LIMIT), YP2(26,LIMIT), EMAXI(26), EC50I(26)
REAL*8 :: YIN1, YIN2, XFIRST, XLAST
REAL*8 :: P1=33.1639330843279D0
REAL*8 :: P2=5.99209862385388D0
REAL*8 :: LAGTIME=0.9D0
REAL*8 :: V=104.3D0

END MODULE SHARED

REAL*8 FUNCTION WEIGHT(TWEIGHT)
USE SHARED
IMPLICIT NONE
REAL*8, INTENT(IN) :: TWEIGHT
REAL*8 :: WEIGHTVALUE, WEIGHTVALUEDIR
CALL spline_linear_val(WEIGHT_POINTS(FILENUM), WEIGHT_X, WEIGHT_Y, TWEIGHT,
WEIGHTVALUE, WEIGHTVALUEDIR)
```

```
WEIGHT=WEIGHTVALUE
END FUNCTION WEIGHT

REAL*8 FUNCTION HGB(T)
USE SHARED
IMPLICIT NONE
REAL*8, INTENT(IN) :: T
REAL*8 :: HGBVALUE, HGBVALUEDIR
CALL spline_linear_val(HGB_POINTS(FILENUM), HGB_X, HGB_Y, T, HGBVALUE,
HGBVALUEDIR)
HGB=HGBVALUE
END FUNCTION HGB

REAL*8 FUNCTION THRESH(T)
USE SHARED
IMPLICIT NONE
REAL*8, INTENT(IN) :: T
REAL*8 :: THRESHVALUE, THRESHVALUEDIR
CALL spline_linear_val(THRESH_POINTS(FILENUM), THRESH_X, THRESH_Y, T,
THRESHVALUE, THRESHVALUEDIR)
THRESH=THRESHVALUE
END FUNCTION THRESH

REAL*8 FUNCTION EPOSPLINE(T) !EXOGENOUS EPO SPLINE
USE SHARED
IMPLICIT NONE
REAL*8, INTENT(IN) :: T
REAL*8 :: EPOSPLINEVALUE, EPOSPLINEVALUEDIR
REAL*8 :: TEMP1(LIMIT), TEMP2(LIMIT)
INTEGER :: i
DO i=1,HGB_SPLINE_POINTS
TEMP1(i)=XP(FILENUM,i)
TEMP2(i)=YP2(FILENUM,i)
END DO
CALL spline_linear_val(HGB_SPLINE_POINTS, TEMP1, TEMP2, T, EPOSPLINEVALUE,
EPOSPLINEVALUEDIR)
EPOSPLINE=EPOSPLINEVALUE*1000D0 !THIS IS USED BECAUSE PD PARAMETERS ARE
IN MU/ML
END FUNCTION EPOSPLINE

REAL*8 FUNCTION HGB_PROD_SPLINE(SUBJECT, T)
USE SHARED
IMPLICIT NONE
INTEGER, INTENT(IN) :: SUBJECT
REAL*8, INTENT(IN) :: T
REAL*8 :: HGBSPLINEVALUE, HGBSPLINEVALUEDIR
REAL*8 :: TEMP1(LIMIT), TEMP2(LIMIT)
INTEGER :: i
DO i=1,HGB_SPLINE_POINTS2(FILENUM)
TEMP1(i)=XP(FILENUM,i)
TEMP2(i)=YP1(FILENUM,i)
END DO
CALL spline_linear_val(HGB_SPLINE_POINTS2(FILENUM), TEMP1, TEMP2, T,
HGBSPLINEVALUE, HGBSPLINEVALUEDIR)
HGB_PROD_SPLINE=HGBSPLINEVALUE
END FUNCTION HGB_PROD_SPLINE
```

```
REAL*8 FUNCTION SD(N, ARRAY)
IMPLICIT NONE
INTEGER :: N
REAL*8 :: ARRAY(N) !THIS ARRAY NEEDS TO BE REAL OR INTEGER BASED ON WHAT SD
IS NEEDED
!INTEGER :: ARRAY(N)
REAL*8 :: MEAN, TEMP1
INTEGER :: i

TEMP1=0D0
MEAN=SUM(ARRAY(1:N))/DBLE(N)
DO i=1,N
TEMP1=TEMP1+(ARRAY(i)-MEAN)**2D0
END DO
SD=SQRT(TEMP1/(N-1))

END FUNCTION SD

REAL*8 FUNCTION PINT(T)
USE SHARED
IMPLICIT NONE
REAL*8, INTENT(IN) :: T
REAL*8 :: PINTVALUE, PINTVALUEDIR
!PINT_X(1)=0D0 ; PINT_Y(1)=10.4D0
!PINT_X(2)=7.99D0 ; PINT_Y(2)=10.4D0
!PINT_X(3)=8D0 ; PINT_Y(3)=9.0D0
!PINT_X(4)=14.99D0 ; PINT_Y(4)=9.0D0
!PINT_X(5)=15D0 ; PINT_Y(5)=7.7D0
!PINT_X(6)=40D0 ; PINT_Y(6)=7.7D0

PINT_X(1)=0D0 ; PINT_Y(1)=10.4D0
PINT_X(2)=6.99D0 ; PINT_Y(2)=10.4D0
PINT_X(3)=7D0 ; PINT_Y(3)=9.0D0
PINT_X(4)=13.99D0 ; PINT_Y(4)=9.0D0
PINT_X(5)=14D0 ; PINT_Y(5)=7.7D0
PINT_X(6)=30D0 ; PINT_Y(6)=7.7D0
CALL spline_linear_val(6, PINT_X, PINT_Y, T, PINTVALUE, PINTVALUEDIR)
PINT=PINTVALUE
END FUNCTION PINT

SUBROUTINE UNALLOCATE !THIS WILL DEALLOCATE ALL VARIABLES FOR EACH FILE
OPEN

USE SHARED

DEALLOCATE(TRX_T)
DEALLOCATE(TRX_H)
DEALLOCATE(TRX_V)
DEALLOCATE(WEIGHT_X)
DEALLOCATE(WEIGHT_Y)
DEALLOCATE(HGB_X)
DEALLOCATE(HGB_Y)
DEALLOCATE(PHLE_X)
DEALLOCATE(PHLE_Y)
DEALLOCATE(PHLE_X_KG)
```

```
DEALLOCATE(PHLE_Y_KG)
DEALLOCATE(SYRINGE)
DEALLOCATE(HGB_M1)
DEALLOCATE(HGB_M2)
DEALLOCATE(HGB_M3)
DEALLOCATE(HGB_M4)
DEALLOCATE(HGB_M5)
DEALLOCATE(HGB_M6)
DEALLOCATE(THRESH_X)
DEALLOCATE(THRESH_Y)
DEALLOCATE(ORIGINAL_DATA)

END SUBROUTINE UNALLOCATE

SUBROUTINE FILE_NAMES

USE SHARED

IMPLICIT NONE

HGB_FILE(1)='DATA\INFANT_302_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(2)='DATA\INFANT_303_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(3)='DATA\INFANT_304_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(4)='DATA\INFANT_305_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(5)='DATA\INFANT_306_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(6)='DATA\INFANT_307_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(7)='DATA\INFANT_308_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(8)='DATA\INFANT_309_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(9)='DATA\INFANT_310_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(10)='DATA\INFANT_311_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(11)='DATA\INFANT_312_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(12)='DATA\INFANT_313_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(13)='DATA\INFANT_314_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(14)='DATA\INFANT_315_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(15)='DATA\INFANT_316_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(16)='DATA\INFANT_317_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(17)='DATA\INFANT_318_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(18)='DATA\INFANT_319_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(19)='DATA\INFANT_320_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(20)='DATA\INFANT_321_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(21)='DATA\INFANT_322_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(22)='DATA\INFANT_323_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(23)='DATA\INFANT_324_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(24)='DATA\INFANT_325_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(25)='DATA\INFANT_326_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'
HGB_FILE(26)='DATA\INFANT_327_HEMOGLOBIN_RBC_CONCENTRATIONS.DAT'

TRX_FILE(1)='DATA\INFANT_302_TRANSFUSIONS.DAT'
TRX_FILE(2)='DATA\INFANT_303_TRANSFUSIONS.DAT'
TRX_FILE(3)='DATA\INFANT_304_TRANSFUSIONS.DAT'
TRX_FILE(4)='DATA\INFANT_305_TRANSFUSIONS.DAT'
TRX_FILE(5)='DATA\INFANT_306_TRANSFUSIONS.DAT'
TRX_FILE(6)='DATA\INFANT_307_TRANSFUSIONS.DAT'
TRX_FILE(7)='DATA\INFANT_308_TRANSFUSIONS.DAT'
TRX_FILE(8)='DATA\INFANT_309_TRANSFUSIONS.DAT'
TRX_FILE(9)='DATA\INFANT_310_TRANSFUSIONS.DAT'
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TRX_FILE(10)='DATA\INFANT_311_TRANSFUSIONS.DAT'
TRX_FILE(11)='DATA\INFANT_312_TRANSFUSIONS.DAT'
TRX_FILE(12)='DATA\INFANT_313_TRANSFUSIONS.DAT'
TRX_FILE(13)='DATA\INFANT_314_TRANSFUSIONS.DAT'
TRX_FILE(14)='DATA\INFANT_315_TRANSFUSIONS.DAT'
TRX_FILE(15)='DATA\INFANT_316_TRANSFUSIONS.DAT'
TRX_FILE(16)='DATA\INFANT_317_TRANSFUSIONS.DAT'
TRX_FILE(17)='DATA\INFANT_318_TRANSFUSIONS.DAT'
TRX_FILE(18)='DATA\INFANT_319_TRANSFUSIONS.DAT'
TRX_FILE(19)='DATA\INFANT_320_TRANSFUSIONS.DAT'
TRX_FILE(20)='DATA\INFANT_321_TRANSFUSIONS.DAT'
TRX_FILE(21)='DATA\INFANT_322_TRANSFUSIONS.DAT'
TRX_FILE(22)='DATA\INFANT_323_TRANSFUSIONS.DAT'
TRX_FILE(23)='DATA\INFANT_324_TRANSFUSIONS.DAT'
TRX_FILE(24)='DATA\INFANT_325_TRANSFUSIONS.DAT'
TRX_FILE(25)='DATA\INFANT_326_TRANSFUSIONS.DAT'
TRX_FILE(26)='DATA\INFANT_327_TRANSFUSIONS.DAT'

