Harmful effects of stored RBC transfusions: Bench ↔ Bedside

March 18, 2011

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Our Interests

- Consequences of RBC clearance
- Iron Status
- Bacterial Infection
- Malarial Infection
- Co-infections
Our Interests

Consequences of RBC clearance

Iron Status

Bacterial Infection
Malarial Infection
Co-infections
Outline

Background

Mouse model

Studies with healthy human volunteers

Unresolved questions

Conclusions & Future Directions
Holy Grail of Transfusion Medicine

Manipulate the composition of blood:

With complete control

Without adverse consequences
Transfusion Medicine

Transfusion of “products”:
  RBC, Plt, WBC, PBSC, FFP

Infusion of recombinant proteins:
  FVIII, FVIIa, ATIII

Prescription of “drugs”:
  Epo, G-CSF, GM-CSF

Removal of “evil humors” (provide “good humors”):
  Apheresis of cells and solutes
Holy Grail of Transfusion Therapy (A corollary)

Transfuse any unit of RBC into any recipient:

With perfect acquisition of the desired effect:
   Normalizing Hct
   Diminishing Hgb SS levels
   Improving $O_2$ delivery

Without adverse consequences:
   Transfusion transmitted diseases (e.g. HIV)
   Transfusion reactions
   Missing the therapeutic target
   Volume overload
Holy Grail of Transfusion Therapy (Another corollary)

Blood products = Pharmaceuticals
White willow bark (Salix alba)

Preparation of white willow bark tea:
1. Fill one tea infuser full of the white willow bark tea herbs
2. Pour one cup of boiling water over the herbs
3. Cover the cup to ensure all the volatile oils & aromas do not escape
4. Allow the herbs to infuse for 3-5 minutes, then sip

Phytochemicals: Apigenin, beta-carotene, catechin, isoquercitrin, lignin, p-coumaric acid, quercitrin, rutin, salicin, salicylic acid, tannin

Nutrients: Calcium, iron, manganese, magnesium, phosphorus, potassium, selenium, zinc, vitamins B1, B2, B3, and C.
Aspirin = Acetylsalicylic acid

“All aspirin is now chemically synthesized. It's not surprising, then, that white willow bark is often called ‘herbal aspirin.’”
Holy Grail of Transfusion Therapy
(Another corollary)

Hemophilia A

Whole Blood

↓

Plasma

↓

Cryoprecipitate

↓

Purified FVIII

↓

Recombinant FVIII
Several non-randomized, observational studies suggest that transfusions of older, stored RBCs cause problems.
The claims regarding RBC storage

Human studies suggest that transfusions of older, stored RBC products are associated with increases in:

- Sepsis
- Pneumonia
- Multi-organ failure
- Myocardial infarction
- Acute renal failure
- Thrombosis
- Length of stay
- Mortality
In patients undergoing cardiac surgery, transfusion of red cells that had been stored for more than 2 weeks was associated with a significantly increased risk of postoperative complications as well as reduced short-term and long-term survival.
## What is the evidence?

