The red blood cell (RBC) storage lesion:
*Is it clinically significant?*

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Red Blood Cell (RBC) Transfusion is a Common Medical Practice

- ~14 Million RBC units are collected/year in US
  - Almost all are transfused (wastage is minimal)

- Complex logistics of procurement and supply
  - Goal is optimal utilization of RBC resources

- RBC units can be stored up to 42 days at 1-6°C prior to transfusion
  - FDA licensure of transfusion technologies (bags, collection solutions, etc) is based on survival of RBCs (not function)
  - Assumption: if RBCs survive, they are working
  - However……
Many biochemical and functional changes occur during RBC storage…

↑ Inflammatory mediators   ↓ RBC deformability
↑ RBC adhesion            ↓ ATP
↑ Immunomodulation         ↓ 2,3-DPG
↑ Hemolysis               ↓ NO

...The critical question is: do these changes sufficiently alter RBC function to produce different outcomes in recipients?

The results will have significant implications for blood supply logistics
20+ major clinical studies addressing this issue

- Decidedly mixed results
  - Some show effects of storage on recipient outcomes
  - Others do not
- Factors complicating analysis
  - Single vs. multiple center
  - Study size ranges from 15 – 400,000 patients
  - Variable RBC processing & storage methods
- How generalizable are results of one study to another patient group?
Koch et al: storage impairs outcome

Retrospective review of cardiac surgery pts
- 8802 units stored median 11d given to 2872 pts
- 10,782 units stored median 19d given to 3130 pts

<table>
<thead>
<tr>
<th></th>
<th>MORTALITY</th>
<th>VENT &gt; 72 H</th>
<th>COMPOSITE</th>
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</thead>
<tbody>
<tr>
<td>RBC: mean 11d</td>
<td>1.7%</td>
<td>5.6%</td>
<td>22.4%</td>
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<tr>
<td>RBC: mean 20d</td>
<td>2.8%</td>
<td>9.7%</td>
<td>25.9%</td>
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<tr>
<td>P-value</td>
<td>0.004</td>
<td>&lt; 0.001</td>
<td>0.001</td>
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405,000 transfusion episodes studied: “Although a small excess mortality was noted in recipients of the oldest RBCs, the risk pattern was more consistent with weak confounding…”
Retrospective studies have problems

- It is almost impossible to remove all biases from retrospective studies.
- Based on research questions that were first examined by retrospective studies, and then prospective RCT:
  - Retrospective studies overstate benefits
  - Retrospective studies understate risks
  - Retrospective studies reach incorrect conclusions > 50% of the time

- "Lies, Damned Lies, and Medical Science"
  - (Atlantic Magazine; November 2010)
Effect of Red Cell Storage Age: 

*Equipoise Exists*

- Results from large retrospective studies are in disagreement
- Until **RECESS** (which has re-started accrual) no large, prospective human RCT has evaluated the effects of transfusion of RBC units stored for different periods on:
  - Clinical outcome
  - Hemodynamic variables & end-organ function
  - Immediate $O_2$ delivery enhancement
  - Microvascular circulatory changes
We are taking another approach: Investigate effects at an “individual patient level”

- Focus on the possible role of alterations in peripheral NO activity following exposure to stored RBCs
- **INOBA** hypothesis: Insufficient **NO Bio-Availability**
  - A hypothesis to explain the adverse effects of aged blood?

Nitric Oxide

- In addition to carrying O$_2$ and CO$_2$, RBCs/Hb can also control local concentrations of NO.
- NO regulation by RBCs is believed to be important for the process of “hypoxic vasodilation”
  - NO concentrations in the periphery help match blood flow (and O$_2$ supply) with O$_2$ need
  - Under low pO$_2$ tissue conditions, more NO is “released” from RBCs → increased blood flow
- Simplest form of Hypothesis: Stored RBCs carry/catalyze less NO for hypoxic vasodilation; transfusion of these cells impairs blood flow.
Nitric Oxide

• **INOBA** hypothesis: **Insufficient NO Bio-Availability**
  - A hypothesis to explain the adverse effects of aged blood?

