Risks of Transfused Older RBCs in Critical Illness

Studies in Canines

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Disclosures

I have no potential conflicts of interest concerning Red Blood Cell Transfusion
RBC Storage Changes Implicated in Toxicity

- Stored RBC units accumulate:
  - Cell free hemoglobin (CFH)
    ✤ associated with nitric oxide (NO) scavenging and vasoconstriction
RBC Storage Changes Implicated in Toxicity

• Stored RBC units accumulate:
  – Cell free hemoglobin (CFH)
    ✤ associated with nitric oxide (NO) scavenging and vasoconstriction
  – Iron
    ✤ essential nutrient promotes bacterial growth and increases risk of infection
Need for a Preclinical Model

• ~108 million units of RBCs are obtained for transfusion yearly worldwide
• ~15 million units of RBCs are obtained for transfusion yearly in the United States
  – ~20% of these units of RBCs are stored 4 – 6 weeks before transfusion
  – These older units have been difficult to study in clinical trials
Do older RBCs increase risks of transfusion in critically ill canines?
Experimental Canine Model

- *S. aureus* pneumonia with mechanical ventilation, sedation, antibiotics and cardiovascular support

- Titrated care similar to ICU patients based on physiologic parameters over 5 days
Transfusion of Old vs. New RBCs During Canine Pneumonia

- Challenged with *S. aureus*
- Transfused universal donor commercially available canine RBCs
  - consistent with stored human RBCs
    ✤ > 60% of canine RBCs remain in circulation after 24h
    ✤ <1% hemolysis in storage bags

Transfusion of Old vs. New RBCs During Canine Pneumonia

- Exchange transfused four times 25% of blood volume (20ml/kg x 4)
  - 42 or 7 day old stored RBCs
  - 4 to 16h after bacterial challenge
  - Replaced 70% of blood volume

Older RBCs Decreased Survival During Canine Pneumonia

Proportion surviving

Time (h) after S. aureus challenge

7 Day Old RBCs
n = 12

42 Day Old RBCs
n = 12

p = 0.0005

Older RBCs Increased Lung Injury During Canine Pneumonia

![Graph showing alveolar arterial oxygen gradient (AaO$_2$) over time for 7 and 42 day old RBCs after S. aureus challenge.](image)

- **42 Day Old RBCs**
  - Time (h) after S. aureus challenge:
    - 0 h: AaO$_2$ = 0 mmHg
    - 4 h: AaO$_2$ = 50 mmHg
    - 16 h: AaO$_2$ = 200 mmHg
    - 24 h: AaO$_2$ = 400 mmHg
    - 48 h: AaO$_2$ = 600 mmHg

- **7 Day Old RBCs**
  - Time (h) after S. aureus challenge:
    - 0 h: AaO$_2$ = 0 mmHg
    - 4 h: AaO$_2$ = 150 mmHg
    - 16 h: AaO$_2$ = 300 mmHg
    - 24 h: AaO$_2$ = 450 mmHg
    - 48 h: AaO$_2$ = 600 mmHg

Statistical significances:
- p = 0.01 for 42 day old RBCs compared to 7 day old RBCs at 48 h.
- p = 0.005 for 42 day old RBCs compared to 7 day old RBCs at 24 h.

Two factors that may explain the increase in lung injury and mortality with older RBCs

- Cell Free Hemoglobin
- Iron
Factor One:
Cell Free Hemoglobin (CFH) Levels

Cell free hemoglobin Level (CFH)

Time (h) after S. aureus challenge

Factor One:
Cell Free Hemoglobin (CFH) Levels

Plasma NO Consumption Capacity

Time (h) after S. aureus challenge

Exchange Transfusion

42 Day Old RBCs

7 Day Old RBCs

Transfused older RBCs increase CFH levels, mostly in the reduced oxyhemoglobin state, reflected by the increase in plasma NO consumption capacity.
Factor Two: Plasma Iron Levels

7 Day Old RBCs

Non transferrin bound iron (NTBI) (~μM)

42 Day Old RBCs

p = 0.03 (vs. 7 Day old)

Abnormal level

Time (h) after S. aureus challenge

Conclusions:
Human Studies and Canine Model
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Human Studies and Canine Model

- Our findings indicate older RBCs increase risks
- RCTs cannot rule out, and observational studies suggest, that older RBCs increase mortality
- Consistent with observational studies, in canines with pneumonia, older RBCs increase mortality associated with:
  - increased hemolysis
  - release of cell free hemoglobin
  - release of iron
Is the volume of transfused older RBCs a critical risk factor during infection?
Is the volume of transfused older RBCs a critical risk factor during infection?