<table>
<thead>
<tr>
<th>Complication</th>
<th>Patients Receiving Newer Blood (N=2872)</th>
<th>Patients Receiving Older Blood (N=3130)</th>
<th>P Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-hospital death</td>
<td>49 (1.7)</td>
<td>88 (2.8)</td>
<td>0.004</td>
</tr>
<tr>
<td>Pulmonary</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventilation &gt;72 hr</td>
<td>160 (5.6)</td>
<td>304 (9.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>81 (2.8)</td>
<td>111 (3.5)</td>
<td>0.11</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>5 (0.2)</td>
<td>7 (0.2)</td>
<td>0.67</td>
</tr>
<tr>
<td>Respiratory insufficiency</td>
<td>177 (6.2)</td>
<td>278 (8.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Renal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal failure</td>
<td>45 (1.6)</td>
<td>84 (2.7)</td>
<td>0.003</td>
</tr>
<tr>
<td>Infectious</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Septicemia or sepsis</td>
<td>80 (2.8)</td>
<td>125 (4.0)</td>
<td>0.01</td>
</tr>
<tr>
<td>Deep sternal wound</td>
<td>25 (0.9)</td>
<td>25 (0.8)</td>
<td>0.76</td>
</tr>
<tr>
<td>Superficial sternal wound</td>
<td>44 (1.5)</td>
<td>62 (2.0)</td>
<td>0.19</td>
</tr>
<tr>
<td>Multiorgan failure</td>
<td>7 (0.2)</td>
<td>23 (0.7)</td>
<td>0.007</td>
</tr>
<tr>
<td>Peripheral vascular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iliac or femoral dissection</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Acute limb ischemia</td>
<td>7 (0.2)</td>
<td>18 (0.6)</td>
<td>0.05</td>
</tr>
<tr>
<td>Composite outcome‡</td>
<td>642 (22.4)</td>
<td>810 (25.9)</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Critique of the Koch study

- Retrospective, non-randomized
- More Group O patients received fresh blood (51% vs. 31%)
- Older RBC group had more abnormal left ventricular function, mitral regurgitation, and peripheral vascular disease
- More leukoreduction in older RBC group
- More large dose transfusions in older RBC group

What happens to RBCs during storage?
The RBC storage lesion
The RBC storage lesion

- Decreased 2,3-DPG and ATP
- Vesiculation and membrane loss
- Increased lysophosphatidylcholine species
- Decreased nitric oxide
- Decreased deformability (~30% irreversibly deformed at 42 days of storage)
- Decreased CD47
- Hemolysis
- Protein oxidation
- Lipid peroxidation of RBC membrane

Decreased RBC survival *in vivo*

Tinmouth et al. Transfusion 2006
How did the FDA decide on the maximal allowable storage time?

- 1979: CPDA-1, FDA-allowed 35 days storage (based on 70% 24-hr survival)
- 70% was picked arbitrarily
- 1985: survival criteria raised to 75%, arbitrarily
- AS-1: FDA originally allowed 49 days storage
RBC Survival Study

- 5-15 mL
- Centrifuge
- Wash
- Incubate 51-Cr Wash
- IV push
- Serial blood draws

Graph showing percent survival over time (hours):
FDA criteria regarding outdate approval

- 20 or more evaluable 24-hr RBC survivals
- Minimum of 2 laboratories
- Sample mean ≥75%
- Standard deviation ≤9%
- Hemolysis <1% at end of storage (95% of the time)
Variability of 24-hr RBC survival in healthy volunteers

Fig. 1. Frequency distribution of 24-hour RBC recovery for RBCs stored for 42 days in AS. n = 641.

Variability of RBC survival in patients (most clearance by 1 hour post-transfusion)

Fig. 1. Individual 1-hour PTR (A) and 24-hour PTR (B) of SS and LS RBCs. SS and LS RBCs that have been transfused into the same patient are connected to each other. Each symbol represents a patient.

What are the consequences of this RBC clearance?
What are the consequences of this RBC clearance?

Are there any consequences of this RBC clearance?
The Blind Men and the Elephant

It’s a Fan!

It’s a Wall!

It’s a Spear!

It’s a Tree!

It’s a Rope!

It’s a Snake!
Consequences of the RBC Storage Lesion

- It’s cytokines
- It’s WBCs
- It’s deformability
- It’s NTBI
- It’s NO
- It’s hemolysis
What are the consequences (if any) of the clearance of stored RBCs?
Hypothesis

Delivery of hemoglobin/iron to the monocyte-macrophage system by clearing a subpopulation of stored RBCs is responsible for the harmful effects of transfusion
Hypothesis