• *Potential problem with the simple hypothesis:*
  • How can a few units of older RBCs (assuming depleted NO) adversely affect outcomes in the presence of other normal RBCs?
  • Alternatively, NO scavenging/buffering may be important…

![Diagram showing the interaction between fresh and stored RBCs and NO]
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- **Aim 1**: determine effects of common RBC storage and processing methods on NO-dependent vasoreactivity using “high throughput” aortic ring models
- **Aim 2**: select most interesting conditions and investigate their effects on NO-dependent vasodilatory function in healthy volunteer transfusion recipients
- **Aim 3**: select 1-3 specific conditions (eg, <10 days vs. >21 days) and investigate in hospitalized patients, correlating with endothelial dysfunction
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If stored RBCs express potent inhibitory activity... relatively small numbers of these cells could exert global inhibition of vascular NO signaling.
Contractility measurements

Determine effects of leukoreduced AS-3 RBC storage time on NO-dependent vasoreactivity
• **When RBCs are added before ACh:**
  - Interfere with ACh-stimulated, NO-mediated vasodilation
  - Inhibitory effect significantly greater with old vs. fresh RBC units
normal protocol

spun (volume reduction) protocol

Aim 1: RBC storage and/or modification alters NO bioavailability in vitro
normal protocol

spun (volume reduction) protocol

% Relaxation

Acetylcholine (M)

Maximum Relaxation (%)

Days of blood storage

Stage I

Stage II

Stage III

no / minimal effect of removing supernatant
ACh Dose for Maximal Relaxation

Blood Dose Response to Elicit Contraction (Reverse Relaxation)

When RBCs are after ACh:

- Reverse ACh-stimulated, NO-mediated vasodilation
- Inhibitory effect significantly greater with old vs. fresh RBC units
Aim 1 - summary

• ACh-stimulated, NO-mediated vasodilation is impaired by RBCs, with inhibition increasing with storage time
  – distinct stages of storage?
• Supernatant (free Hb) removal by centrifugation is ineffective at reversing changes
  – Is washing more effective?
• Maximal ACh-stimulated relaxation can be reversed by addition of RBCs; stored RBCs are more inhibitory than fresh
• Will repeat under hypoxic conditions
Aim 3: Patients with endothelial dysfunction (CVD) are more susceptible to these effects.

Ultrasound:
- Monitor brachial artery diameter

Blood pressure cuff:
- Elicit NO release

Sampling:
- OS markers
- Inflammatory markers
- Circulating EPCs

Monitoring:
- \( O_2 \) delivery
Endothelium-dependent and -independent function

- Flow-mediated vasodilation (FMD)
  - Endothelium-dependent
    - Baseline measurements
    - Reactive Hyperemia
Transfusion of aged RBCs leads to delayed decreased responses to NO as compared to fresh RBCs.

**FMD Percent Change from Baseline**

![Graph showing FMD percent change from baseline with two lines representing fresh and old RBCs.](image)

- **Baseline**: 0%
- **During**: Increase
- **1 hour post**: Increase
- **Next Day**: Decrease

**n = 7 each arm**
Aim 3 - summary

• NO-responsive large arterial vasodilation can be measured in transfused patients using ultrasound FMD measurements

• There are significant changes in FMD that occur following RBC transfusion of anemic patients
  – Acute changes are greater than are seen in most drug studies

• During transfusion, and within 1 hour of completion, fresh (< 7d) and aged (35-42d) RBC transfusion exert similar effects on FMD

• Measured on the day after transfusion, there appears to be a significant arterial constrictive effect of aged vs. fresh RBCs
Aim 2: Transfusion of stored/modified RBCs to healthy recipients inhibits NO-mediated increases in blood flow and $O_2$ delivery.

Infusions:
- L-NMMA (NOS inhibitor)
- TEA (EDHF inhibitor)

Contralateral arm:
- RBC transfusion

Sampling:
- [Nitrite]
- pH
- pO2
- $\text{HbFe}^{2+}\cdot\text{NO}$
- $\text{Hb-SNO}$
- $\text{HbFe}^{3+}$

Monitoring:
- Blood flow
- $O_2$ delivery

Grip exercises
Aim 2: Transfusion of stored/modified RBCs to healthy recipients inhibits NO-mediated increases in blood flow and O₂ delivery.
Summary

• **INOBA** (Insufficient NO bioavailability due to reduced delivery and/or increased scavenging by aged RBCs) may underly adverse effects ascribed to stored RBCs

• In vitro studies show that RBCs interfere with Ach-stimulated NO-mediated vasodilation (aged > fresh)
  – Furthermore, maximal ACh-stimulated relaxation can be reversed by addition of RBCs (again, aged > fresh)

• Preliminary FMD studies in transfused patients also suggest that aged RBCs have a delayed vasoconstrictive effect on the brachial artery

• These results will need to be correlated with clinical outcomes as measured in RECESS and other studies

• This work may lead to improved approaches to RBC storage and/or matching of RBC units to recipients
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