NO!
Hazard Ratios

Favors 7 Day Old RBCs

Equivalent # human RBC Units

Ten to twelve
60-80 mL/kg

Four to Six
20-40 mL/kg

Two to Three
5-10 mL/kg

Favors 42 Day Old RBCs

p > 0.70 (for similar effect)

Hazard Ratio of death (95% Confidence Interval)

Transfusion 2015 Sept 7
Is the mortality risk of transfused older RBCs altered by the presence and severity of infection?
In Healthy Controls, Mortality is **Not** Increased Transfusing Older Stored RBCs

- Dose 0 (no bacteria)
- 7 Day Old RBCs, n=4
- 42 Day Old RBCs, n=4

Survival proportion

Time (h) after saline intra bronchial challenge

*Transfusion 2014; 54: 1712-1724*
Mortality After Transfusion of 7 vs. 42 Day Old RBCs with Increasing S. aureus Challenges

A. Dose 0 (no bacteria)
- n=4

B. Dose 1 (x10⁹CFU/kg)
- 7 Day Old RBCs
  - n=4
- 42 Day Old RBCs
  - n=4

Survival proportion

Time(h) after saline challenge

Time (h) after S. aureus challenge

Transfusion 2014; 54: 1712-1724
Mortality After Transfusion of 7 vs. 42 Day Old RBCs with Increasing S. aureus Challenges

A. Dose 0 (no bacteria)

B. Dose 1 \((x10^9\text{CFU/kg})\)

C. Dose 1.25 \((x10^9\text{CFU/kg})\)

- **7 Day Old RBCs**
  - \(p=0.0005\)
  - \(n=12\)

- **42 Day Old RBCs**
  - \(n=12\)

Time (h) after S. aureus challenge

Survival proportion

\(n=4\)

Transfusion 2014;
54: 1712-1724
Mortality After Transfusion of 7 vs. 42 Day Old RBCs with Increasing *S. aureus* Challenges

A. Dose 0 (no bacteria)

B. Dose 1 ($10^9$CFU/kg)

C. Dose 1.25 ($10^9$CFU/kg)

D. Dose >1.5 ($10^9$CFU/kg)

$\ p=0.0005$
In healthy controls, there was no significant risk of older transfused RBCs. With moderate doses of bacteria, older RBCs increase the mortality risk.
Mortality After Transfusion of 7 vs. 42 Day Old RBCs with Increasing S. aureus Challenges

A. Dose 0 (no bacteria)  
- n=4

B. Dose 1 (x10⁹ CFU/kg)  
- n=4

Risks of older RBCs are dependent on the presence and severity of infection

C. Dose 1.25 (x10⁹ CFU/kg)  
- n=12

D. Dose >1.5 (x10⁹ CFU/kg)  
- n=4

Survival Proportion

Time (h) after S. aureus challenge

Transfusion 2014; 54: 1712-1724
Are the systemic pressure changes associated with older RBCs modified by the presence and severity of infection?
Are the systemic pressure changes associated with older RBCs modified by the presence and severity of infection?

Transfused older RBCs release CFH that scavenges NO causing vasoconstriction
Transfused Older Stored Blood and Vasopressor Requirements in Septic Canines

Shock Score vs Time (h) Before and After S. aureus Challenge

- 7 day old blood
- 42 day old blood

During Transfusion

P = 0.02

P = 0.01

Transfused Older Stored Blood and Vasopressor Requirements in Septic Canines

Transfused older stored blood during transfusion improves, but after 24h worsens septic shock

Time (h) Before and After S. aureus Challenge

Does Older Transfused Stored Blood Have Two Different Effects on Systemic Pressures During Infection?
Bacterial Dose Response Data

- Only Animals Transfused Without Receiving Bacterial Challenges
Shock Scores After Transfused 7 vs 42 Day Old Stored Blood with Increasing Doses of S. aureus In Only Animals Transfused Without Bacterial Challenges

4 h immediately before transfusion

13 h 75% of blood transfused

16 h 2h after transfusion

24 h 10h after transfusion

Dose of S. aureus challenge (x10⁰CFU/kg)

Transfusion, 2014.
Without bacterial infection, transfusion of older blood causes a sustained increase in blood pressure.
Bacterial Dose Response Data

Only Transfused Animals Given Bacterial Challenges
Shock Scores After Transfused 7 vs 42 Day Old Stored Blood with Increasing Doses of *S. aureus* In Only Animals Transfused With Bacterial Challenges

**4 h immediately before transfusion**

**13 h 75% of blood transfused**

**16 h 2h after transfusion**

**24 h 10h after transfusion**

\[ \text{Dose of } S. \text{ aureus challenge (x}10^9\text{CFU/kg)} \]