WEIGHT_FILE(1)='DATA\INFANT_302_BODYWEIGHTS.DAT'
WEIGHT_FILE(2)='DATA\INFANT_303_BODYWEIGHTS.DAT'
WEIGHT_FILE(3)='DATA\INFANT_304_BODYWEIGHTS.DAT'
WEIGHT_FILE(4)='DATA\INFANT_305_BODYWEIGHTS.DAT'
WEIGHT_FILE(5)='DATA\INFANT_306_BODYWEIGHTS.DAT'
WEIGHT_FILE(6)='DATA\INFANT_307_BODYWEIGHTS.DAT'
WEIGHT_FILE(7)='DATA\INFANT_308_BODYWEIGHTS.DAT'
WEIGHT_FILE(8)='DATA\INFANT_309_BODYWEIGHTS.DAT'
WEIGHT_FILE(9)='DATA\INFANT_310_BODYWEIGHTS.DAT'
WEIGHT_FILE(10)='DATA\INFANT_311_BODYWEIGHTS.DAT'
WEIGHT_FILE(11)='DATA\INFANT_312_BODYWEIGHTS.DAT'
WEIGHT_FILE(12)='DATA\INFANT_313_BODYWEIGHTS.DAT'
WEIGHT_FILE(13)='DATA\INFANT_314_BODYWEIGHTS.DAT'
WEIGHT_FILE(14)='DATA\INFANT_315_BODYWEIGHTS.DAT'
WEIGHT_FILE(15)='DATA\INFANT_316_BODYWEIGHTS.DAT'
WEIGHT_FILE(16)='DATA\INFANT_317_BODYWEIGHTS.DAT'
WEIGHT_FILE(17)='DATA\INFANT_318_BODYWEIGHTS.DAT'
WEIGHT_FILE(18)='DATA\INFANT_319_BODYWEIGHTS.DAT'
WEIGHT_FILE(19)='DATA\INFANT_320_BODYWEIGHTS.DAT'
WEIGHT_FILE(20)='DATA\INFANT_321_BODYWEIGHTS.DAT'
WEIGHT_FILE(21)='DATA\INFANT_322_BODYWEIGHTS.DAT'
WEIGHT_FILE(22)='DATA\INFANT_323_BODYWEIGHTS.DAT'
WEIGHT_FILE(23)='DATA\INFANT_324_BODYWEIGHTS.DAT'
WEIGHT_FILE(24)='DATA\INFANT_325_BODYWEIGHTS.DAT'
WEIGHT_FILE(25)='DATA\INFANT_326_BODYWEIGHTS.DAT'
WEIGHT_FILE(26)='DATA\INFANT_327_BODYWEIGHTS.DAT'

PHLE_FILE(1)='DATA\INFANT_302_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(2)='DATA\INFANT_303_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(3)='DATA\INFANT_304_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(4)='DATA\INFANT_305_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(5)='DATA\INFANT_306_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(6)='DATA\INFANT_307_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(7)='DATA\INFANT_308_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(8)='DATA\INFANT_309_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(9)='DATA\INFANT_310_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(10)='DATA\INFANT_311_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(11)='DATA\INFANT_312_PHLEBOTOMY_VOLUMES.DAT'
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PHLE_FILE(12)='DATA\INFANT_313_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(13)='DATA\INFANT_314_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(14)='DATA\INFANT_315_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(15)='DATA\INFANT_316_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(16)='DATA\INFANT_317_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(17)='DATA\INFANT_318_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(18)='DATA\INFANT_319_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(19)='DATA\INFANT_320_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(20)='DATA\INFANT_321_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(21)='DATA\INFANT_322_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(22)='DATA\INFANT_323_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(23)='DATA\INFANT_324_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(24)='DATA\INFANT_325_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(25)='DATA\INFANT_326_PHLEBOTOMY_VOLUMES.DAT'
PHLE_FILE(26)='DATA\INFANT_327_PHLEBOTOMY_VOLUMES.DAT'

PHLE_FILE_KG(1)='DATA\INFANT_302_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(2)='DATA\INFANT_303_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(3)='DATA\INFANT_304_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(4)='DATA\INFANT_305_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(5)='DATA\INFANT_306_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(6)='DATA\INFANT_307_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(7)='DATA\INFANT_308_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(8)='DATA\INFANT_309_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(9)='DATA\INFANT_310_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(10)='DATA\INFANT_311_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(11)='DATA\INFANT_312_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(12)='DATA\INFANT_313_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(13)='DATA\INFANT_314_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(14)='DATA\INFANT_315_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(15)='DATA\INFANT_316_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(16)='DATA\INFANT_317_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(17)='DATA\INFANT_318_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(18)='DATA\INFANT_319_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(19)='DATA\INFANT_320_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(20)='DATA\INFANT_321_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(21)='DATA\INFANT_322_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(22)='DATA\INFANT_323_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(23)='DATA\INFANT_324_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(24)='DATA\INFANT_325_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(25)='DATA\INFANT_326_PHLEBOTOMY_VOLUMES_KG.DAT'
PHLE_FILE_KG(26)='DATA\INFANT_327_PHLEBOTOMY_VOLUMES_KG.DAT'

THRESH_FILE(1)='DATA\THRESH_302.TXT'
THRESH_FILE(2)='DATA\THRESH_303.TXT'
THRESH_FILE(3)='DATA\THRESH_304.TXT'
THRESH_FILE(4)='DATA\THRESH_305.TXT'
THRESH_FILE(5)='DATA\THRESH_306.TXT'
THRESH_FILE(6)='DATA\THRESH_307.TXT'
THRESH_FILE(7)='DATA\THRESH_308.TXT'
THRESH_FILE(8)='DATA\THRESH_309.TXT'
THRESH_FILE(9)='DATA\THRESH_310.TXT'
THRESH_FILE(10)='DATA\THRESH_311.TXT'
THRESH_FILE(11)='DATA\THRESH_312.TXT'
THRESH_FILE(12)='DATA\THRESH_313.TXT'
THRESH_FILE(13)='DATA\THRESH_314.TXT'
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THRESH_FILE(14)='DATA\THRESH_315.TXT'
THRESH_FILE(15)='DATA\THRESH_316.TXT'
THRESH_FILE(16)='DATA\THRESH_317.TXT'
THRESH_FILE(17)='DATA\THRESH_318.TXT'
THRESH_FILE(18)='DATA\THRESH_319.TXT'
THRESH_FILE(19)='DATA\THRESH_320.TXT'
THRESH_FILE(20)='DATA\THRESH_321.TXT'
THRESH_FILE(21)='DATA\THRESH_322.TXT'
THRESH_FILE(22)='DATA\THRESH_323.TXT'
THRESH_FILE(23)='DATA\THRESH_324.TXT'
THRESH_FILE(24)='DATA\THRESH_325.TXT'
THRESH_FILE(25)='DATA\THRESH_326.TXT'
THRESH_FILE(26)='DATA\THRESH_327.TXT'

GA(1)=192
GA(2)=185
GA(3)=179
GA(4)=193
GA(5)=197
GA(6)=189
GA(7)=192
GA(8)=175
GA(9)=184
GA(10)=200
GA(11)=185
GA(12)=194
GA(13)=184
GA(14)=173
GA(15)=175
GA(16)=187
GA(17)=198
GA(18)=186
GA(19)=194
GA(20)=189
GA(21)=170
GA(22)=196
GA(23)=177
GA(24)=195
GA(25)=169
GA(26)=191

EMAXI(1)=0.7187
EMAXI(2)=0.79529
EMAXI(3)=0.57204
EMAXI(4)=0.87313
EMAXI(5)=1.0587
EMAXI(6)=1.5869
EMAXI(7)=1.5901
EMAXI(8)=0.40535
EMAXI(9)=0.47913
EMAXI(10)=0.9972
EMAXI(11)=0.49248
EMAXI(12)=0.82134
EMAXI(13)=1.0126
EMAXI(14)=0.52835
EMAXI(15)=0.30943

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EMAXI(16)=0.27923
EMAXI(17)=0.8056
EMAXI(18)=0.85498
EMAXI(19)=0.8156
EMAXI(20)=0.88296
EMAXI(21)=0.18854
EMAXI(22)=1.434
EMAXI(23)=0.5009
EMAXI(24)=1.0133
EMAXI(25)=0.38503
EMAXI(26)=0.5555

EC50I(1)=61.686
EC50I(2)=97.676
EC50I(3)=7.2515
EC50I(4)=39.38
EC50I(5)=38.268
EC50I(6)=15.68
EC50I(7)=310.84
EC50I(8)=13.265
EC50I(9)=11.768
EC50I(10)=50.091
EC50I(11)=34.117
EC50I(12)=99.772
EC50I(13)=110.15
EC50I(14)=19.684
EC50I(15)=15.563
EC50I(16)=7.3872
EC50I(17)=38.257
EC50I(18)=181.98
EC50I(19)=44.741
EC50I(20)=59.889
EC50I(21)=877.93
EC50I(22)=80.532
EC50I(23)=105.55
EC50I(24)=144.96
EC50I(25)=243.62
EC50I(26)=31.599

END SUBROUTINE FILE_NAMES
```

C.4.3 HGB_CHANGE.F90 (Original Code)

```
SUBROUTINE TRX_PRED

USE SHARED

IMPLICIT NONE

INTEGER :: TOTAL_TRX=0, i, j=0, STATUS, k, final_data_points=0, TRX_USED
REAL*8 :: VOL_TRX_KG(101), HGB_TRX_KG(101), WEIGHT, HGB, TRX_DUR=0.3D0 !THIS IS
THE APPROX DURATION OF A TRX
REAL*8 :: HGB_CHANGE(101), HGB_PER_CHANGE(101), HGB_MAX, HGB_MIN,
VOL_TRX(101), HGB_TRX(101), DUMMY_TRX(101)=7D0 !THIS LAST ARRAY IS GRAPHICAL
MARKER FOR TRX
```

```
REAL*8 :: HGB_MIN_G(1), BLOOD_VOLUME(100)=0D0,
BLOOD_VOLUME_WT(100),BLOOD_VOLUME_AGE(100), BLOOD_VOLUME_PNA(100) !THIS
IS A NEEDED ARRAY JUST FOR GRAPHING
REAL*8 :: FINAL_HB_CHANGE(101), FINAL_AMOUNT_TRX(101),
PRED_HGB_CHANGE(101), PERCENT_ERROR, SD=0D0
REAL*8 :: IDENTITY_X(2), IDENTITY_Y(2) !GRAPHICAL LINE OF IDENTITY
EXTERNAL :: WEIGHT, HGB

