Influence of Transfused RBC Physiology upon Recipient Oxygen Delivery Homeostasis

Allan Doctor, MD
Professor of Pediatrics,
Biochemistry and Molecular Biophysics
Pediatric Critical Care Medicine
Washington University
Saint Louis Children’s Hospital
Branding

- Washington University in St. Louis
- School of Medicine
- Children's Hospital
- St. Louis
- Children's Discovery Institute

Disclosures

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  - Viasys, iNO therapeutics
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  - Novartis
  - Biogen

- **Intellectual Property**
  - S-nitrosothiol assay systems
  - ErythroMer artificial RBC system

- **Equity**
  - KaloCyte, Inc.; Founder & President
Relevant Observations by Smart People

“Sometimes good things fall apart, so better things can fall together.”
- Marilyn Monroe

“That which does not kill us makes us stronger.”
- Friedrich Nietzsche

“Everything should be made as simple as possible... but not simpler.”
- Albert Einstein
Donor (stored) and Native RBCs do not exhibit similar physiology or efficacy. 

... and there's more in the bag than just RBCs (and these 'components' have bioactivities).

These differences progress as a function of storage duration.

These differences impair O₂ delivery to tissue, even by native RBCs.

Transfusion, surprisingly, may harm critically ill anemic patients.

There is sufficient basic, translational, & clinical evidence to prompt consideration of a fundamental practice change in blood banking & transfusion medicine.
RBC Function  

- Oxygen Transport

Transfusion Goal

- Improve Oxygen Transport

Effective Blood Banking and Transfusion Medicine:
Ensure that that happens.
Overview

- Role of RBCs in Regulating $O_2$ Delivery
  - beyond simple $O_2$ loading/unloading
- Physiology of Anemia & $O_2$ Delivery Homeostasis
- RBC Storage Lesion Biology & Transfusion Risk
- Influence of Transfused RBC Physiology upon Recipient Oxygen Delivery Homeostasis
- Transfusion Decision Making
  - Who should get blood?
  - When should they get it?
  - How much should they get?
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Partitioned Functions

RBC-based signaling is fundamental to $O_2$ delivery homeostasis at cellular, tissue, and whole-organism levels.
What governs blood flow distribution (tuning)?

“Perfusion lack must be sensed. Flow must be redistributed. Effective in space and time.”

Relationship between Blood Flow, [Hb] and O$_2$ Delivery

Blood Flow, not O$_2$ Content, is the Principle Determinant of O$_2$ Delivery

A

B

C

D

Erzurum. PNAS 2007;104:17593-17598.
RBCs have context-responsive vasoactivity
this role subserves O₂ delivery homeostasis

- 1 in 1,000 Hb carries NO
  - FeNO (bound to heme)
  - SNOHb (bound to thiol)
  - ~ 450 nM in blood

0.1 - 1 % NO bound to Hb is released during circulatory transit

Stamler. PNAS. 2015;112:6425-30
RBCs are vascular control elements
control achieved by trapping or deploying NO as a function of HbSO₂
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Anemia Tolerance in Healthy Adults

Table

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (n=23)</th>
<th>Volunteers (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin Range</td>
<td>125-134 g/L</td>
<td>45-54 g/L</td>
</tr>
<tr>
<td>SVRI, dynes·cm⁻²·m⁻²</td>
<td>2372 (541)</td>
<td>1001 (176)</td>
</tr>
<tr>
<td>HR, beats per minute</td>
<td>58 (11)</td>
<td>92 (12)</td>
</tr>
<tr>
<td>SVI, mL/m²</td>
<td>52 (9)</td>
<td>62 (8)</td>
</tr>
<tr>
<td>CI, L/m²</td>
<td>3.05 (0.69)</td>
<td>5.71 (0.87)</td>
</tr>
<tr>
<td>VO₂, mL O₂·kg⁻¹·min⁻¹</td>
<td>13.5 (2.7)</td>
<td>10.7 (2.0)</td>
</tr>
<tr>
<td>S.O₂, %</td>
<td>77.1 (3.3)</td>
<td>69.6 (5.6)</td>
</tr>
<tr>
<td>VO₂/TO₂</td>
<td>3.01 (0.42)</td>
<td>3.42 (0.54)</td>
</tr>
<tr>
<td>Plasma lactate, mmol/L</td>
<td>0.77 (0.40)</td>
<td>0.62 (0.19)</td>
</tr>
<tr>
<td>Arterial blood pH</td>
<td>7.395 (0.016)</td>
<td>7.445 (0.025)</td>
</tr>
<tr>
<td>Base-excess, mEq/L</td>
<td>1.3 (1.5)</td>
<td>4.2 (2.2)</td>
</tr>
</tbody>
</table>