*Transfusion, 2014.*
Shock Scores After Transfused 7 vs 42 Day Old Stored Blood with Increasing Doses of *S. aureus*

With established infection early after transfusion, older blood increases the shock score regardless of bacterial dose.
Shock Scores After Transfused 7 vs 42 Day Old Stored Blood with Increasing Doses of S. aureus
In Only Animals Transfused With Bacterial Challenges

4 h immediately before transfusion

13 h 75% of blood transfused

16 h 2h after transfusion

24 h 10h after transfusion

Transfusion, 2014.
Later with established infection after transfusion, older blood decreases the shock score more with increasing bacterial dose.
Shock Scores After Transfused 7 vs 42 Day Old Stored Blood with Increasing Doses of *S. aureus* In Only Animals Transfused With Bacterial Challenges

4 h immediately before transfusion

13 h 75% of blood transfused

16 h 2h after transfusion

24 h 10h after transfusion

Dose of *S. aureus* challenge (x10^9 CFU/kg)

Transfusion, 2014.
Shock Scores After Transfused 7 vs 42 Day Old Stored Blood with Increasing Doses of *S. aureus* In Only Animals Transfused With Bacterial Challenges

- **4 h immediately before transfusion**
- **13 h 75% of blood transfused**
- **16 h 2h after transfusion**
- **24 h 10h after transfusion**

**A.**
- 42 day old
- 7 day old

**B.**

**C.**

**D.**

Transfusion, 2014.
Shock Scores After Transfused 7 vs 42 Day Old Stored Blood with Increasing Doses of S. aureus

Still later with established infection, older blood after transfusion decreases the shock score even more with increasing bacterial dose.
Still later with established infection, older blood after transfusion decreases the shock score even more with increasing bacterial dose.

There is an interaction between age of transfused stored blood and dose of bacterial challenge.
With bacterial infection, older blood after transfusion with time causes a fall in blood pressure that is greater with increasing doses of bacteria.
There are two potential mechanisms of injury associated with transfused older RBCs

– Early vasoconstriction that is independent of bacterial dose

* Main effect comparing 7 vs 42 day old stored blood

** Interaction comparing 7 vs 42 day old stored blood
There are two potential mechanisms of injury associated with transfused older RBCs

- **Early** vasoconstriction that is independent of bacterial dose
- **Later**, a worsening of shock that is dependent on bacterial dose

* Main effect comparing 7 vs 42 day old stored blood
** Interaction comparing 7 vs 42 day old stored blood
Does the severity of infection alter cell free hemoglobin or iron levels?
Does the severity of infection alter cell free hemoglobin or iron levels?

Can these factors explain why older RBCs

• *Early on, raise MAP independent* of the severity of infection and

• *Later on, decrease MAP dependent* on the severity of infection?
Cell Free Hemoglobin (CFH) Levels During Transfusion with Increasing S. aureus Challenges

Main Effect
Comparing 7 vs. 42 day old RBCs

\[ p < 0.0001 \]

Dose of S. aureus challenge (x10^9 CFU/kg)

42 Day Old RBCs
7 Day Old RBCs

Transfusion 2014; 54: 1712-1724
Independent of the severity of infection, increased CFH levels are found in similar amounts in the vascular space for days after transfusing older RBCs.
Iron Levels After Transfusing 7 vs. 42 Day Old RBCs with Increasing S. aureus Challenges

Immediately before transfusion

42 Day Old RBCs
7 Day Old RBCs

Non transferrin bound iron (NTBI) (µM)

Dose of S. aureus challenge (x10⁹CFU/kg)
Iron Levels After Transfusing 7 vs. 42 Day Old RBCs with Increasing S. aureus Challenges

**A.** Immediately before transfusion

- **42 Day Old RBCs**
- **7 Day Old RBCs**

**B.** 2h after transfusion

- *p=0.04*

*interaction comparing 7 vs. 42 day old RBCs*

As bacterial dose increases, iron levels fall faster with older RBCs

Transfusion 2014; 54: 1712-1724
Transfusion of older RBCs, are associated with higher iron levels and more rapid decline as bacterial challenge – dose increases.
Toxicity of Older RBCs: Proposed Role of Cell Free Hemoglobin (CFH)
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- Hemolysis of older transfused RBCs results in elevated CFH over days
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- Hemolysis of older transfused RBCs results in elevated CFH over days
  - The CFH levels are not altered by the presence or severity of infection
Toxicity of Older RBCs: Proposed Role of Cell Free Hemoglobin (CFH)

