Role of nitric oxide and free heme in mediating red cell transfusion toxicity

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Disclosures:

i) Use of nitrite salts for the treatment of cardiovascular conditions (co-inventor)
ii) Sodium nitrite regulation of arteriogenesis and angiogenesis (co-inventor)
RBC Storage Lesion - transfusion toxicity?

If so, what is the problem(s)?
RBC Storage lesion and toxicity- Trauma

- **Leading cause of death and disability in 1-46 y olds**

- **Worldwide 1 in 7 deaths due to physical injury…expected to increase to 1 in 5 in next 15y despite improvements in resuscitation, trauma surgery and critical care**

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Published clinical studies in trauma on the effect of age and amount of transfused RBCs on patient outcomes</td>
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</table>

<table>
<thead>
<tr>
<th>First author</th>
<th>Year(s) of enrollment</th>
<th>Year(s) of study</th>
<th>Study design</th>
<th>Country/continent</th>
<th>Leukoreduction</th>
<th>No. of patients</th>
<th>Outcome measures</th>
<th>Age of old blood criteria</th>
<th>Effect of older RBCs/no. of units</th>
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</thead>
<tbody>
<tr>
<td>Zallen [12]</td>
<td>1999</td>
<td>NR</td>
<td>Retrospective single center</td>
<td>USA</td>
<td>NR</td>
<td>63</td>
<td>MOF</td>
<td>≥21 d</td>
<td>Yes/yes</td>
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<td>Murrell [16]</td>
<td>2005</td>
<td>2001-2003</td>
<td>Retrospective single center</td>
<td>USA</td>
<td>Mix (95%1R)</td>
<td>275</td>
<td>Mortality, LOS</td>
<td>Old &gt;20 d</td>
<td>No/no</td>
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<td>Kiraly [21]</td>
<td>2009</td>
<td>NR</td>
<td>Retrospective single center</td>
<td>USA</td>
<td>NR</td>
<td>32</td>
<td>Tissue oxygenation</td>
<td>≥21 d</td>
<td>Yes</td>
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<td>Vandromme [22]</td>
<td>2009</td>
<td>2004-2007</td>
<td>Retrospective single center</td>
<td>USA</td>
<td>Yes</td>
<td>1183</td>
<td>Pneumonia</td>
<td>≥14 d</td>
<td>Yes</td>
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<td>Weinberg [23]</td>
<td>2010</td>
<td>2000-2009</td>
<td>Retrospective single center</td>
<td>USA</td>
<td>Yes</td>
<td>1647</td>
<td>Mortality</td>
<td>≥14 d</td>
<td>Yes/yes</td>
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<td>Weinberg [26]</td>
<td>2013</td>
<td>2009-2012</td>
<td>Prospective 2 centers</td>
<td>USA</td>
<td>Yes</td>
<td>93</td>
<td>Microvascular response</td>
<td>NR</td>
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</tbody>
</table>

Abbreviations: DVT, deep vein thrombosis; LR, leukoreduction; MOF, multiorgan failure; MV, mechanical ventilation; NR, not reported; TBI, traumatic brain injury.
Over 9.5 years, 1647 trauma patients received exclusively old vs. fresh blood \((old > 14d)\)
RBC Storage lesion and toxicity - Trauma

Massively Transfused Trauma Patients

- Receive \( \geq 10 \) units in the first 24h
- Often get oldest RBCs also
- Retrospective data indicate higher adverse events (2011-2014 at UAB, 14,607 patients)

Kerby et al. unpublished
RBC Storage lesion and toxicity - Trauma
Massively Transfused Trauma Patients

Mostly fresh: >67% <10d RBC
Mostly stored: <33%<10d RBC >33% >14d RBC

184 MT patients: 47% mostly fresh 16% mostly stored
No difference in race, age, sex, injury mechanism. Mostly fresh group less likely to have head injury

Table 2. Risk ratios* (ORs) and associated 95% confidence intervals (CIs) for the association between age of blood category and mortality

