Red Blood Cell (RBC) Transfusion, Anemia, and Necrotizing Enterocolitis (NEC) in Very Low Birth Weight (VLBW) Infants: Are we just chasing our Tails/Tales?

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Cassandra D. Josephson, MD
Professor, Pathology and Pediatrics
Emory University School of Medicine
Director, Transfusion, Tissue, and Apheresis Services
Children’s Healthcare of Atlanta
Disclosures

- Immucor - consultant, speaker
- Biomet Zimmer – consultant
- Octapharma - consultant
Learning Objectives

1. Discuss the association of RBC transfusion and development of NEC in premature infants
2. Explain the increased risk of NEC in premature infants with anemia
3. Define potential mechanisms for why anemia is an independent risk factor for NEC development in premature infants
Background

• NEC is a leading cause of mortality in VLBW (≤ 1500 grams) infants
• NEC is characterized by intestinal inflammation and necrosis although the exact pathogenesis is unknown
• ~ 7% of VLBW infants develop NEC, with a 15-30% mortality rate
• Cause-specific mortality due to NEC has increased from 2000 to 2011.

Neu and Walker, *NEJM* 2011
Bell’s stage classification of NEC

• Stage 1 (suspected)
  – Systemic and gastrointestinal manifestations such as emesis or abdominal distention with ileus on abdominal x-ray

• Stage 2 (definite)
  – Above findings plus pneumatosis intestinalis, portal venous gas or edema in small bowel on abdominal x-ray

• Stage 3 (advanced)
  – Above findings plus deterioration of vital organs, including septic shock or abdominal x-ray shows pneumoperitoneum
  – Patients frequently require surgery

Red cell transfusion, anemia and NEC
1960: NEC described
1970: Bell staging
1978: Outbreak of NEC associated with RBC transfusion
1987: NEC described
1990:

- 1999: Hebert et al.
- 1999: Tx Req Critical Care Adults
- 2000:
- 2000: Josephson et al.
- 2000: Christensen et al.
- 2005: Iowa trial
- 2006: PINT trial
- 2007: TriPICU
- 2006: Mally et al.
- 2007: Blau et al.
- 2010: Paul et al.
- 2010: El-Dib et al.
- 2010: Singh et al.
- 2011: Mohamed & Shah.
- 2012: Mohamed & Shah.
- 2014: DeRienzo et al.
- 2014: Sharma et al.
- 2014: Wallenstein et al.

2008-2011 = increase in RBC NEC-related deaths in < 1000 gm ELBW
2017+: TOP trial

Pre-1960s: NEC described
1960: NEC described
1970: Bell staging
1978: Outbreak of NEC associated with RBC transfusion
1987: NEC described
1990:

- 1999: Hebert et al.
- 1999: Tx Req Critical Care Adults
- 2000:
- 2000: Josephson et al.
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- 2014: Wallenstein et al.

2008-2011 = increase in RBC NEC-related deaths in < 1000 gm ELBW
2017+: TOP trial
Candidate Mechanisms for RBC Transfusion and NEC

- Anemia
  - Decreased oxygen delivery to intestines

Prematurity
Enteral Feeding
Illness severity

Necrotizing Enterocolitis

- Prolonged RBC Storage
- Prolonged Irradiation
- Storage Time

Decreased nitric oxide
↑ Vasoconstriction
Decreased oxygen delivery to intestines
Background

- 60-90% of VLBW infants receive RBC transfusions
- Prolonged RBC storage may decrease donor RBC function (possibly depending on the donor) and viability after transfusion in some populations\(^1\)
- Irradiation of RBCs, a common practice to decrease the risk of graft-vs-host disease in preterm infants, worsens the storage lesion, which may adversely effect clinical outcomes in this population\(^2,3\)

1. Lee JS, Gladwin MT. Nat Med 2010
3. Ran Q et al. PLOS One 2011
Background

• Clinical and biologic determinants of transfusion-related NEC remain poorly understood

• Prior studies limited by:
  – Lack of data regarding donor RBC processing and storage
  – Small numbers of patients and retrospective end-points
  – Lack of evaluation of time-varying exposures and measurement of important confounders