DO FILENUM=1,26
CALL FILE_OPEN
AVE_BV(FILENUM)=0D0

TRX_USED=0
TOTAL_TRX=TOTAL_TRX+TRX_POINTS(FILENUM)

DO i=1,TRX_POINTS(FILENUM)
!IF (TRX_T(i)>28D0) CYCLE !TAKE OUT TRX PAST TIME 28 DAYS
j=j+1
HGB_MAX=0D0
HGB_MIN=1D8
DO k=1,150 !loop to find max hb after trx
IF (HGB(TRX_T(i)+DBLE(K)/100D0)>HGB_MAX)THEN
HGB_MAX=HGB(TRX_T(i)+DBLE(K)/100D0)
END IF
IF (HGB(TRX_T(i)-DBLE(K)/100D0)<HGB_MIN)THEN
IF(TRX_T(i)-DBLE(K)/100D0<=0.1D0)CYCLE !DONT CHECK TIME POINTS BEFORE 0
HGB_MIN=HGB(TRX_T(i)-DBLE(K)/100D0)
END IF
END DO
!WRITE(*,*) HGB_MAX
!READ*
VOL_TRX_KG(j)=TRX_V(i)/WEIGHT(TRX_T(i)) !/(GESTATIONAL_AGE(FILENUM)+TRX_T(i))
HGB_TRX_KG(j)=TRX_H(i)/WEIGHT(TRX_T(i))
VOL_TRX(j)=TRX_V(i)
HGB_TRX(j)=TRX_H(i)
!HGB_CHANGE(j)=HGB(TRX_T(i)+TRX_DUR)-HGB(TRX_T(i))
!HGB_CHANGE(j)=HGB_MAX-HGB(TRX_T(i))
HGB_CHANGE(j)=HGB_MAX-HGB_MIN
HGB_DELTA(FILENUM,i)=HGB_CHANGE(j)
HGB_PER_CHANGE(j)=(HGB_MAX-HGB(TRX_T(i)))/HGB(TRX_T(i))

HGB_MIN_G(1)=HGB_MIN !GRAPHING PURPOSES

IF (j==6 .OR. j==18 .OR. j==22 .OR. j==25 .OR. j==73 .OR. j==81) CYCLE
TRX_USED=TRX_USED+1
final_data_points=final_data_points+1 !THIS GIVES THE FINAL NUMBER OF DATA POINTS
EXCLUDING SEVERAL TRX
BLOOD_VOLUME_PNA(final_data_points)=TRX_T(i)+GA(FILENUM)
BLOOD_VOLUME_AGE(final_data_points)=TRX_T(i)
BLOOD_VOLUME_WT(final_data_points)=WEIGHT(TRX_T(i))
BLOOD_VOLUME(final_data_points)=VOL_TRX_KG(j)*0.283D0/(HGB_CHANGE(j)/100D0)
!WRITE(*,*) 'SUBJECT', FILENUM+301, 'BLOOD VOLUME',
BLOOD_VOLUME(FINAL_DATA_POINTS)
!READ*
```

```
AVE_BV(FILENUM)=AVE_BV(FILENUM)+BLOOD_VOLUME(final_data_points) !NEED TO
CREATE SOMETHING TO KEEP TRACK OF WHEN FILENUM SWITCHES AND GET
AVERAGE
IF (i==TRX_POINTS(FILENUM) .OR. j==17 .OR. j==21 .OR. j==72) THEN !THIS IS LAST TRX
FOR GIVEN FILE
AVE_BV(FILENUM)=AVE_BV(FILENUM)/TRX_USED
END IF
FINAL_HB_CHANGE(final_data_points)=HGB_CHANGE(j)
FINAL_AMOUNT_TRX(final_data_points)=VOL_TRX_KG(j)
END DO

CALL UNALLOCATE

END DO

!CALL ADD_POINTS(BLOOD_VOLUME_WT, BLOOD_VOLUME, final_data_points)
!CALL PLOT_IN_AREA(1,4)
!CALL ADD_POINTS(BLOOD_VOLUME_AGE, BLOOD_VOLUME, final_data_points)
!CALL PLOT_IN_AREA(2,4)
!CALL ADD_POINTS(BLOOD_VOLUME_PNA, BLOOD_VOLUME, final_data_points)
!CALL PLOT_IN_AREA(3,4)
!CALL DISPLAY_PLOT

!OPEN (UNIT=20, FILE='BLOOD_VOLUME.TXT', STATUS='OLD', ACTION= 'WRITE',
IOSTAT=status)
!DO i=1,final_data_points
!WRITE(20,*) BLOOD_VOLUME_WT(i), BLOOD_VOLUME(i)
!END DO
!WRITE(20,*) ''
!DO i=1,final_data_points
!WRITE(20,*) BLOOD_VOLUME_AGE(i), BLOOD_VOLUME(i)
!END DO
!WRITE(20,*) ''
!DO i=1,final_data_points
!WRITE(20,*) BLOOD_VOLUME_PNA(i), BLOOD_VOLUME(i)
!END DO

AVE_BV(20)=93.2D0 !WE CANT GET A BV MEASUREMENT SO USE AVERAGE OF OTHERS

!DO k=1,FINAL_DATA_POINTS
!PRED_HGB_CHANGE(k)=FINAL_AMOUNT_TRX(k)*28.3D0/(SUM(BLOOD_VOLUME(1:100))/
DBLE(FINAL_DATA_POINTS))
!PERCENT_ERROR=PERCENT_ERROR+ABS((PRED_HGB_CHANGE(k)-
FINAL_HB_CHANGE(k))/FINAL_HB_CHANGE(k))*100D0
!SD=SD+(BLOOD_VOLUME(k)-
SUM(BLOOD_VOLUME(1:FINAL_DATA_POINTS))/DBLE(FINAL_DATA_POINTS))**2
!END DO
!IDENTITY_X(1)=0D0; IDENTITY_X(2)=8D0; IDENTITY_Y(1)=0D0; IDENTITY_Y(2)=8D0

END SUBROUTINE TRX_PRED
```

C.4.4 GRAPH_CUM_PHLE2.F90 (Original code)

```
SUBROUTINE GRAPH_CUM_PHLE2
```

```
!THIS VERSION USES INITIAL POINTS INSTEAD OF EXTRAPOLATING BACK TO 0

USE SHARED

IMPLICIT NONE

REAL*8,ALLOCATABLE,DIMENSION(:):: CUM_HGB_REM_KG, CUM_HGB_TRX_KG,
CUM_HGB_REM, CUM_HGB_TRX, CUM_REM_BIRTH, CUM_TRX_BIRTH
REAL*8 :: CUM_TRX_SUM_KG(26), CUM_PHLE_SUM_KG(26), CUM_TRX_SUM(26),
CUM_PHLE_SUM(26), SUM_BIRTH_REM(26), SUM_BIRTH_TRX(26)
REAL*8 :: HGB, WEIGHT, HGB_BIRTH_X(2), HGB_BIRTH_Y(2), SUM_HGB_BIRTH(26), SD
INTEGER :: i
EXTERNAL :: HGB, WEIGHT, SD

HGB_BIRTH_X(1)=0D0; HGB_BIRTH_X(1)=35D0
!CUM_TRX_SUM_KG=0D0; CUM_PHLE_SUM_KG=0D0
!CUM_TRX_SUM=0D0; CUM_PHLE_SUM=0D0

!WRITE(*,*) SD(26, AVE_BV)
!READ*

DO FILENUM=1,26
CALL FILE_OPEN
ALLOCATE(CUM_HGB_REM_KG(PHLE_POINTS_KG(FILENUM)))
ALLOCATE(CUM_HGB_TRX_KG(TRX_POINTS(FILENUM)))
ALLOCATE(CUM_HGB_REM(PHLE_POINTS_KG(FILENUM)))
ALLOCATE(CUM_HGB_TRX(TRX_POINTS(FILENUM)))
ALLOCATE(CUM_REM_BIRTH(PHLE_POINTS_KG(FILENUM)))
ALLOCATE(CUM_TRX_BIRTH(TRX_POINTS(FILENUM)))

CUM_HGB_REM_KG=0D0 ; CUM_HGB_TRX_KG=0D0
CUM_HGB_REM=0D0 ; CUM_HGB_TRX=0D0
CUM_REM_BIRTH=0D0; CUM_TRX_BIRTH=0D0

CUM_HGB_REM_KG(1)=PHLE_Y_KG(1)*HGB(PHLE_X_KG(1))/100D0 ;
CUM_HGB_TRX_KG(1)=TRX_H(1)/WEIGHT(TRX_T(1))
CUM_HGB_REM(1)=PHLE_Y_KG(1)*HGB(PHLE_X_KG(1))/100D0*WEIGHT(PHLE_X_KG(1)) ;
CUM_HGB_TRX(1)=TRX_H(1)
CUM_REM_BIRTH(1)=PHLE_Y_KG(1)*WEIGHT(PHLE_X_KG(1))*HGB(PHLE_X_KG(1))/(WEIG
HT_Y(1)*100D0) ; CUM_TRX_BIRTH(1)=TRX_H(1)/WEIGHT_Y(1)

DO i=2,PHLE_POINTS_KG(FILENUM)
CUM_HGB_REM_KG(i)=CUM_HGB_REM_KG(i-
1)+PHLE_Y_KG(i)*HGB(PHLE_X_KG(i))/100D0
CUM_HGB_REM(i)=CUM_HGB_REM(i-
1)+PHLE_Y_KG(i)*HGB(PHLE_X_KG(i))/100D0*WEIGHT(PHLE_X_KG(i))
CUM_REM_BIRTH(i)=CUM_REM_BIRTH(i-
1)+PHLE_Y_KG(i)*WEIGHT(PHLE_X_KG(i))*HGB(PHLE_X_KG(i))/(WEIGHT_Y(1)*100D0)
END DO
DO i=2,TRX_POINTS(FILENUM)
CUM_HGB_TRX_KG(i)=CUM_HGB_TRX_KG(i-1)+TRX_H(i)/WEIGHT(TRX_T(i))
CUM_HGB_TRX(i)=CUM_HGB_TRX(i-1)+TRX_H(i)
CUM_TRX_BIRTH(i)=CUM_TRX_BIRTH(i-1)+TRX_H(i)/WEIGHT_Y(1)
END DO