<table>
<thead>
<tr>
<th></th>
<th>N (%)</th>
<th>Crude RR (95% CI)</th>
<th>Adjusted† RR (95% CI)</th>
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<tbody>
<tr>
<td><strong>OVERALL DEATH</strong></td>
<td></td>
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<tr>
<td>Mostly fresh (n=84)</td>
<td>29 (34.5)</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Mixed (n=70)</td>
<td>29 (41.4)</td>
<td>1.20 (0.72-2.01)</td>
<td>1.09 (0.64-1.86)</td>
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<tr>
<td>Mostly stored (n=30)</td>
<td>16 (53.3)</td>
<td>1.55 (0.84-2.85)</td>
<td>1.73 (0.90-3.33)</td>
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<tr>
<td><strong>DEATH IN 24 HOURS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mostly fresh (n=84)</td>
<td>18 (21.4)</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Mixed (n=70)</td>
<td>22 (31.4)</td>
<td>1.47 (0.79-2.73)</td>
<td>1.38 (0.71-2.67)</td>
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<tr>
<td>Mostly stored (n=30)</td>
<td>15 (50.0)</td>
<td>2.33 (1.18-4.63)</td>
<td>2.74 (1.28-5.85)</td>
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<tr>
<td><strong>DEATH IN 30 DAYS</strong></td>
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<tr>
<td>Mostly fresh (n=84)</td>
<td>27 (32.1)</td>
<td>Ref</td>
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<tr>
<td>Mixed (n=70)</td>
<td>27 (38.6)</td>
<td>1.20 (0.70-2.05)</td>
<td>1.08 (0.62-1.89)</td>
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<td>Mostly stored (n=30)</td>
<td>16 (53.3)</td>
<td>1.66 (0.89-3.08)</td>
<td>1.80 (0.93-3.51)</td>
</tr>
</tbody>
</table>

* Estimated from Cox regression assuming equal time at risk
† Adjusted for sex, number of packed red blood cell units transfused in first 24 hours, Glasgow Coma Scale score at emergency department presentation, and base deficit at emergency department presentation

RANDOMISED, BLINDED, CONTROLLED STUDY

Kerby et al unpublished
Stored RBC and Trauma-hemorrhage

Transfusion-Related Acute Lung Injury in a Rat Model of Trauma-Hemorrhage

Susannah E. Nicholson, MD, Robert A. Johnson, PhD, Teresa Craig, John G. Myers, MD, William Durante, PhD, Ronald M. Stewari, MD, and Frucsina K. Johnson, MD

Microparticles from Stored Red Blood Cells Activate Neutrophils and Cause Lung Injury after Hemorrhage and Resuscitation

Ritha M Belizaire, MD, Priya S Prakash, MD, Jillian R Richter, PhD, Bryce R Robinson, MD, Michael J Edwards, MD, FACS, Charles C Caldwell, PhD, Alex B Lentsch, PhD, Timothy A Pritts, MD, PhD, FACS

Published in final edited form as:

Erythrocyte storage increases rates of NO- and Nitrite scavenging: Implications for transfusion related toxicity

Ryan Stapley1, Benjamin Y. Owusu1, Angela Brandon1, Marianne Cusick2, Clina Rodriguez2, Marisa B. Marques1, Jeffrey D. Kerby2, Scott R. Barnum3, Jordan A. Weinberg8, Jack R. Lancaster Jr4,5,6,7, and Rakesh P. Patel1,7,*

Resuscitation With Aged Blood Exacerbates Liver Injury in a Hemorrhagic Rat Model

Idit Matot, MD; Miriam Katz, MD; Orit Pappo, MD; Orly Zelig, MD; Nathalie Corchia, MSc; Shaul Yedgar, PhD; Gregory Barshtein, PhD; Elliot Bennett Guerrero, MD; Rinat Abramovitch, PhD