*De < P<

Anemia Tolerance in Healthy Children

Table 2. Hemoglobin, Hematocrit, and Acid-Base Status During Hemodilution and Reinfusion

<table>
<thead>
<tr>
<th>Variable</th>
<th>T₀</th>
<th>T₁</th>
<th>T₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hct (%)</td>
<td>29.5±4.8</td>
<td>9.0±2.2*</td>
<td>16.7±3.1*</td>
</tr>
<tr>
<td>(Range)</td>
<td>(20.3–36.1)</td>
<td>(6.3–13.3)</td>
<td>(12.2–21.6)</td>
</tr>
<tr>
<td>Hgb (g/dL)</td>
<td>10.0±1.6</td>
<td>3.0±0.8*</td>
<td>5.6±1.0*</td>
</tr>
<tr>
<td>(Range)</td>
<td>(7.0–12.4)</td>
<td>(2.1–4.5)</td>
<td>(4.1–7.1)</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>1.3±0.2</td>
<td>1.4±0.5</td>
<td>1.5±0.5</td>
</tr>
<tr>
<td>(Range)</td>
<td>(1.0–1.5)</td>
<td>(0.9–1.95)</td>
<td>(1.0–2.2)</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.42±0.05</td>
<td>7.33±0.08*</td>
<td>7.37±0.06</td>
</tr>
<tr>
<td>(Range)</td>
<td>(7.33–7.50)</td>
<td>(7.25–7.49)</td>
<td>(7.26–7.45)</td>
</tr>
<tr>
<td>Venous pH</td>
<td>7.39±0.05</td>
<td>7.28±0.07*</td>
<td>7.33±0.06</td>
</tr>
<tr>
<td>(Range)</td>
<td>(7.34–7.46)</td>
<td>(7.22–7.42)</td>
<td>(7.22–7.42)</td>
</tr>
<tr>
<td>P₅CO₂</td>
<td>34.1±6.5</td>
<td>37.9±3.4</td>
<td>39.2±4.1</td>
</tr>
<tr>
<td>(Range)</td>
<td>(25.1–46.1)</td>
<td>(32.1–42.7)</td>
<td>(33.6–45.0)</td>
</tr>
<tr>
<td>P₅CO₂</td>
<td>38.3±4.0</td>
<td>43.4±2.5*</td>
<td>44.8±4.3</td>
</tr>
<tr>
<td>(Range)</td>
<td>(32.5–43.3)</td>
<td>(39.3–45.9)</td>
<td>(39.2–50.8)</td>
</tr>
<tr>
<td>ABE (mmol/L)</td>
<td>−1.1±2.3</td>
<td>−5.0±3.6*</td>
<td>−1.8±2.5</td>
</tr>
<tr>
<td>(Range)</td>
<td>(−6.1–0.6)</td>
<td>(−8.4–2.4)</td>
<td>(−5.4–2.1)</td>
</tr>
<tr>
<td>VBE (mmol/L)</td>
<td>−0.6±1.1</td>
<td>−6.0±3.5*</td>
<td>−2.3±2.5*</td>
</tr>
<tr>
<td>(Range)</td>
<td>(−2.5–1.0)</td>
<td>(−8.9–1.4)</td>
<td>(−5.5–2.1)</td>
</tr>
</tbody>
</table>
Underlying Condition Alters Anemia Tolerance
the degree of lost resilience is condition-specific