CUM_TRX_SUM_KG(FILENUM)=CUM_HGB_TRX_KG(TRX_POINTS(FILENUM))
```

```
CUM_PHLE_SUM_KG(FILENUM)=CUM_HGB_REM_KG(PHLE_POINTS_KG(FILENUM))

CUM_TRX_SUM(FILENUM)=CUM_HGB_TRX(TRX_POINTS(FILENUM))
CUM_PHLE_SUM(FILENUM)=CUM_HGB_REM(PHLE_POINTS_KG(FILENUM))

SUM_BIRTH_REM(FILENUM)=CUM_REM_BIRTH(PHLE_POINTS_KG(FILENUM))
SUM_BIRTH_TRX(FILENUM)=CUM_TRX_BIRTH(TRX_POINTS(FILENUM))

HGB_BIRTH_Y(1)=AVE_BV(FILENUM)*WEIGHT_Y(1)*HGB_Y(1)/100D0;
HGB_BIRTH_Y(2)=HGB_BIRTH_Y(1) !THIS IS HB AMOUNT AT BIRTH
!HGB_BIRTH_Y(1)=AVE_BV(FILENUM)*HGB_Y(1)/100D0;
HGB_BIRTH_Y(2)=HGB_BIRTH_Y(1) !THIS IS HGB/KG BIRTH WEIGHT
SUM_HGB_BIRTH(FILENUM)=HGB_BIRTH_Y(1)
CUM_HGB_REM=CUM_HGB_REM/HGB_BIRTH_Y(1)*100D0 !THIS PUTS HB AMOUNT IN %

!CALL ADD_POINTS(PHLE_X_KG, CUM_HGB_REM_KG, PHLE_POINTS_KG(FILENUM))
!CALL INCLUDE_POINTS_RIGHT(TRX_T, CUM_HGB_TRX_KG, TRX_POINTS(FILENUM),1)
!CALL ADD_CURVE(HGB_BIRTH_X, HGB_BIRTH_Y, 2)
CALL ADD_POINTS(PHLE_X_KG, CUM_HGB_REM, PHLE_POINTS_KG(FILENUM))
!CALL INCLUDE_POINTS_RIGHT(TRX_T, CUM_HGB_TRX, TRX_POINTS(FILENUM),1)
!CALL ADD_POINTS(PHLE_X_KG, CUM_REM_BIRTH, PHLE_POINTS_KG(FILENUM))
!CALL INCLUDE_POINTS_RIGHT(TRX_T, CUM_TRX_BIRTH, TRX_POINTS(FILENUM),1)
CALL TITLE_WITH_INTEGER_NUMBER_ADDED('SUBJECT',filenum+301)
CALL X_LABEL ('DAY OF LIFE')
!CALL LEFT_LABEL('CUM HGB REM PER KG')
!CALL RIGHT_LABEL('CUM HGB TRX PER KG')
!CALL LEFT_LABEL('CUM HGB REM')
CALL LEFT_LABEL('PERCENT BIRTH HB REM')
!CALL RIGHT_LABEL('CUM HGB TRX')
!CALL LEFT_LABEL('CUM HGB REM PER KG BW')
!CALL RIGHT_LABEL('CUM HGB TRX PER KG BW')
CALL BEGIN_X_AT(0D0)
CALL END_X_AT(30D0)
CALL BEGIN_LEFT_AT(0D0)
CALL END_LEFT_AT(120D0)
!CALL BEGIN_RIGHT_AT(0D0)
!CALL END_RIGHT_AT(35D0)
CALL PLOT_IN_AREA(FILENUM,26)

DEALLOCATE(CUM_HGB_REM_KG)
DEALLOCATE(CUM_HGB_TRX_KG)
DEALLOCATE(CUM_HGB_REM)
DEALLOCATE(CUM_HGB_TRX)
DEALLOCATE(CUM_REM_BIRTH)
DEALLOCATE(CUM_TRX_BIRTH)
CALL UNALLOCATE
END DO

!WRITE(*,*) 'AVE BLOOD TRX PER KG', CUM_TRX_SUM_KG/26D0
!WRITE(*,*) 'AVE BLOOD PHLE PER KG', CUM_PHLE_SUM_KG/26D0
!WRITE(*,*) 'AVE BLOOD TRX', CUM_TRX_SUM/26D0
!WRITE(*,*) 'AVE BLOOD PHLE', CUM_PHLE_SUM/26D0
!WRITE(*,*) 'AVE BLOOD TRX PER KG BIRTH WEIGHT',SUM_BIRTH_TRX/26D0
!WRITE(*,*) 'AVE BLOOD PHLE PER KG BIRTH WEIGHT',SUM_BIRTH_REM/26D0
!WRITE(*,*) 'AVE BIRTH HGB AMOUNT', SUM_HGB_BIRTH/26D0
```

```
WRITE(*,*) 'AVE BLOOD TRX PER KG', SUM(CUM_TRX_SUM_KG(1:26))/26D0, SD(26,
CUM_TRX_SUM_KG)
WRITE(*,*) 'AVE BLOOD PHLE PER KG', SUM(CUM_PHLE_SUM_KG(1:26))/26D0, SD(26,
CUM_PHLE_SUM_KG)
WRITE(*,*) 'AVE BLOOD TRX', SUM(CUM_TRX_SUM(1:26))/26D0, SD(26, CUM_TRX_SUM)
WRITE(*,*) 'AVE BLOOD PHLE', SUM(CUM_PHLE_SUM(1:26))/26D0, SD(26,
CUM_PHLE_SUM)
WRITE(*,*) 'AVE BLOOD TRX PER KG BIRTH WEIGHT',SUM(SUM_BIRTH_TRX(1:26))/26D0,
SD(26, SUM_BIRTH_TRX)
WRITE(*,*) 'AVE BLOOD PHLE PER KG BIRTH WEIGHT',SUM(SUM_BIRTH_REM(1:26))/26D0,
SD(26, SUM_BIRTH_REM)
WRITE(*,*) 'AVE BIRTH HGB AMOUNT', SUM(SUM_HGB_BIRTH(1:26))/26D0, SD(26,
SUM_HGB_BIRTH)

CALL DISPLAY_PLOT

END SUBROUTINE GRAPH_CUM_PHLE2
```

C.4.5 GRAPH_PINT.F90 (Original code)

```
SUBROUTINE GRAPH_PINT

USE SHARED

IMPLICIT NONE

REAL*8,ALLOCATABLE,DIMENSION(:):: HGB_WHEN_TRX, PINT, PINT_HIGH, PINT_MOD,
PINT_HIGH_MOD
REAL*8 :: HGB, IDENTITY_X(2), IDENTITY_Y(2), PINT_CAP(8), PINT_CEN(8),
HGB_WHEN_TRX_CAP(8), HGB_WHEN_TRX_CEN(8)!THIS SHOULD ADJUST GRAPHING
FOR DIFF POINTS
INTEGER :: i, j, CURRENT_DRAW, k, CAP, CEN
INTEGER:: ML1=0, ML2=0, ML3=0, ML4=0
EXTERNAL :: HGB

IDENTITY_X(1)=6D0
IDENTITY_X(2)=16D0
IDENTITY_Y(1)=6D0
IDENTITY_Y(2)=16D0

DO FILENUM=1,26
CALL FILE_OPEN
ALLOCATE(HGB_WHEN_TRX(TRX_POINTS(FILENUM)))
ALLOCATE(PINT(TRX_POINTS(FILENUM)))
ALLOCATE(PINT_HIGH(TRX_POINTS(FILENUM)))
ALLOCATE(PINT_MOD(TRX_POINTS(FILENUM)))
ALLOCATE(PINT_HIGH_MOD(TRX_POINTS(FILENUM)))

PINT_CAP=0D0; PINT_CEN=0D0; CAP=0; CEN=0

DO i=1,TRX_POINTS(FILENUM)
!HGB_WHEN_TRX(i)=HGB(TRX_T(i))
HGB_WHEN_TRX(i)=THRESH_Y(i+1)
!DO k=1,50
!IF (HGB(TRX_T(i)-DBLE(k))< HGB_WHEN_TRX(i)) THEN
```

```
!HGB_WHEN_TRX(i)=HGB(TRX_T(i)-DBLE(k)/100D0)
!END IF
!END DO
IF (TRX_T(i)<=7D0) THEN
        DO j=1,HGB_POINTS(FILENUM)
        IF (HGB_X(j)<TRX_T(i)) THEN
        CURRENT_DRAW=SYRINGE(j)
        END IF
        END DO
PINT(i)=10.4D0
PINT_HIGH(i)=12.2D0
PINT_MOD(i)=10.4D0+1.1D0*ABS((DBLE(CURRENT_DRAW)-1D0))
PINT_HIGH_MOD(i)=12.2D0+1.3D0*ABS((DBLE(CURRENT_DRAW)-1D0))
ELSE IF (TRX_T(i) <= 14D0) THEN
PINT(i)=9D0
PINT_HIGH(i)=10.9D0
PINT_MOD(i)=9D0+1D0*ABS((DBLE(CURRENT_DRAW)-1D0))
PINT_HIGH_MOD(i)=10.9D0+1.1D0*ABS((DBLE(CURRENT_DRAW)-1D0))
ELSE
PINT(i)=7.7D0
PINT_HIGH(i)=9.0D0
PINT_MOD(i)=7.7D0+0.8D0*ABS((DBLE(CURRENT_DRAW)-1D0))
PINT_HIGH_MOD(i)=9.0D0+1D0*ABS((DBLE(CURRENT_DRAW)-1D0))
END IF
IF (HGB_WHEN_TRX(i)>PINT(i))  ML1=ML1+1 !WE HAVE A MORE LIBERAL TRX
IF (HGB_WHEN_TRX(i)>PINT_HIGH(i))  ML2=ML2+1 !WE HAVE A MORE LIBERAL TRX
IF (HGB_WHEN_TRX(i)>PINT_MOD(i))  ML3=ML3+1 !WE HAVE A MORE LIBERAL TRX
IF (HGB_WHEN_TRX(i)>PINT_HIGH_MOD(i))  ML4=ML4+1 !WE HAVE A MORE LIBERAL TRX
IF (PINT_MOD(i)==10.4D0 .OR. PINT_MOD(i)==9D0 .OR. PINT_MOD(i)==7.7D0) THEN !WE
HAVE A CENTRAL POINT
CEN=CEN+1
PINT_CEN(CEN)=PINT_MOD(i)
HGB_WHEN_TRX_CEN(CEN)=HGB_WHEN_TRX(i)
ELSE
CAP=CAP+1
PINT_CAP(CAP)=PINT_MOD(i)
HGB_WHEN_TRX_CAP(CAP)=HGB_WHEN_TRX(i)
END IF
END DO