Original Contribution
Red blood cell washing, nitrite therapy, and antihemec therapies prevent stored red blood cell toxicity after trauma–hemorrhage

Ryan Stapley1, Clina Rodriguez2, Joo-yeun Oh2, Jaidip Honavar2, Angela Brandon4, Brant M. Wagener5, Marisa B. Marques1, Jordan A. Weinberg8, Jeffrey D. Kerby2, Jean-Francois Pfitz1,2, Rakesh P. Patel1,7,*

TRANSFUSION COMPLICATIONS

CHEWSAM

Red blood cells stored 35 days or more are associated with adverse outcomes in high-risk patients

Ruchika Goel,1, Daniel J. Johnson,2 Andrew V. Scott,2, Aaron A.R. Tobian,7 Paul M. Ness,1 Enika Nagababu,2 and Steven M. Frank7

Published in final edited form as:

Transfusion of Older Stored Blood Worsens Outcomes in Canines Depending on the Presence and Severity of Pneumonia

Dong Wang1, Irene Cortés-Puch1, Junfeng Sun1, Steven B. Solomon1, Tamir Kanas2, Kenneth E. Remy1, Jing Feng1, Meghna Alimchandani3, Martha Quezado4, Christine Helms4, Andreas Periegas4, Mark T. Gladwin5, Daniel B. Kim-Shapiro6, Harvey G. Klein6, and Charles Natanson1

Circulation

Haptoglobin or Hemopexin Therapy Prevents Acute Adverse Effects of Resuscitation After Prolonged Storage of Red Cells

Mechanisms and the two hit hypothesis for RBC Storage toxicity

TH - Shock
Pro-Inflammatory state (receiving transfusion)

Storage Lesion (Aged Red Blood Cells)

• Increased Inflammation
• Microcirculatory dysfunction
• Increased infection
Stord RBC and toxicity in Trauma Hemorrhage 2-hit model and RBC storage age dependence

C57Bl/6 male mice 60% blood loss

LR stored RBC (+ plasma 1:1) (vary age and # units)

BAL protein (mg/ml)

BAL cells ($\times 10^4$ / ml)

Percent survival

Stapley et al FRBM 2015
Mediators and mechanisms: continuum of leukoreduced RBC derived species during cold storage that mediate post-transfusion toxicity

- Unstirred layer
- Intrinsic membrane barrier
- Healthy RBC
- Aged RBC
- Microparticles
- Hemoglobin
- Heme
- Free Iron

Decreased NO

Increased inflammation

Oxidative stress

Infection susceptibility
Mediators and mechanisms: continuum of leukoreduced RBC derived species during cold storage
Storage induced hemolysis and increased rates of NO-scavenging by free hemoglobin

Nitric oxide scavenging
Nitrite oxidation
Loss of ATP release / SNO
Inhibition of NO-signaling

Stapley et al Biochem J 2012
Liu et al Redox Biol, 2013
Inhibition of NO and stored RBC ked RBC scavenge NO faster with increasing storage time.

- Inhibition of ex vivo vessel dilation (Alexander et al 2012)
- Inhibition of FMD in humans (Hayek et al 2015, Risbano et al 2015)
Effects of NO-repletion using nitrite on stored RBC toxicity

NO-repletion prevents stored RBC dependent injury

Stapley et al 2015
Mediators and mechanisms: continuum of leukoreduced RBC derived species during cold storage

- Unstirred layer
- Intrinsic membrane barrier
- Healthy RBC
- Aged RBC
- Microparticles
- Hemoglobin
- Heme
- Free Iron

Increased inflammation
Oxidative stress
Infection susceptibility
Erythrocyte DAMPs and sterile inflammation

Mechanism IV. Molecular signalling effects of hemin

Gladwin MT, and Ofori-Acquah SF Blood 2014;123:3689-3690

Schaer DJ et al 2013 Blood 121(8):1276
Free heme in stored RBC and post transfusion - methods!!