• Authors of meta-analysis emphasized the need for prospective study of association between transfusion and NEC¹

¹. Mohamed and Shah, Pediatrics 2012
Transfusion and NEC: Meta-analysis

First Meta-Analysis done in 2012 demonstrated an association between RBC transfusion and NEC

A. **Unadjusted: Odds ratio** (RBC vs No RBC) \( tx = 3.91 \) (95% CI 2.97-5.14)

B. **Adjusted: Odds ratio** (RBC vs No RBC) \( tx = 2.01 \) (95% CI 1.61-2.50)

In this meta-analysis of RCTs for transfusion thresholds **no difference** in the incidence of NEC was found in the liberal versus conservative RBC transfusion approaches. (note: study was not powered to determine this secondary outcome)

Kirpalani H, Zupancic JA. *Semin Perinatol.* 2012
Anemia and NEC

- Risk of NEC increases with increasing anemia
- Odds ratio (per 1% drop in HCT) 1.10 (95% CI 1.02-1.18)

Primary objective: To test the hypothesis that the risk of NEC is greater in VLBW infants exposed to RBC transfusion compared to non-transfused VLBW infants

Secondary objective: To determine if exposure to severe anemia (hemoglobin ≤8g/dL) is an independent risk factor for NEC in VLBW infants
Causal framework

Participating Metro-Atlanta Hospitals
Study design

- Secondary analysis of a prospective, multicenter observational birth-cohort study (TT-CMV Study)
- 3 Atlanta-area hospitals
- Each RBC exposure systematically measured
- Active surveillance by research nurses for NEC for all enrolled infants
- Adjudication of NEC cases (minimize ascertainment bias)

Patel RM et al. JAMA. 2016
Study design

• Inclusion criteria:
  – 1) Birth weight <1500g
  – 2) Postnatal age ≤ 5 days

• Exclusion criteria:
  – 1) Not expected to survive >7 days
  – 2) Severe congenital anomaly
  – 3) Transfusion prior to admission
  – 4) Maternal refusal

• Infants followed from birth to 90 days, transfer, discharge or death

Patel RM et al. JAMA. 2016
Exposures, Outcomes, and Statistical Analysis

• Primary exposure RBC transfusions
• Secondary exposure was severe anemia, defined \textit{a priori} as 8 grams/dl or less
• Both exposures were evaluated as time-varying covariates at weekly intervals.
• Hb values at 8 scheduled assessments were recorded with widening windows over time.
• If Hb level was not measured in a given week, prior value was carried forward and imputed
Severe anemia in a given week (time-varying):

- Severe anemia
- Not severely anemic
- Not severely anemic
- Severe anemia

Best aligned with what is actually observed

Severe anemia (time-varying):

Ever with severe anemia (fixed):
Sample Size and Statistical Analysis

- Sample size calculated assuming a NEC incidence of 7%, a final sample size of 535 enrolled VLBW infants was calculated to achieve 80% statistical power to detect a Hazard Ratio (HR) for NEC at ~2.5 for transfused VLBW infants compared to non-transfused VLBW infants (assuming proportional hazards), at a significance level of 0.05
- Competing Risk analysis to estimate the cause-specific hazard ratio (CSHR) for NEC and Mortality using Cox proportional-hazards regression model
Patient Enrollment

1652 Newborns assessed for eligibility

471 Excluded
398 Did not meet inclusion criteria
333 Birth weight >1500 g
( November 2010-February 2014)
37 Birth weight >1250 g
( January 2010-October 2010)
26 Aged >5 d
2 Met >1 of above criteria
73 Met exclusion criteria
59 Not expected to survive >7 d
10 Congenital anomaly
2 Received a transfusion before screening
2 Met >1 of above criteria