IF (FILENUM ==26) CALL TITLE_WITH_INTEGER_NUMBER_ADDED('LOW THRESH',ML1)
CALL X_LABEL ('HGB WHEN TRX ACTUAL')
CALL LEFT_LABEL('HGB WHEN TRX PINT')
CALL BEGIN_X_AT(6D0)
CALL END_X_AT(16D0)
CALL BEGIN_LEFT_AT(6D0)
CALL END_LEFT_AT(16D0)
CALL INCLUDE_POINTS (HGB_WHEN_TRX, PINT, TRX_POINTS(FILENUM),1)
CALL ADD_CURVE(IDENTITY_X, IDENTITY_Y, 2)
CALL PLOT_IN_AREA(1,4)

IF (FILENUM ==26) CALL TITLE_WITH_INTEGER_NUMBER_ADDED('HIGH THRESH',ML2)
CALL X_LABEL ('HGB WHEN TRX ACTUAL')
CALL LEFT_LABEL('HGB WHEN TRX PINT')
CALL BEGIN_X_AT(6D0)
CALL END_X_AT(16D0)
```

```
CALL BEGIN_LEFT_AT(6D0)
CALL END_LEFT_AT(16D0)
CALL INCLUDE_POINTS (HGB_WHEN_TRX, PINT_HIGH, TRX_POINTS(FILENUM),1)
CALL ADD_CURVE(IDENTITY_X, IDENTITY_Y, 2)
CALL PLOT_IN_AREA(2,4)

IF (FILENUM ==26) CALL TITLE_WITH_INTEGER_NUMBER_ADDED('LOW THRESH W
ADJUSTMENT',ML3)
CALL X_LABEL ('HGB WHEN TRX ACTUAL')
CALL LEFT_LABEL('HGB WHEN TRX PINT')
CALL BEGIN_X_AT(6D0)
CALL END_X_AT(16D0)
CALL BEGIN_LEFT_AT(6D0)
CALL END_LEFT_AT(16D0)
!CALL INCLUDE_POINTS (HGB_WHEN_TRX, PINT_MOD, TRX_POINTS(FILENUM),1)
CALL INCLUDE_POINTS (HGB_WHEN_TRX_CEN, PINT_CEN, CEN,1)
CALL INCLUDE_POINTS (HGB_WHEN_TRX_CAP, PINT_CAP, CAP,2)
CALL ADD_CURVE(IDENTITY_X, IDENTITY_Y, 2)
CALL PLOT_IN_AREA(3,4)

IF (FILENUM ==26) CALL TITLE_WITH_INTEGER_NUMBER_ADDED('HIGH THRESH W
ADJUSTMENT',ML4)
CALL X_LABEL ('HGB WHEN TRX ACTUAL')
CALL LEFT_LABEL('HGB WHEN TRX PINT')
CALL BEGIN_X_AT(6D0)
CALL END_X_AT(16D0)
CALL BEGIN_LEFT_AT(6D0)
CALL END_LEFT_AT(16D0)
CALL INCLUDE_POINTS (HGB_WHEN_TRX, PINT_HIGH_MOD, TRX_POINTS(FILENUM),1)
CALL ADD_CURVE(IDENTITY_X, IDENTITY_Y, 2)
CALL PLOT_IN_AREA(4,4)

!CALL ADD_CURVE(HGB_X, HGB_Y, HGB_POINTS(FILENUM))
!CALL ADD_POINTS(TRX_T, HGB_WHEN_TRX, TRX_POINTS(FILENUM))
!CALL PLOT_IN_AREA(FILENUM,26)

DEALLOCATE(HGB_WHEN_TRX)
DEALLOCATE(PINT)
DEALLOCATE(PINT_HIGH)
DEALLOCATE(PINT_MOD)
DEALLOCATE(PINT_HIGH_MOD)
CALL UNALLOCATE
END DO

CALL DISPLAY_PLOT

END SUBROUTINE GRAPH_PINT
```

C.5 Fortran subroutines for Chapter 6

C.5.1 MAIN_V_5.F90 (Original Code)

```
PROGRAM PHLE_RED
```

```
!THIS IS VERSION 5 WHICH DOES THE GRAPHING FOR DIFFERENT PHLE REDUCTION
PERCENTAGES

USE SHARED

IMPLICIT NONE

INTEGER :: i,j,k, status, VENOUS=0, PHLEBOTOMIES(26)=0, VLBW_AVOID=0,
ELBW_AVOID=0, VLBW_AVOID_PINT=0, ELBW_AVOID_PINT=0
INTEGER :: LOOP
INTEGER, PARAMETER :: NAT_HB_DAYS=30, PHLE_RED_POINTS=101
REAL*8 :: WEIGHT, ALIFESPAN=120D0, ILIFESPAN=65.8D0, PHLE_RED_FACTOR !THIS IS
PERCENT OF REMOVED BLOOD THAT WILL STAY IN FOR SIMULATION
REAL*8 :: VOL_KG_ADDED, AMOUNT_HGB_ADDED, HGB, THRESH, PINT,
SIM_TRX_HGB=83.1D0/3D0
REAL*8 :: SIM_TRX_X(10), SIM_TRX_Y(10)=7.0D0, SIM_TRX_X_PINT(10),
TRX_LENGTH=5D0/24D0
REAL*8 :: TEMP1, TEMP2, SD1, SD2, MEAN1, MEAN2, SD, TEMP_PHLE_PROD,
DELTA_HGB, HGB_NAT(26,NAT_HB_DAYS), HGB_NAT_AVE_X(NAT_HB_DAYS),
HGB_NAT_AVE_Y(NAT_HB_DAYS)
REAL*8 :: HGB_NAT_AVE_Y_PLUS(NAT_HB_DAYS),
HGB_NAT_AVE_Y_MINUS(NAT_HB_DAYS), TEMP_SD(26), TEMP_SD_WEIGHT(26),
WEIGHT_SUM(NAT_HB_DAYS), CONS_PROD_HB(NAT_HB_DAYS),
WEIGHT_AVE_Y(NAT_HB_DAYS)
REAL*8 :: WEIGHT_AVE_Y_PLUS(NAT_HB_DAYS),
WEIGHT_AVE_Y_MINUS(NAT_HB_DAYS), WEIGHT_SUM_ALL(26,NAT_HB_DAYS)
REAL*8 :: HGB_PROD_SPLINE, HGB_AMT_AVE(NAT_HB_DAYS), HGB_AMT_SEN_X(2),
HGB_AMT_SEN_Y(2)
REAL*8 :: GRAPH_PER_RED_X(PHLE_RED_POINTS),
VLBW_RBCTX_PER_INFANT_PINT_Y(PHLE_RED_POINTS),
VLBW_RBCTX_PER_INFANT_Y(PHLE_RED_POINTS),
VLBW_AVOID_Y(PHLE_RED_POINTS), VLBW_AVOID_PINT_Y(PHLE_RED_POINTS)
REAL*8 :: ELBW_RBCTX_PER_INFANT_PINT_Y(PHLE_RED_POINTS),
ELBW_RBCTX_PER_INFANT_Y(PHLE_RED_POINTS),
ELBW_AVOID_Y(PHLE_RED_POINTS), ELBW_AVOID_PINT_Y(PHLE_RED_POINTS)
EXTERNAL :: WEIGHT, HGB, THRESH, PINT, SD, HGB_PROD_SPLINE

CALL FILE_NAMES
CALL TRX_PRED !THIS WILL GIVE THE HGB CHANGE VALUES
CALL EPO_PROFILE !THIS WILL GET THE HB PRODUCED FOR GIVEN SUBJECT NO YET
PHLE ADJUSTED
!CALL GRAPH_PINT
!CALL GRAPH_CUM_PHLE2
WEIGHT_SUM=0D0


DO99: DO LOOP=1,PHLE_RED_POINTS !DIFFERENT PHLE RED FACTOR FOR EACH LOOP
PHLE_RED_FACTOR=DBLE(LOOP)/100D0-0.01D0
DO1: DO FILENUM=1,26
CALL FILE_OPEN

!WRITE(*,*) 'SUBJECT',301+FILENUM, TRX_POINTS(FILENUM)
!READ*

!JUST MODIFY THRESH POINTS BECAUSE ALREADY ALLOCATED CORRECTLY
```

```
DO i=2,THRESH_POINTS(FILENUM)-1
THRESH_Y(i)=HGB(THRESH_X(i))
END DO

DO i=1,PHLE_POINTS(FILENUM)
IF(PHLE_X(i)<=30D0) PHLEBOTOMIES(FILENUM)=PHLEBOTOMIES(FILENUM)+1
END DO

ORIGINAL_DATA=HGB_Y

DO2: DO i=1,TRX_POINTS(FILENUM)
        DO3: DO j=1,HGB_POINTS(FILENUM)
                TEMP_PHLE_PROD=1D0
                DO k=1, PHLE_POINTS_KG(FILENUM)
                        IF (PHLE_X_KG(k)>TRX_T(i) .AND. PHLE_X_KG(k)<HGB_X(j)) THEN
!THIS SHOULD MULTIPLY ALL PHLE AFTER TRX AND BEFORE CURRENT CORRECTION
                        TEMP_PHLE_PROD=TEMP_PHLE_PROD*(1D0-
PHLE_Y_KG(k)/AVE_BV(FILENUM))
                        END IF
                END DO
        IF (HGB_X(j)>TRX_T(i)) THEN !WE HAVE A HGB POINT AFTER THE TRX
                DELTA_HGB=TRX_H(i)
                IF (HGB_X(j)>TRX_T(i)+TRX_LENGTH) THEN
                HGB_Y(j)=(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*HGB_Y(j)/100D0-
DELTA_HGB*TEMP_PHLE_PROD*(1D0-(HGB_X(j)-
TRX_T(i))/(ALIFESPAN)))/(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*0.01D0)
                ELSE !POINT OCCURS DURING TRX
                HGB_Y(j)=(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*HGB_Y(j)/100D0-
DELTA_HGB*((HGB_X(j)-TRX_T(i))/TRX_LENGTH)*TEMP_PHLE_PROD*(1D0-(HGB_X(j)-
TRX_T(i))/(ALIFESPAN)))/(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*0.01D0)
                END IF
        END IF
        END DO DO3
END DO DO2
HGB_M1=HGB_Y !ONCE Y MODIFIED AGAIN MAKE HGB_M2=HGB_Y