Fresh unit → Oxidative damage → Infectious risk

Old unit → NTBI → Exacerbation of SIRS
A little arithmetic

5 L total blood volume
RBC lifespan ~120 days
1/120th of RBCs gets cleared in 24 hr = 40 mL/24 hr
40 mL/24 hr x 50% Hematocrit = 20 mL/24 hr
~1 mL RBC/hour = ~1x10^10 RBC = ~1mg Fe

1 unit transfusion at outdate = 300 mL
25% cleared, most within 1 hour = 75 mL/hr
75 mL/hr x 70-80% hematocrit = ~60 mL RBC/hr
~60 ml RBC/hour = ~6x10^11 RBC = ~60mg Fe
A little arithmetic

5 L total blood volume
RBC lifespan ~120 days
1/120th of RBCs gets cleared in 24 hr = 40 mL/24 hr
40 mL/24 hr x 50% Hematocrit = 20 mL/24 hr
~1 mL RBC/hour = \( \sim 1 \times 10^{10} \) RBC = \( \sim 1 \text{mg Fe} \)

1 unit transfusion at outdate = 300 mL
25% cleared, most within 1 hour = 75 mL/hr
75 mL/hr x 70-80% hematocrit = \( \sim 60 \) mL RBC/hr
~60 ml RBC/hour = \( \sim 6 \times 10^{11} \) RBC = \( \sim 60 \text{mg Fe} \)
How are we studying this issue?
Mouse Blood Bank model

- Collect blood by aseptic cardiac puncture
- Pre-storage leukoreduction (Pall filter)
- CPDA-1 as preservative
- 60-80% hematocrit at 1-6°C
- Aerobic blood culture; monitored for 5 days
- Transfuse into recipient mice
Survival of stored mouse RBCs

Where do the cleared RBCs go?
Liver

Fresh RBCs

Stored RBCs

H&E

F4/80
Transfused stored RBCs are cleared by splenic macrophages
Liposomal clodronate infusions deplete hepatic and splenic macrophages
Liposomal clodronate infusions deplete hepatic and splenic macrophages.
Macrophage depletion improves survival of transfused stored RBCs
Stored mouse RBCs are ingested by mouse macrophages *in vitro*.

- J774.1 cells + Fresh RBCs
- J774.1 cells + stored RBCs
What are the consequences of RBC clearance?
Hepatic macrophage (i.e. Kupffer cell) phagocytosis
Hepatic macrophage (i.e. Kupffer cell) phagocytosis and cytokine secretion
Cytokines

• Signaling molecules
• Often secreted by immune cells
  – Response to pathogens
• Interleukin (IL)-6 = pyrogen, acute phase reactant
• TNF-α, IL-1β, MCP-1, MIP-1α, etc.
Does transfusion of older, stored RBCs induce a pro-inflammatory cytokine response in mice?
Transfusion of older, stored RBCs induces a pro-inflammatory cytokine response in mice

Fresh or Stored RBC transfusion

Plasma collected

Plasma level (pg/mL)

IL-6

Untransfused Fresh (1u) Fresh (2u) Stored (1u) Stored (2u)

<24hr 14d

* **
Transfusion of older, stored RBCs induces an acute phase response

SAA1 promoter → luciferase

Fresh or Stored RBC transfusion

Luciferin injection

Time (hr)

0
0.5
2
4
6
24

Fresh

Stored

Caliper: Drs. Zhang, Ansaldi, & Francis
What is responsible for the inflammation?
The RBCs or something else?
Only transfusion of washed stored RBCs induces the pro-inflammatory response.
What is Non-Transferrin Bound Iron (NTBI)?