1181 Met enrollment criteria

581 Mothers declined consent

600 Enrolled

2 Excluded from follow-up
1 Incorrectly enrolled (did not meet enrollment criteria)
1 Mother withdrew consent

598 Underwent 90-day follow-up until hospital discharge, transfer to a non-study-affiliated hospital, or death

598 Included in the analysis
### Baseline characteristics of cohort

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>RBC exposed N=319</th>
<th>RBC unexposed N=279</th>
<th>NEC N=44</th>
<th>No NEC N=554</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total VLBW infants</strong></td>
<td>N=598</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gestational age in weeks, mean (SD)</strong></td>
<td>26.4 (2.1)</td>
<td>29.5 (2.2)</td>
<td>26.6 (2.3)</td>
<td>28.0 (2.6)</td>
</tr>
<tr>
<td><strong>Birth weight in grams, mean (SD)</strong></td>
<td>864 (240)</td>
<td>1189 (193)</td>
<td>820 (250)</td>
<td>1031 (269)</td>
</tr>
<tr>
<td>Male gender</td>
<td>163 (51.1%)</td>
<td>139 (49.8%)</td>
<td>20 (45.5%)</td>
<td>282 (50.9%)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>199 (62.4%)</td>
<td>147 (52.7%)</td>
<td>31 (70.5%)</td>
<td>315 (56.9%)</td>
</tr>
<tr>
<td>White</td>
<td>96 (30.1%)</td>
<td>104 (37.3%)</td>
<td>11 (25%)</td>
<td>189 (34.1%)</td>
</tr>
<tr>
<td>Asian</td>
<td>9 (2.8%)</td>
<td>16 (5.7%)</td>
<td>2 (4.5%)</td>
<td>23 (4.2%)</td>
</tr>
<tr>
<td>More than one race</td>
<td>12 (3.8%)</td>
<td>11 (3.9%)</td>
<td>0</td>
<td>23 (4.2%)</td>
</tr>
<tr>
<td>Other&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3 (0.9%)</td>
<td>1 (0.4%)</td>
<td>0</td>
<td>4 (0.7%)</td>
</tr>
<tr>
<td>Hispanic ethnicity</td>
<td>27 (8.5%)</td>
<td>23 (8.2%)</td>
<td>1 (2.3%)</td>
<td>49 (8.8%)</td>
</tr>
<tr>
<td>Singleton birth</td>
<td>231 (72.4%)</td>
<td>180 (64.5%)</td>
<td>34 (77.3%)</td>
<td>377 (68.1%)</td>
</tr>
<tr>
<td>Small for gestational age&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62 (19.4%)</td>
<td>76 (27.2%)</td>
<td>14 (31.8%)</td>
<td>124 (22.4%)</td>
</tr>
<tr>
<td>Born outside of study hospital</td>
<td>5 (1.6%)</td>
<td>2 (0.7%)</td>
<td>0</td>
<td>7 (1.3%)</td>
</tr>
</tbody>
</table>

Results

• 44 (7.4%) developed NEC
• 32 (5.4%) infants died (all cause)
• 53% of infants (319) received a total of 1430 RBC transfusions
• Unadjusted Cumulative Incidence of NEC at 8 weeks: RBC exposed 9.9% (95% CI, 6.9%-14.2%) vs RBC unexposed 4.6% (95% CI, 2.6%-8.0%)
• Cumulative Incidence NEC at 8 wks: 7.7% (95% CI, 5.7%-10.3%)
Cumulative Incidence Mortality at 8 wks: 3.3% (95% CI, 2.0%-5.0%)
# Multivariate Analysis

Anemia, NEC and RBC transfusion


<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>NEC (Cause-Specific HR (95% CI))</th>
<th>P Value</th>
<th>% Reliability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight, per 100-g increase</td>
<td>0.72 (0.62-0.84)</td>
<td>&lt;.001</td>
<td>98</td>
</tr>
<tr>
<td>Received RBC transfusion in a given week</td>
<td>0.44 (0.17-1.12)</td>
<td>.09</td>
<td>45</td>
</tr>
<tr>
<td>Severe anemia in a given week (hemoglobin ≤8 g/dL)</td>
<td>5.99 (2.00-18.0)</td>
<td>.001</td>
<td>70</td>
</tr>
<tr>
<td>Days of breast milk feeding in first 10 days of life, per 1-day increase</td>
<td>1.10 (1.01-1.21)</td>
<td>.04</td>
<td>37</td>
</tr>
<tr>
<td>SNAP on day of birth, per 1-point increase</td>
<td>1.00 (0.93-1.07)</td>
<td>.99</td>
<td>8</td>
</tr>
<tr>
<td>Days of antibiotic treatment in first 10 days of life, per 1-day increase</td>
<td>1.04 (0.93-1.16)</td>
<td>.50</td>
<td>8</td>
</tr>
</tbody>
</table>