DO6: DO i=1,PHLE_POINTS_KG(FILENUM)
        DO7: DO j=1, HGB_POINTS(FILENUM)
                        TEMP_PHLE_PROD=1D0
                        DO k=1, PHLE_POINTS_KG(FILENUM)
                        IF (PHLE_X_KG(k)>PHLE_X_KG(i) .AND. PHLE_X_KG(k)<HGB_X(j))
THEN !THIS SHOULD MULTIPLY ALL PHLE AFTER CURRENT PHLE AND BEFORE
CURRENT CORRECTION
                        TEMP_PHLE_PROD=TEMP_PHLE_PROD*(1D0-
PHLE_Y_KG(k)/AVE_BV(FILENUM))
                        END IF
                        END DO
        IF (HGB_X(j)>PHLE_X_KG(i)) THEN !WE HAVE A POINT AFTER THE PHLE
        DELTA_HGB=PHLE_RED_FACTOR*(PHLE_Y_KG(i)*WEIGHT(PHLE_X_KG(i))*HGB(P
HLE_X_KG(i))*0.01D0)
        IF (DELTA_HGB < 0D0) DELTA_HGB=0D0 !MAKE SURE NO PHLE CORRECTION
FOR NEG HGB
        HGB_Y(j)=(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*HGB_Y(j)/100D0+DELTA_HGB*TE
MP_PHLE_PROD*(1D0-(HGB_X(j)-
PHLE_X_KG(i))/(ILIFESPAN)))/(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*0.01D0)
        END IF
```

```
        END DO DO7
END DO DO6
DO j=1, HGB_POINTS(FILENUM)
HGB_Y(j)=(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*HGB_Y(j)/100D0+HGB_PROD_SPLINE(FI
LENUM,HGB_X(j))*TEMP_PHLE_PROD)/(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*0.01D0)
!THIS WILL ADD IN HB FROM EPO
END DO
HGB_M2=HGB_Y

DO i=1,NAT_HB_DAYS !THIS LOOP SHOULD COME UP WITH NATURAL AVERAGE HB
VALUES
HGB_NAT(FILENUM,i)=MAX(HGB(DBLE(i)-0.9D0),0D0)
HGB_NAT_AVE_X(i)=DBLE(i)-0.9D0
WEIGHT_SUM(i)=WEIGHT_SUM(i)+WEIGHT(DBLE(i)-0.9D0)
WEIGHT_SUM_ALL(FILENUM,i)=WEIGHT(DBLE(i)-0.9D0)
IF (FILENUM==26) THEN !LAST HB
HGB_NAT_AVE_Y(i)=SUM(HGB_NAT(1:FILENUM,i))/26D0
CONS_PROD_HB(i)=HGB_NAT_AVE_Y(1)*(WEIGHT_SUM(1)/WEIGHT_SUM(i))
WEIGHT_AVE_Y(i)=WEIGHT_SUM(i)/26D0
HGB_AMT_AVE(i)=HGB_NAT_AVE_Y(i)*93.2D0*WEIGHT_AVE_Y(i)/100D0 !THIS IS
AVERAGE HGB AMT WITH NO PERTERBATIONS
DO j=1,26
TEMP_SD(j)=HGB_NAT(j,i)
TEMP_SD_WEIGHT(j)=WEIGHT_SUM_ALL(j,i)
END DO
HGB_NAT_AVE_Y_PLUS(i)=HGB_NAT_AVE_Y(i)+SD(26,TEMP_SD)
HGB_NAT_AVE_Y_MINUS(i)=HGB_NAT_AVE_Y(i)-SD(26,TEMP_SD)
WEIGHT_AVE_Y_PLUS(i)=WEIGHT_AVE_Y(i)+SD(26,TEMP_SD_WEIGHT)
WEIGHT_AVE_Y_MINUS(i)=WEIGHT_AVE_Y(i)-SD(26,TEMP_SD_WEIGHT)
END IF
END DO
HGB_AMT_SEN_X(1)=0D0
HGB_AMT_SEN_X(2)=ILIFESPAN
HGB_AMT_SEN_Y(1)=HGB_AMT_AVE(1)
HGB_AMT_SEN_Y(2)=0D0

DO10: DO i=1, HGB_POINTS(FILENUM)
DELTA_HGB=15D0*WEIGHT(HGB_X(i))*SIM_TRX_HGB*0.01D0 !THIS WILL BE FIXED FOR A
GIVEN BLOOD VOLUME
        IF (HGB_Y(i)<=THRESH(HGB_X(i))) THEN !HGB GOES BELOW TRX THRESH
        SIM_TRX_NUM(FILENUM)=SIM_TRX_NUM(FILENUM)+1
        SIM_TRX_X(SIM_TRX_NUM(FILENUM))=HGB_X(i)
        DO11: DO j=i, HGB_POINTS(FILENUM)
                        TEMP_PHLE_PROD=1D0
                        DO k=1, PHLE_POINTS_KG(FILENUM) !A SIMULATED TRX HAS
OCCURED B/C INSIDE IF STATEMENT
                         IF (PHLE_X_KG(k)>HGB_X(i) .AND. PHLE_X_KG(k)<HGB_X(j)) THEN
!THIS SHOULD MULTIPLY ALL PHLE AFTER TRX AND BEFORE CURRENT CORRECTION
!                         TEMP_PHLE_PROD=TEMP_PHLE_PROD*(1D0-
PHLE_Y_KG(k)/AVE_BV(FILENUM))
                         TEMP_PHLE_PROD=TEMP_PHLE_PROD*(1D0-
PHLE_Y_KG(k)*(1D0-PHLE_RED_FACTOR)/AVE_BV(FILENUM))
                         END IF
                        END DO
```

```
        HGB_Y(j)=(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*HGB_Y(j)/100D0+DELTA_HGB*TE
MP_PHLE_PROD*(1D0-(HGB_X(j)-
HGB_X(i))/(ALIFESPAN)))/(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*0.01D0) !ADDS TRX
        END DO DO11
        END IF
END DO DO10
HGB_M3=HGB_Y
HGB_Y=HGB_M2 !RESET Y SO WE CAN APPLY A DIFFERENT TRX CRITERIA

DO12: DO i=1, HGB_POINTS(FILENUM)
DELTA_HGB=15D0*WEIGHT(HGB_X(i))*SIM_TRX_HGB*0.01D0 !THIS WILL BE FIXED FOR A
GIVEN BLOOD VOLUME
IF (SYRINGE(i)==0) VENOUS=VENOUS+1
!       IF (SYRINGE(i)==0) HGB_Y(i)=HGB_Y(i)*0.9D0
        IF (HGB_Y(i)<=PINT(HGB_X(i))) THEN !HGB GOES BELOW PINT CRITERIA
        SIM_TRX_NUM_PINT(FILENUM)=SIM_TRX_NUM_PINT(FILENUM)+1
        SIM_TRX_X_PINT(SIM_TRX_NUM_PINT(FILENUM))=HGB_X(i)
        DO13: DO j=i, HGB_POINTS(FILENUM)
                        TEMP_PHLE_PROD=1D0
                        DO k=1, PHLE_POINTS_KG(FILENUM) !A SIMULATED TRX HAS
OCCURED B/C INSIDE IF STATEMENT
                         IF (PHLE_X_KG(k)>HGB_X(i) .AND. PHLE_X_KG(k)<HGB_X(j)) THEN
!THIS SHOULD MULTIPLY ALL PHLE AFTER TRX AND BEFORE CURRENT CORRECTION
!                         TEMP_PHLE_PROD=TEMP_PHLE_PROD*(1D0-
PHLE_Y_KG(k)/AVE_BV(FILENUM))
                         TEMP_PHLE_PROD=TEMP_PHLE_PROD*(1D0-
PHLE_Y_KG(k)*(1D0-PHLE_RED_FACTOR)/AVE_BV(FILENUM))
                         END IF
                        END DO
        HGB_Y(j)=(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*HGB_Y(j)/100D0+DELTA_HGB*TE
MP_PHLE_PROD*(1D0-(HGB_X(j)-
HGB_X(i))/(ALIFESPAN)))/(AVE_BV(FILENUM)*WEIGHT(HGB_X(j))*0.01D0) !ADDS TRX
        END DO DO13
        END IF
END DO DO12
HGB_M4=HGB_Y

!CALL ADD_POINTS(HGB_X, HGB_M2, HGB_POINTS(FILENUM))
!!CALL ADD_POINTS(SIM_TRX_X, SIM_TRX_Y, SIM_TRX_NUM(FILENUM))
!!CALL ADD_POINTS(SIM_TRX_X_PINT, SIM_TRX_Y, SIM_TRX_NUM_PINT(FILENUM))
!!CALL ADD_CURVE(HGB_X, HGB_M1, HGB_POINTS(FILENUM))
!!CALL INCLUDE_CURVE(HGB_X, HGB_M2, HGB_POINTS(FILENUM),2)
!!CALL INCLUDE_CURVE(HGB_X, HGB_M3, HGB_POINTS(FILENUM),1)
!!CALL ADD_CURVE(HGB_X, HGB_M6, HGB_POINTS(FILENUM))
!!CALL ADD_POINTS(THRESH_X, THRESH_Y, THRESH_POINTS(FILENUM))
!CALL ADD_POINTS(TRX_T, SIM_TRX_Y, TRX_POINTS(FILENUM))
!CALL ADD_CURVE(HGB_X, ORIGINAL_DATA, HGB_POINTS(FILENUM))
!CALL X_LABEL ('DAY OF LIFE')
!CALL TITLE_WITH_INTEGER_NUMBER_ADDED('SUBJECT',filenum+301)
!CALL LEFT_LABEL('HGB')
!CALL PLOT_IN_AREA(FILENUM,26)

IF (FILENUM==26) THEN
!WRITE(*,*) HGB_NAT_AVE_Y(NAT_HB_DAYS), HGB_NAT_AVE_Y_PLUS(NAT_HB_DAYS)-
HGB_NAT_AVE_Y(NAT_HB_DAYS)
!READ*
```