Conceptual Evolution: Hormesis & Salutary effects of Anemia? *metabolic response to diminished O₂ delivery improves resilience to other physiologic challenges (preconditioning)*

![Diagram showing the relationship between O₂ delivery and response to anemia, with thresholds for adverse response and regions of homeostasis highlighted.](Lelubre.Critical Care.2016;20:152)
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RBC Storage Lesion
native and stored RBCs demonstrate dissimilar physiology

- **RBC metabolism**
  - Abnormal glycolysis & energy metabolism
    - ATP depletion (ion pumps fail)
    - 2,3 – DPG depletion (p50 falls, O₂ loading/delivery altered)
    - Reducing equivalent (NADPH, GSH) depletion (oxidative stress)
    - (↓ pH, ↑ lactate)

- **Generation of Cytokines and Bioreactive agents**
  - Elaboration of RBC-derived microvesicles (vasoactive & pro-inflammatory)
  - Soluble CD40 ligand, lyso-PCs (activate primed PMNs)

- **RBC rheology**
  - Progressive loss of deformability
  - Progressive adhesion to activated endothelium

- **RBC control of regional blood flow**
  - Failed context-responsive control of vasoactive effectors in plasma
  - Disruption of RBC NO metabolism
Does RBC Transfusion during ECMO improve tissue oxygenation or outcome?

- 1984-2011; 827 ECMO runs Grp 1: non-leukoreduced, Grp II: leukoreduced
- Group I each Tx of 10 mL/kg/d of pRBC ~ 33% increase in mortality (P <0.05)
- Group II each Tx of 10 mL/kg/d of pRBC ~ 21% increase in mortality (P = 0.07)

Anemia, Transfusion and Cardiac Surgery

Anemia is bad, transfusion is bad; does transfusion treat anemia?

Goal Directed Hemodynamic Support After Cardiac Surgery does not require transfusion and may confer outcome benefit.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>EGDT Events</th>
<th>Total Events</th>
<th>Weight</th>
<th>Odds Ratio M-H, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kapoor</td>
<td>1</td>
<td>13</td>
<td>3.2%</td>
<td>0.50 [0.04, 6.28]</td>
</tr>
<tr>
<td>McKendry</td>
<td>17</td>
<td>89</td>
<td>41.9%</td>
<td>0.58 [0.29, 1.17]</td>
</tr>
<tr>
<td>Mythen</td>
<td>0</td>
<td>30</td>
<td>2.4%</td>
<td>0.06 [0.00, 1.15]</td>
</tr>
<tr>
<td>Osawa</td>
<td>26</td>
<td>62</td>
<td>39.6%</td>
<td>0.41 [0.20, 0.83]</td>
</tr>
<tr>
<td>Polonen</td>
<td>2</td>
<td>196</td>
<td>9.0%</td>
<td>0.15 [0.03, 0.66]</td>
</tr>
<tr>
<td>Smetkin</td>
<td>1</td>
<td>20</td>
<td>3.9%</td>
<td>0.21 [0.02, 2.08]</td>
</tr>
</tbody>
</table>

Total (95% CI) 410 415 100.0% 0.40 [0.26, 0.63]

Total events 47 92

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 4.87$, df = 5 ($P = 0.43$); $I^2 = 0$

Test for overall effect: $Z = 3.94$ ($P < 0.0001$)

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● Transfusion Decision Making
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NO content is rapidly depleted in stored RBCs. Capacity for hypoxia-responsive vasodilation is lost.
Stored RBCs fail to support HVD in hypoxic myocardium; capacity for hypoxia-responsive vasodilation is lost

- Mongrel dogs (n = 7)
- Doppler flow probe on L circumflex a.
- Cannulated L main coronary a.
  - Serial infusion of SNO-depleted or renitrosylated RBCs (1 mL)
  - 30 min interval between infusions

- FiO₂ reduced to 5%
- Baseline LCx flow ↑ 67%
- hypoxic vasodilation
- RBC Infusions repeated
  - 90 min intervals

RBC Derived Microparticles Appear to Disrupt O₂ Delivery Homeostasis

[Graphs and data showing the effects of microparticles on oxygen delivery]