**Primary outcome:** RBC transfusion in a given week was not significantly related to the rate of NEC
Multivariate Analysis
Anemia, NEC and RBC transfusion

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<tbody>
<tr>
<td>Model 1—Primary Analysis (N = 598)</td>
<td></td>
<td></td>
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<td>Birth weight, per 100-g increase</td>
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</table>

**Secondary Outcome:** Based on evaluation of 4565 longitudinal measurements of Hb (median 7 per infant), the rate of NEC was significantly increased among VLBW infants with severe anemia in a given week compared with those who did not have severe anemia.

Anemia, NEC and RBC transfusion

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>NEC Cause-Specific HR (95% CI)(^d)</th>
<th>(P) Value</th>
<th>% Reliability(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1—Primary Analysis (N = 598)(^d)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight, per 100-g increase</td>
<td>0.72 (0.62-0.84)</td>
<td>&lt;.001</td>
<td>98</td>
</tr>
<tr>
<td>Received RBC transfusion in a given week(^e)</td>
<td>0.44 (0.17-1.12)</td>
<td>.09</td>
<td>45</td>
</tr>
<tr>
<td>Severe anemia in a given week (hemoglobin (\leq) 8 g/dL)(^e)</td>
<td>5.99 (2.00-18.0)</td>
<td>.001</td>
<td>70</td>
</tr>
<tr>
<td>Days of breast milk feeding in first 10 days of life, per 1-day increase</td>
<td>1.10 (1.01-1.21)</td>
<td>.04</td>
<td>37</td>
</tr>
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<td>8</td>
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</table>

**Model 2—Study Exposures + Confounders With Reliability \(\geq\) 50% (N = 598)\(^d\)**

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>NEC Cause-Specific HR (95% CI)(^d)</th>
<th>(P) Value</th>
<th>% Reliability(^c)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.72 (0.62-0.84)</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>Received RBC transfusion in a given week</td>
<td>0.43 (0.18-1.04)</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Severe anemia in a given week</td>
<td>5.49 (1.81-16.6)</td>
<td>69</td>
<td></td>
</tr>
</tbody>
</table>
## Risk factors among RBC transfused infants

N=319 with 44 NEC events

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>NEC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CSHR</td>
</tr>
<tr>
<td>Birth weight (per 100g increase)</td>
<td>0.67</td>
</tr>
<tr>
<td>Received RBC transfusion in a given week&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>Nadir Hb in a given week&lt;sup&gt;b&lt;/sup&gt; (per 1 g/dL decrease)</strong></td>
<td><strong>1.65</strong></td>
</tr>
<tr>
<td>Days of breast milk feeding in 1st 10 days (per 1 day increase)</td>
<td>1.13</td>
</tr>
<tr>
<td>SNAP score on day of birth (per 1 point increase)</td>
<td>0.97</td>
</tr>
<tr>
<td>Days of antibiotic treatment in 1&lt;sup&gt;st&lt;/sup&gt; 10 days (per 1 day increase)</td>
<td>1.05</td>
</tr>
</tbody>
</table>
The overall risk of developing NEC within 48 hours after RBC transfusion was 0.49% (7 events following 1,430 RBC transfusions).
Updated meta-analysis of association between RBC transfusion and NEC

Hay S et al. Semin Perinatol. 2016 (in press)
**TOP Trial - Transfusion thresholds**

<table>
<thead>
<tr>
<th>Week of life</th>
<th>High Threshold</th>
<th>Low Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.0 / 38</td>
<td>12.0 / 35</td>
</tr>
<tr>
<td>2</td>
<td>12.5 / 37</td>
<td>11.0 / 32</td>
</tr>
<tr>
<td>≥ 3</td>
<td>11.0 / 32</td>
<td><strong>10.0</strong> / 29</td>
</tr>
</tbody>
</table>

Hemoglobin (g/dl) / Hematocrit (%)