```
!CALL TITLE('AVERAGE NATURAL HGB')
!CALL X_LABEL ('DAY OF LIFE')
!CALL LEFT_LABEL('HGB')
!CALL ADD_CURVE(PINT_X, PINT_Y, 6)
!!CALL ADD_CURVE(HGB_NAT_AVE_X, CONS_PROD_HB, NAT_HB_DAYS)
!CALL ADD_CURVE(HGB_NAT_AVE_X, HGB_NAT_AVE_Y, NAT_HB_DAYS)
!!CALL INCLUDE_CURVE(HGB_NAT_AVE_X, HGB_NAT_AVE_Y_PLUS, NAT_HB_DAYS,2)
!!CALL INCLUDE_CURVE(HGB_NAT_AVE_X, HGB_NAT_AVE_Y_MINUS, NAT_HB_DAYS,2)
!CALL BEGIN_LEFT_AT(0D0)
!CALL END_LEFT_AT(18D0)
!CALL PLOT_IN_AREA(1,2)
!CALL ADD_CURVE(HGB_NAT_AVE_X, WEIGHT_AVE_Y, NAT_HB_DAYS)
!CALL INCLUDE_CURVE(HGB_NAT_AVE_X, WEIGHT_AVE_Y_PLUS, NAT_HB_DAYS,1)
!CALL INCLUDE_CURVE(HGB_NAT_AVE_X, WEIGHT_AVE_Y_MINUS, NAT_HB_DAYS,1)
!CALL X_LABEL ('DAY OF LIFE')
!CALL LEFT_LABEL('AVERAGE WEIGHT')
!CALL PLOT_IN_AREA(2,2)
!CALL ADD_CURVE(HGB_NAT_AVE_X, HGB_AMT_AVE, NAT_HB_DAYS)
!CALL ADD_CURVE(HGB_AMT_SEN_X, HGB_AMT_SEN_Y, 2)
!CALL X_LABEL ('DAY OF LIFE')
!CALL LEFT_LABEL('AVERAGE HGB AMT')
!CALL PLOT_IN_AREA(3,4)
!!WRITE(*,*) SUM(SIM_TRX_NUM(1:26)) !TOTAL TRX NUM
!!WRITE(*,*) SUM(SIM_TRX_NUM_PINT(1:26)) !TOTAL TRX NUM
!CALL DISPLAY_PLOT
!!CALL GRAPH_TRX_DIST
!!WRITE(*,*) SUM(PHLE_POINTS(1:26))/26D0
!!WRITE(*,*) SD(26, PHLE_POINTS)
!!WRITE(*,*) VENOUS
END IF

IF (SIM_TRX_NUM(FILENUM)==0 .AND. WEIGHT_Y(1)>1.0D0) THEN !NO TRX PREDICTED
AND VLBW
VLBW_AVOID=VLBW_AVOID+1
ELSE IF (SIM_TRX_NUM(FILENUM)==0 .AND. WEIGHT_Y(1)<1.0D0) THEN !NO TRX
PREDICTED AND ELBW
ELBW_AVOID=ELBW_AVOID+1
END IF

IF (SIM_TRX_NUM_PINT(FILENUM)==0 .AND. WEIGHT_Y(1)>1.0D0) THEN !NO TRX
PREDICTED AND VLBW
VLBW_AVOID_PINT=VLBW_AVOID_PINT+1
ELSE IF (SIM_TRX_NUM_PINT(FILENUM)==0 .AND. WEIGHT_Y(1)<1.0D0) THEN !NO TRX
PREDICTED AND ELBW
ELBW_AVOID_PINT=ELBW_AVOID_PINT+1
END IF

CALL UNALLOCATE

!WRITE(*,*) AVE_BV(FILENUM)

!WRITE(*,*) 'SUBJECT', 301+FILENUM, SIM_TRX_NUM(FILENUM), TRX_POINTS(FILENUM)
!WRITE(*,*) 'SUBJECT', 301+FILENUM, SIM_TRX_NUM(FILENUM)-TRX_POINTS(FILENUM)
!WRITE(*,*) 'SUBJECT', 301+FILENUM, SIM_TRX_NUM_PINT(FILENUM),
TRX_POINTS(FILENUM)
```

```
END DO DO1
!WRITE(*,*) 'IOWA VLBW',SUM(SIM_TRX_NUM(VLBW))/DBLE(VLBW_NUM),
DBLE(VLBW_AVOID)/DBLE(VLBW_NUM)*100D0
!WRITE(*,*) 'PINT VLBW',SUM(SIM_TRX_NUM_PINT(VLBW))/DBLE(VLBW_NUM),
DBLE(VLBW_AVOID_PINT)/DBLE(VLBW_NUM)*100D0
!WRITE(*,*) 'IOWA ELBW',SUM(SIM_TRX_NUM(ELBW))/DBLE(ELBW_NUM),
DBLE(ELBW_AVOID)/DBLE(ELBW_NUM)*100D0
!WRITE(*,*) 'PINT ELBW',SUM(SIM_TRX_NUM_PINT(ELBW))/DBLE(ELBW_NUM),
DBLE(ELBW_AVOID_PINT)/DBLE(ELBW_NUM)*100D0

GRAPH_PER_RED_X(LOOP)=PHLE_RED_FACTOR
VLBW_RBCTX_PER_INFANT_Y(LOOP)=SUM(SIM_TRX_NUM(VLBW))/DBLE(VLBW_NUM)
VLBW_RBCTX_PER_INFANT_PINT_Y(LOOP)=SUM(SIM_TRX_NUM_PINT(VLBW))/DBLE(VLB
W_NUM)
VLBW_AVOID_Y(LOOP)=DBLE(VLBW_AVOID)/DBLE(VLBW_NUM)*100D0
VLBW_AVOID_PINT_Y(LOOP)=DBLE(VLBW_AVOID_PINT)/DBLE(VLBW_NUM)*100D0
ELBW_RBCTX_PER_INFANT_Y(LOOP)=SUM(SIM_TRX_NUM(ELBW))/DBLE(ELBW_NUM)
ELBW_RBCTX_PER_INFANT_PINT_Y(LOOP)=SUM(SIM_TRX_NUM_PINT(ELBW))/DBLE(ELB
W_NUM)
ELBW_AVOID_Y(LOOP)=DBLE(ELBW_AVOID)/DBLE(ELBW_NUM)*100D0
ELBW_AVOID_PINT_Y(LOOP)=DBLE(ELBW_AVOID_PINT)/DBLE(ELBW_NUM)*100D0

SIM_TRX_NUM=0
SIM_TRX_NUM_PINT=0
VLBW_AVOID=0
VLBW_AVOID_PINT=0
ELBW_AVOID=0
ELBW_AVOID_PINT=0
END DO DO99

CALL ADD_CURVE(GRAPH_PER_RED_X*100D0, VLBW_RBCTX_PER_INFANT_Y,
PHLE_RED_POINTS)
CALL INCLUDE_CURVE(GRAPH_PER_RED_X*100D0,
VLBW_RBCTX_PER_INFANT_PINT_Y, PHLE_RED_POINTS, 2)
CALL BEGIN_LEFT_AT(-1.0D0)
CALL END_LEFT_AT(5D0)
CALL X_LABEL ('PERCENT PHLE REDUCTION')
CALL LEFT_LABEL('RBCTX PER INFANT')
CALL TITLE ('VLBW WITH PHLE RED AND EPO')
CALL PLOT_IN_AREA(1,4)

CALL ADD_CURVE(GRAPH_PER_RED_X*100D0, VLBW_AVOID_Y, PHLE_RED_POINTS)
CALL INCLUDE_CURVE(GRAPH_PER_RED_X*100D0, VLBW_AVOID_PINT_Y,
PHLE_RED_POINTS, 2)
CALL BEGIN_LEFT_AT(-10.0D0)
CALL END_LEFT_AT(100D0)
CALL X_LABEL ('PERCENT PHLE REDUCTION')
CALL LEFT_LABEL('PERCENT AVOIDED RBCTX')
CALL TITLE ('VLBW WITH PHLE RED AND EPO')
CALL PLOT_IN_AREA(3,4)

CALL ADD_CURVE(GRAPH_PER_RED_X*100D0, ELBW_RBCTX_PER_INFANT_Y,
PHLE_RED_POINTS)
CALL INCLUDE_CURVE(GRAPH_PER_RED_X*100D0,
ELBW_RBCTX_PER_INFANT_PINT_Y, PHLE_RED_POINTS, 2)
CALL BEGIN_LEFT_AT(-1.0D0)
```

```
CALL END_LEFT_AT(5D0)
CALL X_LABEL ('PERCENT PHLE REDUCTION')
CALL LEFT_LABEL('RBCTX PER INFANT')
CALL TITLE ('ELBW WITH PHLE RED AND EPO')
CALL PLOT_IN_AREA(2,4)

CALL ADD_CURVE(GRAPH_PER_RED_X*100D0, ELBW_AVOID_Y, PHLE_RED_POINTS)
CALL INCLUDE_CURVE(GRAPH_PER_RED_X*100D0, ELBW_AVOID_PINT_Y,
PHLE_RED_POINTS, 2)
CALL BEGIN_LEFT_AT(-10.0D0)
CALL END_LEFT_AT(100D0)
CALL X_LABEL ('PERCENT PHLE REDUCTION')
CALL LEFT_LABEL('PERCENT AVOIDED RBCTX')
CALL TITLE ('ELBW WITH PHLE RED AND EPO')
CALL PLOT_IN_AREA(4,4)
CALL DISPLAY_PLOT