Influence of RBC storage upon Capacity for Vasodilation
fundamental component of O₂ delivery homeostasis

A. Vasorelaxation
- Intra-arterial Ach infusion
  - ↑ Ach
  - Muscarinic Receptor
  - ↑ Ca²⁺
  - L-Arginine → ↑ NO
  - Arg-1
  - NOS → L-Citrulline
  - Endothelial Cell
  - ↑ NO Synthesis
  - ↑ Availability of NO
  - GTP → ↑ NO
  - GC
  - eGMP
  - Smooth Muscle Relaxation

B. Vasoconstriction
- RBC Hemolysis
  - ↑ Cell Free Hb
  - NO Scavenging
  - Methemoglobin
  - Intra-arterial Ach infusion
  - ↑ Ach
  - Muscarinic Receptor
  - ↑ Ca²⁺
  - L-Arginine → ↓ NO
  - Arg-1
  - NOS → ↓ L-Citrulline
  - Endothelial Cell
  - ↓ NO Synthesis
  - ↓ Availability of NO
  - GTP → ↓ NO
  - GC
  - ↓ cGMP
  - NO Signaling, ↓ GC Signaling
  - ↓ Smooth Muscle Relaxation

Pre Blood D42
Pre Blood D5
Post Blood D42

Oxygen Delivery & Consumption Relationships
Dynamic and Vary with Physiologic State
StO₂ Measurement During Occlusion Stress
Emergent Variables Elucidate Microcirculatory Function

**Method**
- Cuff inflated 30-50 mmHg > systolic BP
- Monitor StO₂ for 5 minutes, or to StO₂ < 40%
- Release cuff

**RdecStO₂ (%/s):** the rate of the decrease in StO₂ during the ischemic period
- Vascular occlusion eliminates flow, fixing blood volume and [Hb]
- RdecStO₂ monitors O₂ content of Hb & Mb and redox state of CytOx
- Only 1st 25% of slope is linear
- Varies with tissue VO₂, O₂ content, barriers to O₂ transfer

**RincStO₂ (%/s):** the rate of the increase in StO₂ during the reperfusion phase
- Preventing arterial inflow creates regional hypoxia and supra-physiologic accumulation of hypoxic signaling
- RincStO₂ interrogates complex myogenic, endothelial and erythrocytic signaling intended to recruit bloodflow to ischemic tissue
- Varies with: Cl, perfusion pressure, regional impedance to bloodflow, [Hb], SaO₂

**Δ StO₂ (%):** difference between maximum StO₂ value during the reperfusion period and baseline StO₂
- Post-Ischemic Hyperemia
  - Overshoot reflects "reserve" capacity to recruit blood flow in setting of perfusion insufficiency
  - Varies with factors influencing RincStO₂ as well as barriers to O₂ transfer

**Post-Ischemic Hyperemia**
- Evaluates functional variation in O₂ delivery following ischemic stress
- Other methods for assay: plethysmography, laser doppler flometry, transcutaneous pO₂, infrared imaging

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Anemia Tolerance in Critical Illness

[Hb] alone does not determine clinical severity and therefore, decisions should not be based upon [Hb] alone

- Determinants of Clinical Significance
  - Magnitude of reduction in O₂ content (Hb & O₂%Saturation)
  - Change in blood volume
  - Rate at which above two factors occur
  - Capacity of cardiopulmonary systems to maintain O₂ delivery by improving cardiac output/blood flow
  - Sufficiency of dynamic matching between O₂ consumption/delivery
  - Underlying disorder(s) [overall metab., prior I/R, focal perfusion, etc.]

![Graph showing risk of mortality vs. Hb level]

![Diagram showing relationship between Hb, Oxygen saturation, and pH]

![Table showing mortality stratified by postoperative Hb level]


RBC transfusion is indicated when the following conditions are met:

- \( O_2 \) delivery fails to meet metabolic need (or failure is impending),
- \( O_2 \) delivery failure is of sufficient magnitude to injure or threaten injury,
- the risk and impact of this injury exceeds risk and impact of harm anticipated from transfusion, and...