Resp support: Vent/CPAP, NC >1L or FiO2>0.35

**Enrollment as of Today:** 1796 of 1824

28 patients away from completion

Role of assessing oxygen delivery
Near-infrared spectroscopy

700-1100nm NIR range spectrum

The INVOS™ system uses two depths of light penetration to subtract out surface data, resulting in a regional oxygenation value for deeper tissues.
Pre-transfusion mesenteric NIRS
Expected response to RBC transfusion

**RESPONDER:**
Increase in MES-rSO2 post-transfusion

Indicates RBC transfusion (time condensed)
Paradoxical response to RBC transfusion

NON-RESPONDER: Paradoxical decrease in MES-rSO2 post-transfusion
Impact of anemia on MES-rSO$_2$ response by area under the curve (AUC) to RBC transfusion

Anemic: Hb $\leq$ 9 g/dL
Do donor RBC characteristics mediate some of the variability in responses to RBC transfusion?
Variation in Donor RBCs prepared for Infant Transfusion

Donor

Variability: Age, gender, CMV status

Pre-storage RBC unit

Variability: Anticoagulant, preservative, timing of leukoreduction

Storage

Variability: When to expire unit

Volume Reduction

Variability: When to volume reduce and method of reduction.

Aliquoting

Variability: Time to outdated after aliquoting and method of aliquoting (syringe or bag)

Irradiation

Variability: Site of irradiation (supplier or hospital) and when to expire unit after irradiation.

Washing

Variability: When to wash, based on age of blood, age after irradiation or patient characteristics

Effect of storage of red cells on survival

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of Deaths</th>
<th>Total No.</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fresher Blood</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bennett-Guerrero et al, 2009</td>
<td>1</td>
<td>12</td>
<td>2.77 (0.12-61.65)</td>
</tr>
<tr>
<td>Aubron et al, 2012</td>
<td>5</td>
<td>25</td>
<td>2.60 (0.55-12.19)</td>
</tr>
<tr>
<td>Schulman et al, 2002</td>
<td>4</td>
<td>8</td>
<td>2.25 (0.55-9.17)</td>
</tr>
<tr>
<td>Hébert et al, 2005</td>
<td>5</td>
<td>26</td>
<td>1.49 (0.45-4.98)</td>
</tr>
<tr>
<td>Steiner et al, 2015</td>
<td>23</td>
<td>538</td>
<td>0.83 (0.48-1.41)</td>
</tr>
<tr>
<td>Kor et al, 2012</td>
<td>17</td>
<td>50</td>
<td>0.77 (0.47-1.27)</td>
</tr>
<tr>
<td>Heddle et al, 2012</td>
<td>35</td>
<td>309</td>
<td>1.12 (0.75-1.65)</td>
</tr>
<tr>
<td>Lacroix et al, 2015</td>
<td>448</td>
<td>1211</td>
<td>1.05 (0.94-1.17)</td>
</tr>
<tr>
<td>Subtotal</td>
<td>538</td>
<td>2179</td>
<td>1.04 (0.95-1.15)</td>
</tr>
</tbody>
</table>

| **Standard Issue Blood**        |               |           |             |
| No. of Deaths | Total No. | RR (95% CI) |
| Adults                          | 0             | 11        |             |
| Aubron et al, 2012              | 2             | 26        |             |
| Schulman et al, 2002            | 2             | 9         |             |
| Hébert et al, 2005              | 4             | 31        |             |
| Steiner et al, 2015             | 29            | 560       |             |
| Kor et al, 2012                 | 22            | 50        |             |
| Heddle et al, 2012              | 61            | 601       |             |
| Lacroix et al, 2015             | 430           | 1219      |             |
| Subtotal                        | 550           | 2507      |             |

Heterogeneity: $\tau^2 = 0; \chi^2 = 5.47; P = .60; I^2 = 0\%$
Tests for overall effect: $z$ score = 0.85; $P = .40$