!BREAK

!CALL ADD_CURVE(GRAPH_PER_RED_X*100D0, VLBW_RBCTX_PER_INFANT_Y,
PHLE_RED_POINTS)
!CALL INCLUDE_CURVE(GRAPH_PER_RED_X*100D0,
VLBW_RBCTX_PER_INFANT_PINT_Y, PHLE_RED_POINTS, 2)
!CALL BEGIN_LEFT_AT(-1.0D0)
!CALL END_LEFT_AT(5D0)
!CALL X_LABEL ('PERCENT PHLE REDUCTION')
!CALL LEFT_LABEL('RBCTX PER INFANT')
!CALL TITLE ('VLBW WITH PHLE RED ONLY')
!CALL PLOT_IN_AREA(1,4)
!
!CALL ADD_CURVE(GRAPH_PER_RED_X*100D0, VLBW_AVOID_Y, PHLE_RED_POINTS)
!CALL INCLUDE_CURVE(GRAPH_PER_RED_X*100D0, VLBW_AVOID_PINT_Y,
PHLE_RED_POINTS, 2)
!CALL BEGIN_LEFT_AT(-10.0D0)
!CALL END_LEFT_AT(100D0)
!CALL X_LABEL ('PERCENT PHLE REDUCTION')
!CALL LEFT_LABEL('PERCENT AVOIDED RBCTX')
!CALL TITLE ('VLBW WITH PHLE RED ONLY')
!CALL PLOT_IN_AREA(3,4)
!
!CALL ADD_CURVE(GRAPH_PER_RED_X*100D0, ELBW_RBCTX_PER_INFANT_Y,
PHLE_RED_POINTS)
!CALL INCLUDE_CURVE(GRAPH_PER_RED_X*100D0,
ELBW_RBCTX_PER_INFANT_PINT_Y, PHLE_RED_POINTS, 2)
!CALL BEGIN_LEFT_AT(-1.0D0)
!CALL END_LEFT_AT(5D0)
!CALL X_LABEL ('PERCENT PHLE REDUCTION')
!CALL LEFT_LABEL('RBCTX PER INFANT')
!CALL TITLE ('ELBW WITH PHLE RED ONLY')
!CALL PLOT_IN_AREA(2,4)
!
!CALL ADD_CURVE(GRAPH_PER_RED_X*100D0, ELBW_AVOID_Y, PHLE_RED_POINTS)
!CALL INCLUDE_CURVE(GRAPH_PER_RED_X*100D0, ELBW_AVOID_PINT_Y,
PHLE_RED_POINTS, 2)
!CALL BEGIN_LEFT_AT(-10.0D0)
!CALL END_LEFT_AT(100D0)
```

```
!CALL X_LABEL ('PERCENT PHLE REDUCTION')
!CALL LEFT_LABEL('PERCENT AVOIDED RBCTX')
!CALL TITLE ('ELBW WITH PHLE RED ONLY')
!CALL PLOT_IN_AREA(4,4)
!CALL DISPLAY_PLOT

!WRITE(*,*) 'IOWA ALL',SUM(SIM_TRX_NUM(1:26))/27D0 !, SD1
!WRITE(*,*) 'PINT ALL',SUM(SIM_TRX_NUM_PINT(1:26))/27D0 !, SD2

END PROGRAM PHLE_RED
```

C.5.2 SHARED.F90 (Same as Appendix C.4.2)

C.5.3 EPO_PK_PD.F90 (Original Code)

```
SUBROUTINE EPO_PROFILE

USE SHARED
IMPLICIT NONE

INTEGER :: i

DO FILENUM=1,26
CALL FILE_OPEN

DOSE_X(1)=0.112237691000215D0; DOSE_Y(1)=600D0
DOSE_X(2)=0.113131565094521D0; DOSE_Y(2)=600D0
DOSE_X(3)=2.17264353305690D0; DOSE_Y(3)=600D0
DOSE_X(4)=2.74917762575167D0; DOSE_Y(4)=600D0
DOSE_X(5)=3.62076985911502D0; DOSE_Y(5)=600D0
DOSE_X(6)=4.73534901008724D0; DOSE_Y(6)=600D0
DOSE_X(7)=6.60263097500551D0; DOSE_Y(7)=600D0
DOSE_X(8)=13.1665927242332D0; DOSE_Y(8)=600D0
DOSE_X(9)=13.6195124926136D0; DOSE_Y(9)=600D0
DOSE_X(10)=14.2916803864258D0; DOSE_Y(10)=600D0
DOSE_X(11)=14.3778065537930D0; DOSE_Y(11)=600D0
DOSE_X(12)=25.3625314679754D0; DOSE_Y(12)=600D0

        YIN1=0D0; YIN2=DOSE_Y(1)/V; XFIRST=DOSE_X(1); XLAST=DOSE_X(2)-1D-8; CALL
DIFFEQ
        DO i=2,DOSES-1
        YIN1=YP1(FILENUM,HGB_SPLINE_POINTS);
YIN2=DOSE_Y(i)/V+YP2(FILENUM,HGB_SPLINE_POINTS); XFIRST=DOSE_X(i);
XLAST=DOSE_X(i+1)-1D-8; CALL DIFFEQ
        END DO
        YIN1=YP1(FILENUM,HGB_SPLINE_POINTS);
YIN2=DOSE_Y(DOSES)/V+YP2(FILENUM,HGB_SPLINE_POINTS);
XFIRST=DOSE_X(DOSES); XLAST=30D0; CALL DIFFEQ
!CALL TITLE_WITH_INTEGER_NUMBER_ADDED('SUBJECT',filenum+301)
!CALL ADD_CURVE (XP, YP1, HGB_SPLINE_POINTS)
!CALL PLOT_IN_AREA(FILENUM, 26)
HGB_SPLINE_POINTS2(FILENUM)=HGB_SPLINE_POINTS
```

```
HGB_SPLINE_POINTS=0
!CALL DISPLAY_PLOT

CALL UNALLOCATE
END DO

END SUBROUTINE EPO_PROFILE

SUBROUTINE DIFFEQ

USE SHARED
IMPLICIT REAL*8 (A-H,O-Z)
REAL :: DURATION(2) !THIS IS SINGLE PERCISION BECAUASE OF INTINSIC FUNCTION
PARAMETER (N=3) !SET ICONT ICOMP AND ICOMP TO BE (3)
PARAMETER (NDGL=n,NGRID=11,LWORK=8*NDGL+11+NGRID,LIWORK=10)
PARAMETER (NRDENS=NDGL,LRCONT=50000,LICONT=NRDENS+1)
INTEGER :: J, G, POINTSH
DIMENSION Y(NDGL),WORK(LWORK),IWORK(LIWORK)
COMMON/STATRE/NFCN,NSTEP,NACCPT,NREJCT
COMMON /CORER/RCONT(LRCONT)
COMMON /COREI/NRDS,ICONT(LICONT)
EXTERNAL FCN,SOLOUT

!Y(1)=YIN1; Y(2)=YIN2*1000D0/(V); X=XFIRST; XEND=XLAST !ALL INITIAL VALUES SET IN
MAIN
Y(1)=YIN1; Y(2)=YIN2; X=XFIRST; XEND=XLAST !ALL INITIAL VALUES SET IN MAIN
!IF (XEND<X) THEN
!X=XLAST
!XEND=XFIRST
!END IF

IOUT=1
ITOL=0
RTOL=1.0D-3 !THIS IS A HIGH ERROR TOLERANCE RESULTS MAY BE SKEWED IF NOT
SET HIGH ENOUGH
ATOL=RTOL
DO I=1,10
IWORK(I)=0
WORK(I)=0.D0
END DO
IWORK(5)=NRDENS
ICONT(2)=2
!IWORK(6)=NGRID
DO I=1,NGRID-1
WORK(10+I)=I
WORK(10+NGRID)=20.D0
END DO

CALL
RETARD(N,FCN,X,Y,XEND,RTOL,ATOL,ITOL,SOLOUT,IOUT,WORK,LWORK,IWORK,LIWORK
,LRCONT,LICONT,IDID)
!WRITE(*,*) 'IDID', IDID
!READ*

END SUBROUTINE DIFFEQ
```

```
SUBROUTINE SOLOUT (NR,XOLD,X,Y,N,IRTRN)
USE SHARED
IMPLICIT REAL*8 (A-H,O-Z)
DIMENSION Y(N)
EXTERNAL PHI
COMMON /INTERN/XOUT
IF (NR.EQ.1) THEN
ELSE
HGB_SPLINE_POINTS=HGB_SPLINE_POINTS+1
XP(FILENUM,HGB_SPLINE_POINTS)=X
YP1(FILENUM,HGB_SPLINE_POINTS)=Y(1)
YP2(FILENUM,HGB_SPLINE_POINTS)=Y(2)
END IF
RETURN
END

SUBROUTINE FCN(N,X,Y,F)
USE SHARED
IMPLICIT REAL*8 (A-H,O-Z)
DIMENSION Y(N),F(N)
REAL*8 :: EPOTOTAL_LAG, EPOSPLINE
EXTERNAL :: PHI, WEIGHT, EPOSPLINE

IF (X>LAGTIME+DOSE_X(1)) THEN !WE HAVE A POINT PAST EPO DOSE AND LAG TIME
EPOTOTAL_LAG=EPOSPLINE(X-LAGTIME)!*0.1D0
ELSE
EPOTOTAL_LAG=0D0
END IF

EMAXT=EMAXI(FILENUM)*(WEIGHT(X)**0.75D0)  !THIS IS THE MODEL WITH INDIVIDUAL
PARAMETERS
ET = EMAXT*(EPOTOTAL_LAG)/(EC50I(FILENUM)+EPOTOTAL_LAG)

F(1)=ET
F(2)=-P1*Y(2)/(P2+Y(2)) !THIS EQUATION IS EPO PROFILE

RETURN
END SUBROUTINE FCN

FUNCTION PHI(I,X)
IMPLICIT REAL*8 (A-H,O-Z)
IF (I.EQ.2) PHI=0.1D0
RETURN
END
```

APPENDIX D. PUBLICATIONS AND SUBMITTED MANUSCRIPTS

1. Rosebraugh M, Widness JA, Veng-Pedersen P 2011 Receptor-based dosing optimization of erythropoietin in juvenile sheep after phlebotomy. *Drug Metab Dispos* 39:1214-1220

2. Rosebraugh MR, Widness JA, Veng-Pedersen P 2012 Multidose optimization simulation of erythropoietin treatment in preterm infants. *Pediatr Res* 71:332-337.

3. Rosebraugh M, Nalbant D, Widness JA, Veng-Pedersen P 2012 Combining accurate patient data with mathematical modeling to precisely quantitate the role of phlebotomy losses and need for transfusions in neonatal anemia. *Trans* [Accepted for Publication]

4. Rosebraugh MR, Nalbant D, Cress G, Widness JA, Veng-Pedersen P 2012 Modeled Erythropoietin Optimization Combined With Phlebotomy Reduction Eliminates Blood Transfusions in Selected Preterm Infants. *Pediatr Res* [Submitted for Publication]

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