- tx is appropriately sequenced with other interventions (based upon principles of integrative physiology, potential morbidity, likelihood to optimize \( O_2 \) delivery, and context specific to individual patient trajectories. (e.g. precision medicine)).

Once the decision to transfuse has been made, use a titrated approach to administering RBCs to maintain the risk of transfusion as low as reasonably achievable, while monitoring for resolution of anemia intolerance and improvement in \( O_2 \) delivery.

Doctor and Hovmand. WFPIC 2016. Toronto.
Transfusion and Anemia Expertise Initiative (TAXI)

**Assess Anemia Tolerance**
Current and Prospective Trajectory (of anemia, of illness)

**Ischemic vital organ**
(brain, heart, kidney, liver)

**Injury**

- **Heart**
  - ECG (ST Δ; HRV)
  - Troponin
  - Kidney & Liver
  - Regional StO₂ (NIRS)

- **Brain**
  - Cognitive Impairment
  - Cerebral StO₂ (NIRS) & PbO₂ (Fiberoptic)
  - Jugular Bulb HbO₂%
  - CBF (XeCT, fMRI)
  - iEEG

**O₂ delivery homeostasis failure**
(e.g. O₂ supply dependency, anaerobic metabolism is present)

**Compromised O₂ delivery homeostasis reserve**

**Physiologic Metric**
- Heart Rate (& HRV)
- Blood Pressure (& BRS)
- Respiratory Rate/Dyspnea
- Core – Peripheral ΔT gradient
- StO₂ (NIRS & Dynamic NIRS)
- ScvO₂
- DO₂ & O₂ER
- Functional Capillary Density

**Biomarker**
- methHb%

**Correct anemia**

**support compensatory physiology**

**blunt O₂ consumption**

**support compensatory physiology**

**Correct anemia**
High Resolution Risk Analytic Engine (iBET) reports dO$_2$ effectiveness and integrated cardiopulmonary performance failure signals for system components can be tracked.
Summary

- RBCs comprise a key node in regulation of O₂ delivery
  - Match regional blood flow and tissue respiration
  - Participate in signaling that supports O₂ delivery homeostasis on a cellular, tissue and organism level
- Stored RBCs strongly influence above physiology & signaling and paradoxically, may impair O₂ delivery homeostasis
- Transfusion Decision Making (and ability to study transfusion efficacy) requires precise understanding of:
  - Anemia tolerance (physiologic reserve, supply dependency, specific vital organ threats)
  - Illness trajectory & likelihood of transfusion harms
  - Sequencing of transfusion with other interventions supporting O₂ delivery
  - Ability to monitor O₂ delivery (components) as well as the dynamic reflexes that comprise homeostasis
    - Enables titration of transfusion to lowest effective, least harmful dose
Grateful Tribute
those who had the good ideas, actually did the work and provided $$:

- **Washington University**
  - **Doctor Lab**
    - Stephen Rogers, PhD
    - Vered Gazit, PhD
    - Xue Lin MD, PhD
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    - David Timm, PhD (cand)
    - Chris Markham, MD (PCCM fellow)
    - Brett Olsen, PhD student
    - Marc Sherman, MD, PhD student
    - Jerlinda Ross, MD student
    - Melanie Ernst, BME student
    - Dylan McLaughlin, BS student

- **Institute of Public Health**
  - **Center for Implementation & Dissemination**
    - Enola Proctor, PhD

- **Social Systems Design Lab**
  - Peter Hovmand, PhD

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  - Sara Small, MS
  - Julie Hoerr, RN, APN
  - Micki Eaton, RN

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  - Scot Bateman, MD and Stacey Valentine, MD (chairs)

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  - Andrew Argent, MBBCh, MD
  - Jeff Carson, MD
  - University of Montreal
  - University of Cape Town
  - Rutgers (RWJ)

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  - Dimiter Baronov, PhD
  - Evan Butler, MS
  - Mel Almodovar, MD
  - Peter Laussen, MD
  - Avihu Gazit, MD
  - Etiometry
  - Etiometry
  - Harvard
  - U Toronto
  - WUSM

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