**Neonates, Infants, and Children**

<table>
<thead>
<tr>
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<th>No. of Deaths</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dhabangi et al, 2013</td>
<td>1</td>
<td>37</td>
<td>3.00 (0.13-71.34)</td>
</tr>
<tr>
<td>Strauss et al, 1996</td>
<td>0</td>
<td>21</td>
<td>0.30 (0.01-7.02)</td>
</tr>
<tr>
<td>Dhabangi et al, 2015</td>
<td>7</td>
<td>143</td>
<td>1.40 (0.45-4.31)</td>
</tr>
<tr>
<td>Fernandes da Cunha et al, 2005</td>
<td>9</td>
<td>26</td>
<td>0.90 (0.44-1.85)</td>
</tr>
<tr>
<td>Fergusson et al, 2012</td>
<td>30</td>
<td>188</td>
<td>0.97 (0.61-1.54)</td>
</tr>
<tr>
<td>Subtotal</td>
<td>47</td>
<td>415</td>
<td>0.99 (0.69-1.42)</td>
</tr>
</tbody>
</table>

Heterogeneity: $\tau^2 = 0; \chi^2 = 1.46; P = .83; I^2 = 0\%$
Tests for overall effect: $z$ score = 0.66; $P = .96$

**Overall**

<table>
<thead>
<tr>
<th>No. of Deaths</th>
<th>Total No.</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>597</td>
<td>2921</td>
<td>1.04 (0.95-1.14)</td>
</tr>
</tbody>
</table>

Heterogeneity: $\tau^2 = 0; \chi^2 = 7.00; P = .86; I^2 = 0\%$
Tests for overall effect: $z$ score = 0.81; $P = .42$
Tests for subgroup differences: $\chi^2 = 0.08; P = .78; I^2 = 0\%$

Carson JL et al. JAMA. 2016
Variation in irradiation and storage

- Survey of 29 hospitals part of the NICHD neonatal research network
- Most (93%) irradiate cellular blood products before transfusion
- Irradiator on site in 66%, 34% receive irradiated products from blood center
**Objective**: Investigate the kinetic changes in the patterns of metabolites that occur during donor RBC storage with and without prior irradiation.
Red Blood Cell Storage Lesion: A Metabolically Active Process

D'Alessandro A. et al Transfusion 2015
Red Blood Cell Storage Lesion: Metabolomic Patterns

ATP
Membrane protein spots
DPG
Glucose (supernatant)
pH
NO
S-NO-Hb
Deformability
GSSG
Vesiculation
Lactate
Inflammatory Lipids (8-isoprostane)
K⁺ (supernatant)
Hemolysis
Hb1Ac
Malondialdehyde
Irreversible morphology alterations
Carbonylation
ROS
Prdx2
Ca²⁺

D’Alessandro A. et al Transfusion 2015
Patterns of metabolites by storage age and gamma irradiation status

- >10,000 m/z features in each sample
- 600 metabolites differentiated b/t storage time and/or irradiation using msPLSDA (multi-level sparse partial least squares discriminant analysis)
- Reduces high dimensional data into 3 principal components
- Score Plot A: Across PC1 most variability, distinct separation b/t fresh and saRBC
- saRBC 2-7d stored clustered together, 10-35 days shifted to left
- 2-7 stored no clear effect of irradiation
- In contrast RBC samples stored > 10 days separated from each other based on irradiation across PC2, esp ones stored 10-14 days

Patel RM et al. Transfusion. 2015
Separation of metabolites by irradiation status

- Score Plot (figure B) comparing PC1 to PC3 there was near complete separation between irradiated and non-irradiated RBC units

- Differences in metabolite patterns between irradiated and control samples increased as the storage age increased and were seen as early as 7 days after storage.

- To validate the findings two-way hierarchical clustering was done to compare patterns of metabolites b/t storage conditions and found 12 distinct clusters of RBC metabolites

Patel RM et al. Transfusion. 2015
# Metabolic pathways altered by storage and irradiation