- Hemolysis of older transfused RBCs results in elevated CFH over days
  - The CFH levels are not altered by the presence or severity of infection
  - CFH is vasoconstrictive and at sites of injury can potentially cause additional ischemic damage worsening lung injury and mortality
Toxicity of Older RBCs: Proposed Role of Non Transferrin Bound Iron

- Transfused older RBCs hemolyze releasing iron an essential nutrient that can promote bacterial growth
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  - In healthy controls transfused older RBC produce high iron levels without increasing risks
Toxicity of Older RBCs: Proposed Role of Non Transferrin Bound Iron

- Transfused older RBCs hemolyze releasing iron an essential nutrient that can promote bacterial growth
  - In healthy controls transfused older RBC produce high iron levels without increasing risks
  - Iron released during pneumonia is associated with increased risks and, as the bacterial challenge dose gets greater, iron levels disappear faster
Do older transfused RBCs increase risks in shock without infection?
Hemorrhagic Shock Model:
Shock, Inflammation and Reperfusion Injury in the Absence of Infection

- Animals bled 55 ml/kg
- Shock maintained for 2.5 hours
- Then transfused an equivalent amount of either 7 or 42 day old RBCs
Iron Levels Increase after Transfusion of Older RBCs During Hemorrhage/Reperfusion Injury

Non transferrin bound iron (μM)

42 Day Old RBCs

7 Day Old RBCs

Time (h) after starting hemorrhage (0 h)

RBCs Withdrawn 55ml/kg

Old vs. New RBCs Transfused 55ml/kg

p = 0.01

p = 0.004

n = 6

Abnormal levels

Transfusion 2015 Jul 15.
CFH Levels Increase after Transfusion of Older RBCs During Hemorrhage/Reperfusion Injury

Cell free hemoglobin (CFH) level (µM)

42 Day Old RBCs

7 Day Old RBCs

Time (h) after starting hemorrhage (0 h)

RBCs Withdrawn 55ml/kg
Old vs. New RBCs Transfused 55ml/kg

p = 0.05

p < 0.0001

n = 6

Transfusion 2015 Jul 15.
Survival Unchanged after Transfusion of Older RBCs During Hemorrhage/Reperfusion Injury

Proportion surviving

Time (h) after starting hemorrhage (0 h)

42 Day Old RBCs
n = 6

7 Day Old RBCs
n = 6

Transfusion 2015 Jul 15.
Survival Unchanged after Transfusion of Older RBCs During Hemorrhage/Reperfusion Injury

- Transfusion of older RBCs during hemorrhage/reperfusion injury:
  - Increased cell free hemoglobin and iron levels
  - but did not worsen mortality

This was a significantly different effect than during infection
Potential Mechanism of “Benefit” of Older RBCs During Hemorrhage/Reperfusion Injury

- Cell free hemoglobin released by transfused older RBCs scavenges nitric oxide reducing levels and the doses of norepinephrine required to maintain MAP
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- Cell free hemoglobin released by transfused older RBCs scavenges nitric oxide reducing levels and the doses of norepinephrine required to maintain MAP

- Decreased norepinephrine dosing resulted in lowered cardiac outputs which decreased reperfusion and its attendant injury
Example of Liver Injury Found in Non-surviving Canines Receiving Fresh Blood
Liver Infarct of Right Middle Lobe

Figure 1. Example of the gross morphologic changes in liver found in all three nonsurviving animals that were hemorrhaged and then transfused with 7-day-old blood. (Left) Median liver lobe with hemorrhagic infarct changes in situ. (Right) Same liver infarct once separated from the rest of the liver parenchyma.

Transfusion 2015 Jul 15.
During Pneumonia with Acute Mild Anemia Risks of Transfusion (Fresh RBCs) vs. Iron Therapy
During Pneumonia with Acute Mild Anemia Risks of Transfusion (Fresh RBCs) vs. Iron Therapy

Can Standard Iron Therapy Alone Increase Risks in Animals Receiving Bacterial Challenges?
Survival Transfusion vs. Iron Therapy

Time after *S. aureus* intrabronchial challenge

Proportion Surviving

- Transfusion
- Iron Sucrose (*p*=0.01)
- Ferumoxytol (*p*=0.04)

*Compared to transfusion, log rank test

Manuscript in preparation.
Lung Injury Transfusion vs. Iron Therapy

Alveolar-arterial Oxygen Gradient

Time after S. aureus challenge

Ferumoxytol

Iron Sucrose

Transfusion

*p=0.007
*p=0.02
*p=0.001
*p=0.001

*Compared to transfusion

Manuscript in preparation.
Iron Levels Transfusion vs. Iron Therapy

Non-transferrin bound iron plasma levels (log µM)

