Measurement of Circulating Red Cell Volume
and Survival of Red Blood Cells
in Anemic Low Birth Weight Infants

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No conflicts of interest to disclose

Funding, Collaborators, & Research Teams
NIH P01 HL046925, Thrasher Research Fund, Sysmex

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Laboratory Team
“RBC Kinetics” encompasses \textit{in vivo} RBC survival (RCS) & the time dependent circulating RBC volume (RCV; aka, “red cell mass”).

- RBC kinetics reflects the balance in RBC production & elimination.
- Hence, RBC kinetic production & elimination are major determinants of anemia management decisions.
Significance of RBC Kinetics

- Anemia
- Reduced circulating RBC volume & reduced O₂ delivery
- RBC senescence
- Optimal RBC transfusion products in blood banking & transfusion medicine
- There have been no RBC survival studies in infants since the early 1970’s to establish a “best practice.”

Neonatal Anemia Models

Mechanistic PK/PD models for studying anemia in premature infants and clinical variables affecting erythropoiesis require information on transfusions, phlebotomies, RBC life span, iron availability, erythropoietic reserve, and blood volume expansion during growth.

Explaining the variability in responses to endogenous and exogenous EPO requires an understanding of Red Cell Survival that can be incorporated into the mathematical models.
Factors influencing Anemia Management Decisions

In addition to gestational age, other markers are important predictors of response to endogenous /recombinant human EPO (rhEPO). There are many conditions reported as being associated with rhEPO resistance, (e.g., inflammation, oxidative stress, and iron deficiency as reflected in Ret Hb – Functional Iron Deficiency).

Plasma levels of C-reactive protein, IL-6, soluble IL-2, and elastase have all been described as inflammatory markers for rhEPO hyporesponsiveness in hemodialysis patients

Reticulocyte counts are reflective of erythropoietic function and are included in the assessments

Red Cell Survival Studies

- \(^{51}\)Cr is the accepted method
- FDA
- PTR\(_{24}\)

But …

- Ethical and acceptance problems
- Analytical problems
Special Considerations Apply in Research Studies of “Vulnerable” Patient Populations

$^{51}$Cr is radioactive.
- Safety/Ethics/Participation
- Sample assay volume $\geq 100$ µL

Background: RBC Survival Parameters Following “Population Labeling”

Data from steady-state erythropoietic conditions in adults

- Post-transfusion RBC recovery at 24 h ($PTR_{24}$): very short-term RBC survival ($^{51}$Cr works!)
- Time to disappearance of 50% of labeled RBCs ($T_{50}$): Common index of long-term RBC survival
- Mean Potential Life Span (MPL): Index of very long-term RBC survival. Linear portion extrapolated to time axis
Illustration of Potential Range of $^{51}$Cr Elution from RBCs in an Adult Study Subject Also Studied with BioRBCs*

<table>
<thead>
<tr>
<th>BioRBC (flow)</th>
<th>$^{51}$Cr Uncorrected</th>
<th>$^{51}$Cr Corrected†</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{50}$ (days)</td>
<td>50.8</td>
<td>35.4</td>
</tr>
<tr>
<td>MPL (days)</td>
<td>107.7</td>
<td>84.2</td>
</tr>
</tbody>
</table>

- Example taken from Mock et al. *Transfusion* 39:156-162, 1999
- † Daily $^{51}$Cr elution correction recommended by the International Committee for Standardization in Hematology (1980)

Characteristics of an ideal RBC population label

- Label a representative sample of the entire population of RBCs
- Quantification without interference from the unlabeled RBCs
- Survival of the labeled RBCs must have the same survival properties before and after the labeling.
Characteristics of an ideal RBC population label

- No radiation exposure
- No label loss
- Label and independently identify, quantify, and track multiple populations
- Minimal blood sample volume

Iatrogenic Exsanguination?
Partitioning of Cumulative Total Laboratory Phlebotomy Loss in Ventilated VLBW Infants in 1st 28 d of Age

BW = 890±250 g; Gest Age=26.6±1.6 Wk

~97% of blood samples were weighed
Laboratory requested volume was 74% of total loss

Rosebraugh et al. Transfusion 2013

EX VIVO BIOTIN “RBC Population” LABELING

Using readily available commercial reagents

Washed x2 Red Blood Cells (RBCs) + S-NHS → Biotinylation Reagent (permanently binds to epsilon amino group of lysine)

NHS

30 min @ 21°C

Washed x2

The procedure takes 2-3 h
Representative Flow Cytometric Histogram Showing that 4 Different Blood Samples Can Be Biotinylated at 4 Different Labeled BioRBC Populations to Study Concurrent RBC Survival

- Compare RBC storage media, anti-infective treatments, prolonged storage, etc.
- 3-fold concentration increases of biotinylating reagent
- Can be applied to both allogeneic & autologous RBCS

Biotin vs. $^{51}$Cr

<table>
<thead>
<tr>
<th>Features</th>
<th>BioRBCs</th>
<th>$^{51}$Cr</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy of measurement allows extended study time</td>
<td>$&gt;120+ \text{ d}$</td>
<td>$\sim 40 \text{ d}$</td>
<td>Dependent on counting error</td>
</tr>
<tr>
<td>Required sample volume (mL)</td>
<td>0.01</td>
<td>0.1 to 1.0</td>
<td>BioRBC volumes ideal for infants (leftover blood)</td>
</tr>
<tr>
<td>Susceptible to artifact from hemoconcentration or hemodilution?</td>
<td>No</td>
<td>Yes</td>
<td>Capillary blood sampling possible for BioRBC method</td>
</tr>
</tbody>
</table>
### Biotin vs. $^{51}$Cr