**PBS**
- Hb Kit: \( y = 1.01x + 1.4, r^2 = 0.97 \)
- Heme Kit: \( y = 1.03x + 1.3, r^2 = 0.97 \)

**PBS**
- Hb Kit: \( y = 1.02x + 0.49, r^2 = 0.99 \)
- Heme Kit: \( y = 1.1x + 0.43, r^2 = 1 \)

**Levels in transfused Units (segments)**
- \( n = 8-25 \)

**Day 35 Paired**
- Heme or oxy-Hb (\( \mu M \))
- Bag: 20, Segment: 40
- Bag: 30, Segment: 60

**Concentration (\( \mu M \))**
- oxyHb: Non MTT, MTT
- heme: Non MTT, MTT

*Stapley et al FRBM 2015; Oh et al Transfusion 2015; Oh et al Redox Biol 2016*
Stored RBC derived microvesicle heterogeneity and heme

[Bar graph showing nmol/# particle (x 10^10) for different conditions]

<table>
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<th>Ctrl</th>
<th>RBC</th>
<th>MP</th>
<th>Exo</th>
<th>Heme</th>
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<tr>
<td></td>
<td>0.5µM</td>
<td>10µM</td>
<td>50µM</td>
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</table>
Role of free heme in stored RBC toxicity after TH

C57Bl/6 male mice 60% blood loss

Hemorrhage

Shock (25 ± 5 mmHg)

Resuscitation

24h ALI survival

LR mRBC 0-14d
1-3 units (1:1 plasma)

Live
Dead

Percent

Hemopexin

0 20 40 60 80 100

d0 d14 d0 d14 d14
1 unit 3 units

BAL Cells (x 10^4 / ml)

Day 0

Hpx TAK-242 TLR4-/-

Day 14

Stapley et al FRBM 2015
Pittet et al unpublished
Role of free heme in stored RBC-dependent infection risk after TH

Pittet et al. unpublished
Mediators and mechanisms: continuum of leukoreduced RBC derived species during cold storage

Interactions: additive / synergistic?

Unstirred layer
Intrinsic membrane barrier
Healthy RBC
Aged RBC
Microparticles
Hemoglobin
Heme
Free Iron

Decreased NO

Oxidative stress
Mediators and mechanisms: continuum of leukoreduced RBC derived species during cold storage

What else is in the bag - functional
Gain of toxic or loss of protective mechanisms?
Precursor chromatograph of 463.2332 ± 0.025 Da, m/z

Stored human RBC supernatant

GTFATLSELHC(DHA)DK

- Day 7
- Day 35

Cysteine

Dehydroalanine (DHA)

Nucleophilic

Electrophilic

Effect on post-transfusion toxicity?

Not observed in stored C57Bl/6
Peroxiredoxin-2: significant antioxidant protein in RBC

Monomer (reduced) → Dimer (oxidized) → Monomer

A

H₂O₂ (µM) for 10 min

0 0.5 1 2 5 10 50 100 200

Prx-2

Dimer

Prx-2

Monomer

B

RBC-PRx-2 and PMN-derived Oxidant scavenging

(LPS-C57Bl/6)

Low et al 2007
Bayer et al 2013
Prx-2 still reacts with hydrogen peroxide but recycling is inhibited during storage.

Rate = 1

Rate = 0.57
- Many putative mediators
- Model and protocol dependent
- Interactions between mediators
- Gain of toxic
- Loss or protective effects

Decreased NO

Increased inflammation

Oxidative stress

Infection susceptibility
Acknowledgements

UAB Pathology
Joo-Yeun Oh, PhD
Ryan Stapley, PhD
Jaideep Honavar, BS
Victoria Harper, BS
Jennifer Hamm, MD
Karina Ricart, PhD
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Jeff Kerby MD, PhD
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Russel Griffin, PhD

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Brant Wagener, MD, PhD
Angela Brandon, BS

UAB Emergency Medicine
Henry Wang MD