Time after S. aureus challenge

Iron Sucrose

- *p<0.0001
- *p=0.002
- *p=0.0006

Ferumoxytol

- *p=0.04
- *p=0.004

Transfusion

- *p=0.02
- *p=0.04

*Compared to transfusion

Manuscript in preparation.
PCWP, Cl, and HgB Transfusion vs. Iron Therapy

A. PCWP

B. Cl

- **Transfusion**
- **Ferumoxytol**
- **Iron Sucrose**

**Pulmonary Capillary Wedge Pressure (mmHg)**

**Cardiac Index (L/min/m²)**

Time after *S. aureus* challenge
PCWP, CI, and HgB Transfusion vs. Iron Therapy

A. PCWP

B. CI

C. HgB

- Transfusion
- Ferumoxytol
- Iron Sucrose
Conclusions: Transfusion vs. Iron Therapy

- Iron Therapy during pneumonia with mild acute anemia vs. transfusion
Conclusions:
Transfusion vs. Iron Therapy

- Iron Therapy during pneumonia with mild acute anemia vs. transfusion
  - Increased mortality
Conclusions:
Transfusion vs. Iron Therapy

- Iron Therapy during pneumonia with mild acute anemia vs. transfusion
  - Increased mortality
  - Increased lung injury
Conclusions: Transfusion vs. Iron Therapy

- Iron Therapy during pneumonia with mild acute anemia vs. transfusion
  - Increased **mortality**
  - Increased **lung injury**
  - Increased **iron levels**
Conclusions: Transfusion vs. Iron Therapy

- Iron Therapy during pneumonia with mild acute anemia vs. transfusion
  - Increased mortality
  - Increased lung injury
  - Increased iron levels

- These risks were independent of the type of iron therapy (Iron Sucroce vs. Ferumoxytol)
Conclusions: Transfusion vs. Iron Therapy

• Iron Therapy during pneumonia with mild acute anemia vs. transfusion
  – Increased mortality
  – Increased lung injury
  – Increased iron levels

• These risks were independent of the type of iron therapy (Iron Sucrose vs. Ferumoxytol)

Transfusion of fresh blood is associated less risks than iron therapy during mild anemia with pneumonia
Conclusions:
Transfusion vs. Iron Therapy

- Iron Therapy during pneumonia with mild acute anemia vs. transfusion
  - Increased mortality
  - Increased lung injury
  - Increased iron levels

- These risks were independent of the type of iron therapy (Iron Sucrose vs. Ferumoxytol)

Further raising iron levels alone independent of cell free Hgb during mild anemia with pneumonia can increase risks
Conclusions:
Animal Model Older RBCs

- Older RBCs are associated with
  - increased hemolysis
  - release of cell free hemoglobin
  - release of iron
Conclusions:

Animal Model Older RBCs

• Older RBCs are associated with
  – increased **hemolysis**
  – release of **cell free hemoglobin**
  – release of **iron**

• In pneumonia these abnormalities are associated with increased lung injury and mortality
Conclusions:
Animal Model Older RBCs

- Older RBCs are associated with
  - increased hemolysis
  - release of cell free hemoglobin
  - release of iron

- In pneumonia these abnormalities are associated with increased lung injury and mortality

- This increased risk of older blood exists even at commonly used transfusion volumes (2 units)
Conclusions:
Animal Model Older RBCs

- The importance of the RBC storage lesion is critically dependent on the clinical setting and age of RBCs.
Conclusions: Animal Model Older RBCs

- The importance of the RBC storage lesion is critically dependent on the clinical setting and age of RBCs

- Older transfused RBCs do not exacerbate and may improve hemorrhagic shock/reperfusion injury
Conclusions: Animal Model and Human Studies

- If transfusion risks are dependent on RBC storage age as well as presence of infection, then RCTs evaluating relatively fresh cells or mostly uninfected patients are unlikely to detect these effects
Conclusions: Animal Models and RBC Quality

- Animal models could help with preclinical determination of stored RBCs quality
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- Animal models could help with preclinical determination of stored RBCs quality.
- For example, animal models may have real relevance in determining safety of stored RBCs in various clinical situations affected differently by RBCs of different ages.
Conclusions: Animal Models and RBC Quality

• Animal models could help with preclinical determination of stored RBCs quality

• For example animal models may have real relevance in determining safety of stored RBCs in various clinical situations affected differently by RBCs of different ages

• This more than simple lab determinations would link biomarkers with clinical outcomes
Critical Care Medicine’s Applied Physiology Laboratory at the Clinical Center, NIH