- Undetectable in healthy humans
- Oxidative damage
  - Fenton chemistry:
    \[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^- \]
- Cytotoxicity
- Enhanced endothelial expression of adhesion molecules
- Promotes pathogen growth
NTBI in transfused mice

2-hr

Fresh or Stored RBC transfusion

Plasma collected

NTBI (μM)

Fresh
 Stored
 Washed
 SN
 Ghosts
Plasma, after transfusion of older, stored RBCs, enhances bacterial growth

Fresh or Stored RBC transfusion

2 hours

Plasma collected

Plasma, after transfusion of older, stored RBCs, enhances bacterial growth
Are there clinical consequences to transfusion of older stored RBCs in mice?
Transfusion of older, stored RBCs exacerbates LPS-induced inflammation (24 hrs)
Mice infected with *Salmonella* have shortened survival when transfused with old RBCs.
Hypothesis

Fresh unit

Old unit

Fe

NTBI

Oxidative damage

Infectious risk

Inflammatory cytokines

Exacerbation of SIRS

Infectious risk
But, mice aren’t human....
Protocol Schematic

Pre-storage leukoreduction, autologous
# 14 Volunteers: Baseline Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age – yr (mean ± s.d.)</td>
<td>30.4 ± 9.1</td>
</tr>
<tr>
<td>Female – no.</td>
<td>4</td>
</tr>
<tr>
<td>Blood type: A, B, O, AB</td>
<td>6, 5, 3, 0</td>
</tr>
<tr>
<td>Race/ethnicity – no.</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>9</td>
</tr>
<tr>
<td>Black</td>
<td>1</td>
</tr>
<tr>
<td>Asian</td>
<td>2</td>
</tr>
<tr>
<td>Hispanic</td>
<td>2</td>
</tr>
<tr>
<td>Height – inches (mean ± s.d.)</td>
<td>70.5 ± 3.7</td>
</tr>
<tr>
<td>Weight – pounds (mean ± s.d.)</td>
<td>193 ± 38</td>
</tr>
<tr>
<td>Baseline Hemoglobin – g/dL (mean ± s.d.)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15.3 ± 1.2</td>
</tr>
<tr>
<td>Female</td>
<td>14.2 ± 0.8</td>
</tr>
</tbody>
</table>
Transfusions were well tolerated

- No adverse events identified
- No deviations from protocol
- All volunteers remained afebrile & vital signs were stable throughout
- No transfusion reactions
Complete Blood Counts
“Therapeutic Effect”
“Therapeutic Effect”

P = 0.1443

Hemoglobin (g/dL)

Pre-transfusion

Time post-transfusion (hr)

0 2 4 24 72

Old
Fresh
"Therapeutic Effect"
Markers of Hemolysis
What happens to cleared RBCs?

Andrews, NC. Nature Reviews Genetics, 2000
Total Bilirubin

![Graph showing total bilirubin levels over time post-transfusion. The graph compares 'Old' and 'Fresh' samples, with a significant difference indicated by P=0.0003.](image-url)
Direct Bilirubin

![Graph showing Direct Bilirubin levels over time post-transfusion, with old blood and fresh blood comparisons.]

- **Old** blood
- **Fresh** blood

P-value: 0.0043

**Time post-transfusion (hr)**

- Pre-transfusion
- 0
- 2
- 4
- 24
- 72

**Direct Bilirubin (mg/dL)**

- 0.0
- 0.5
- 1.0
- 1.5

*** indicates statistical significance.
Iron Parameters
Transferrin Saturation

![Graph showing transferrin saturation over time post-transfusion for fresh and old blood samples. The graph includes multiple lines representing different samples, with time points at 0, 4, 24, and 72 hours.](Image)
NTBI

ΔNon-transferrin-bound iron (μM)

P=0.001

Old
Fresh

Pre-transfusion
Time post-transfusion (hr)

0
1
2
4
24
72

***
Ferritin

ΔFerritin (ng/mL)

Time post-transfusion (hr)

Pre-transfusion

Old

Fresh

P=0.0013

***

72

24
Hepcidin

P=0.7522

Old
Fresh

ΔHepcidin (ng/mL)

Pre-transfusion
Time post-transfusion (hr)
Markers of inflammation
Absolute Neutrophil Count

P = 0.7415

Old
Fresh

Absolute Neutrophil Count (#/μL) vs. Time post-transfusion (hr)
Absolute Neutrophil Count

- **Fresh**
  - Graph showing the absolute neutrophil count over time post-transfusion for fresh samples.