**TABLE 1. Candidate pathways of metabolites disrupted by RBC storage and irradiation**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Raw p value</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arachidonic acid metabolism</td>
<td>3.28 x 10^-33</td>
<td>2.62 x 10^-31</td>
</tr>
<tr>
<td>Linoleic acid metabolism</td>
<td>1.61 x 10^-11</td>
<td>5.00 x 10^-10</td>
</tr>
<tr>
<td>Steroid hormone biosynthesis</td>
<td>1.88 x 10^-11</td>
<td>5.00 x 10^-10</td>
</tr>
<tr>
<td>α-Linolenic acid metabolism</td>
<td>1.75 x 10^-5</td>
<td>0.00035</td>
</tr>
<tr>
<td>Retinol metabolism</td>
<td>0.078191</td>
<td>1</td>
</tr>
<tr>
<td>One carbon pool by folate</td>
<td>0.24998</td>
<td>1</td>
</tr>
<tr>
<td>Sphingolipid metabolism</td>
<td>0.51166</td>
<td>1</td>
</tr>
<tr>
<td>Primary bile acid biosynthesis</td>
<td>0.57811</td>
<td>1</td>
</tr>
<tr>
<td>Glycerophospholipid metabolism</td>
<td>0.61518</td>
<td>1</td>
</tr>
<tr>
<td>Terpenoid backbone biosynthesis</td>
<td>0.70368</td>
<td>1</td>
</tr>
<tr>
<td>Biotin metabolism</td>
<td>0.71347</td>
<td>1</td>
</tr>
<tr>
<td>Lysine degradation</td>
<td>0.75885</td>
<td>1</td>
</tr>
<tr>
<td>Glycosylphosphatidylinositol-anchor biosynthesis</td>
<td>0.79645</td>
<td>1</td>
</tr>
<tr>
<td>Cyanoamino acid metabolism</td>
<td>0.83799</td>
<td>1</td>
</tr>
<tr>
<td>Sulfur metabolism</td>
<td>0.87107</td>
<td>1</td>
</tr>
<tr>
<td>Pyrimidine metabolism</td>
<td>0.89969</td>
<td>1</td>
</tr>
<tr>
<td>Caffeine metabolism</td>
<td>0.90851</td>
<td>1</td>
</tr>
<tr>
<td>Drug metabolism: cytochrome P450</td>
<td>0.91359</td>
<td>1</td>
</tr>
<tr>
<td>Ether lipid metabolism</td>
<td>0.92723</td>
<td>1</td>
</tr>
<tr>
<td>Inositol phosphate metabolism</td>
<td>0.93327</td>
<td>1</td>
</tr>
</tbody>
</table>

* The top 20 known metabolites identified through pathway enrichment analysis from 599 detected metabolites are shown.

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Pathway enrichment analysis utilizing a false detection rate (FDR) of 5%
Potential donor RBC biomarkers

Candidate Metabolites Identified by Pathway Enrichment Analysis

- Arachidonic acid, linolenic acid, steroid biosynthesis, and alpha-linolenic acid pathways were significantly affected by irradiation storage.
- 4 pathways identified are important in cellular membrane metabolism and RBC membrane deterioration which are key in RBC storage lesion.
- Dysfunction of the RBC membrane through alterations in these pathways may lead to RBC stiffening with loss of deformability and contribute to the clinical effects of the storage lesion and potentially dysfunction of stored and/or irradiated RBCs associated with NEC.

Planned prospective cohort study

ELBW Infant (n=220)
Enrolled within 5 days of birth

Specific Aim 1
NIRS: mesSO2 for 48hr
RBC irradiation storage (IST)
Evaluate all RBC transfusions
COMPARE
Responder Non-responder
Metabolomics profile (MP) of RBC unit
IST (n=110) no IST (n=110)
RBC units transfused to infants with TR-NEC
RBC units transfused to closely matched non-NEC control infants

Specific Aim 2
TR-NEC Infants (n=20)
Aortic ring
Reaches NEC “window” (PMA 29-34 weeks) (n=30)
COMPARE
Anemia No Anemia
NIRS: mesSO2 every week
If PRBC transfusion

Specific Aim 3
If PRBC transfusion
COMPARE
Anemia No Anemia
NIRS: mesSO2 every week
Conclusions

• Severe anemia, rather than RBC transfusion, may be an important risk factor for NEC.
• Near infrared spectroscopy (NIRS) may be useful in understanding infant-specific responses to transfusion.
• Additional study is needed to determine if NIRS can help identify infants at risk for developing NEC.
• Additional studies are necessary to determine if preventing severe anemia (degree and/or duration) is more important than minimizing RBC transfusion.
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