<table>
<thead>
<tr>
<th>Features</th>
<th>BioRBCs</th>
<th>$^{51}$Cr</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost of reagents</td>
<td>~$50</td>
<td>~$650</td>
<td>+ Tech time</td>
</tr>
<tr>
<td>Radioactive waste?</td>
<td>No</td>
<td>Yes</td>
<td>Both hazard &amp; expense</td>
</tr>
<tr>
<td>Complexity of labeling procedure</td>
<td>4-6 wash steps</td>
<td>No wash steps</td>
<td>Biotinylation requires 2 wash steps x2 &amp; sterile hood</td>
</tr>
<tr>
<td>Ab to labeled RBCs?</td>
<td>Rarely</td>
<td>No</td>
<td>1°? No 2°? Yes</td>
</tr>
</tbody>
</table>

### Validation of Biotin Method

<table>
<thead>
<tr>
<th>Species</th>
<th>BioRBCs</th>
<th>Reference</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>Single &amp; multiple</td>
<td>$^{14}$C-cyanate</td>
<td>RCV</td>
</tr>
<tr>
<td>Sheep</td>
<td>Single &amp; multiple</td>
<td>$^{14}$C-cyanate</td>
<td>RCS</td>
</tr>
<tr>
<td>Human adult</td>
<td>Single &amp; multiple</td>
<td>$^{51}$Cr</td>
<td>RCV</td>
</tr>
<tr>
<td>Human adult</td>
<td>Single &amp; multiple</td>
<td>$^{51}$Cr</td>
<td>RCS</td>
</tr>
<tr>
<td>Human infant</td>
<td>Single &amp; multiple</td>
<td>Minor Antigen</td>
<td>RCV and RCS</td>
</tr>
</tbody>
</table>
Group Results of Autologous Multi-density BioRBC Survival in 8 Normal Adult Subjects

(Mock et al. Transfusion 51: 1047-1057, 2011)

Iowa Multidensity BioRBC Paired Survival Data (n=8)

Decreasing RBC survival as biotin density increases & close agreement of the 2 lowest densities

Concurrent “Tracking” of Adult Multi-density Biotin Labeled Allogeneic RBCs in VLBW Infants Compared to Non-labeled Allogeneic RBCs

Identical concurrent tracking of the “Reference” unlabeled Kidd minor antigen RBCs with the 3 lowest biotin densities
**Objective**
To concurrently compare survival of autologous neonatal RBCs with that of allogeneic adult donor RBCs.

**Hypothesis**
In VLBW infants the survival of autologous neonatal RBC will be <50% of the concurrently administered allogeneic adult donor RBC.

**Background**

<table>
<thead>
<tr>
<th>T&lt;sub&gt;50&lt;/sub&gt; (d)</th>
<th>8 Adults* (Biotin)</th>
<th>51 Term Neonates**(&lt;sup&gt;51&lt;/sup&gt;Cr)</th>
<th>31 Preterm Neonates**(&lt;sup&gt;51&lt;/sup&gt;Cr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60</td>
<td>23</td>
<td>17</td>
</tr>
</tbody>
</table>

Note: Determinations based on autologous labeled RBC transfusion

*Mock et al, 2011  
**Pearson, 1967
**Study Protocol**

Subjects Ventilated VLBW Infants without Major Anomalies Receiving Their First Transfusion

![Diagram showing study protocol](image)

**VLBW Infant Study Demographics**

Number of Infants: 7

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA (wk)</td>
<td>25.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Birth wt (g)</td>
<td>839</td>
<td>184</td>
</tr>
<tr>
<td>Study age (d)</td>
<td>4.1</td>
<td>4.5</td>
</tr>
<tr>
<td>Study wt (g)</td>
<td>784</td>
<td>164</td>
</tr>
<tr>
<td>RBC Tfs During Study</td>
<td>3.4</td>
<td>1.5</td>
</tr>
</tbody>
</table>
"Tracking" of BioRBC Survival (ie, no modeling) in the 7 VLBW Study Subjects

Non-steady State Confounders

1. Phlebotomy leading to loss of BioRBCs & unlabeled RBCs
2. Transfusion leading to a dilution in BioRBCs
3. Growth leading to a dilution in BioRBCs as a result of new RBCs & increased blood vol

Modeling adjusts for these confounders.
Intensive supporting blood counts are needed.
(Thanks to Sysmex.)
Concurrent Unmodeled vs Modeled Survival of Neonatal Autologous & Adult Allogeneic RBCs in VLBW Infants

- **Unmodeled** Raw Enrichment Data
- **Modeled** enrichment data corrected for transfusion & growth, with modeling accounting for phlebotomy

Concurrent Post-Transfusion *Modeled* Lifespan of Neonatal Autologous & Adult Donor RBCs

*Paired t-test*

- *P* = 0.10, *n* = 7
Summary

• In 7 VLBW infants, we concurrently tracked the survival of neonatal & adult BioRBCs using discarded blood (<10 µL)
• Modeling of RBC survival corrected for confounders and permitted determination of RBC survival in traditional steady-state parameters.
• No safety issues

• *Contrary to our hypothesis, we observed similar survival of transfused autologous neonatal and adult allogeneic RBCs*

New Knowledge

A similar infant…

… 6 yrs later

- The environment of RBCs, rather than the origin, is the primary determinant of RBC survival.
- Despite the current understanding, developmental factors matter little.
**Implications**

- Delayed cord clamping (or milking) provides the newborn with RBCs that will survive about as long as adult donor RBCs & therefore should generally be encouraged.

- With respect to neonatal jaundice, transfused adult donor RBCs contribute equivalently (per g of Hb) to autologous neonatal RBCs.

- Mathematical models for PK/PD incorporate red cell survival information in the quest for management optimization in anemia of prematurity.

That’s all folks!