- **Old**
  - Graph showing the absolute neutrophil count over time post-transfusion for old samples.

The y-axis represents the absolute neutrophil count (cells/μL), and the x-axis represents time post-transfusion (hours). The graph compares the neutrophil count in fresh and old samples over the first 24 hours post-transfusion.
CRP

P = 0.6991

C-reactive protein (mg/L)

Time post-transfusion (hr)

Pre-transfusion
CRP

C-reactive protein (mg/L)

Time post-transfusion (hr)

Fresh

Old

3
4
5
6
7
8
9
10
11
12
13
14
Conclusions from studies with human volunteers

- Responses to stored and fresh RBC transfusions differ

- Stored RBC transfusions are associated with significant rises in:
  - Total bilirubin
  - Serum iron
  - Transferrin saturation
  - Non-Transferrin Bound Iron (NTBI)
  - Serum ferritin

- With possible exceptions, transfusions of 1 unit of stored RBCs do NOT induce an inflammatory response
Bacterial growth *in vitro*

Area under curve of bacterial growth 
(\(\Delta OD_{600} \times hr\))

- Old
- Fresh
- Old - Fresh

P = 0.059

Pre-transfusion

Time post-transfusion (hr)
Potential explanations for muted pro-inflammatory response

• Humans are not mice
Potential explanations for muted pro-inflammatory response

- Humans are not mice
- Dose effect
Potential explanations for muted pro-inflammatory response

- Humans are not mice
- Dose effect
- Rate effect
Potential explanations for muted pro-inflammatory response

- Humans are not mice
- Dose effect
- Timing effect
- Hepcidin effect
Potential explanations for muted pro-inflammatory response

- Humans are not mice
- Dose effect
- Timing effect
- Hepcidin effect
- Need to be ill
Other weaknesses of human study

Didn’t measure RBC recovery

Didn’t measure non-protein inflammatory mediators

Probably missed hepcidin peak
Future Directions
(in humans)
Sickle cell disease & β-thalassemia

Year 1

2x RBC donation

Recipient A

Transfusion event #1

Fresh Old

Transfusion event #2
(Chelation treatment arm)

Fresh Old

Transfusion event #3
(Washed RBC arm)

Washed Old

Transfusion event #4
(Cryopreservation arm)

Cryopreserved Fresh

Year 2

2x RBC donation

1 unit cryopreserved

Donor A

= Hold chelation therapy for 1 week prior to transfusion
Final thought

- 56 yo M, no PMHx, here for “elective” transfusion
- 4 hours after transfusion labs are drawn:
  - Hb = 12.8 → 12.8 g/dL
  - WBC = 5.5 → 7.4 x10^9/L
  - T. bilirubin = 0.7 → 1.7 mg/dL
  - Haptoglobin = 69 → 72 mg/dL
  - Iron = 110 → 327 µg/dL
  - Transferrin sat. = 37 → 87%
  - NTBI = 0 → ~8 µM
Conclusions

Older RBC transfusions:
Are harmful in mice
Have side-effects in humans
Can mimic a hemolytic transfusion reaction
May result in unnecessary testing
May lead to transfusion delays
Does iron exacerbate infectious risk?
Do risks outweigh benefits?

We have frozen aliquoted samples…
Columbia
Eldad Hod, M.D.
Boguslaw Wojczyk, Ph.D.
Richard Francis, M.D., Ph.D.

Gary Brittenham, M.D.
Sujit Sheth, M.D.
Genia Billotte, R.N.
Phyllis Della-Latta, Ph.D.
Susan Whittier, Ph.D.

Emory
James Zimring, M.D., Ph.D.
Jeanne Hendrickson, M.D.

Yale
Stephanie Eisenbarth, M.D., Ph.D.

New York Blood Center
Yelena Ginzburg, M.D.