Predictive Clinical Value of In Vitro Measures of Red Cell Quality

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Canadian Blood Services
it's in you to give
What does a “Quality” blood product mean?

“We should be attentive to the quality of the many millions of RBC units that are transfused so casually by physicians who can only assume that what they receive from the blood bank is a worthy replacement for the blood their patient has lost”.

Hemolysis in Red Cell Concentrates

- biconcave discoid
- crenation spicule formation
- ATP ↓ K⁺/Na⁺
- swelling
- ROS methHb
- externalization of phosphatidylserine
- microvesicle formation
- cytoskeletal changes
- hemolysis

Method of Manufacturing Affects Hemolysis

<table>
<thead>
<tr>
<th>Method</th>
<th>Hemolysis (%)</th>
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<tbody>
<tr>
<td></td>
<td>Fresh</td>
<td>Expiry</td>
<td></td>
</tr>
<tr>
<td>SAGM, RBC LR</td>
<td>0.10 ± 0.01</td>
<td>0.25</td>
<td>0.01</td>
</tr>
<tr>
<td>SAGM, WB LR</td>
<td>0.12 ± 0.01</td>
<td>0.36</td>
<td>0.02</td>
</tr>
<tr>
<td>AS-1, NLR</td>
<td>0.13 ± 0.02</td>
<td>0.72</td>
<td>0.36</td>
</tr>
<tr>
<td>AS-1, LR</td>
<td>0.14 ± 0.05</td>
<td>0.36</td>
<td>0.05</td>
</tr>
<tr>
<td>MCS+</td>
<td>0.22 ± 0.05</td>
<td>0.33</td>
<td>0.16</td>
</tr>
<tr>
<td>Trima</td>
<td>0.25 ± 0.05</td>
<td>0.38</td>
<td>0.09</td>
</tr>
<tr>
<td>Alyx</td>
<td>0.18 ± 0.03</td>
<td>0.34</td>
<td>0.09</td>
</tr>
</tbody>
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Age and Sex of Donor Affect Hemolysis

Female RBCs hemolyze less than male RBCs in all tested age groups.

N=28,227

Kanias et al. Transfusion (2016). In press
How Hemolysis is Measured is Important

Hemolysis at d 43
Mean 0.22% (0.16% – 0.32%)

©: g force increase

Biochemical Parameters
- ATP, 2,3-DPG concentration
- pH, glucose, lactate
- K⁺, Na⁺ concentrations
- p50, pO₂, pCO₂
- oxidative injury (metHb, TBARS, ROS, lipid peroxidation)
- phosphatidylserine inversion, CD47 expression
- lipidomic analysis
- metabolomic profile

Biophysical Parameters
- morphology index
- **hemolysis**, supernatant Hb
- MCV, MCHC, MCH, % retics
- osmotic fragility, membrane permeability
- extracellular vesicle size, concentration and composition (lipidomics)
- deformability, aggregability
- adherence to endothelium

Unit Characteristics
- **total Hb**, unit volume, hct
- residual leukocytes, plasma
- sterility, blood group

What’s in the Bag?

In vitro Measures of RBC Quality

Microfluidic Cell Analyzer

Izon qNano

ImageStream X

Stanford MagDense

Photoacoustics
All RCCs tested met internal QC and regulatory requirements and could be issued for transfusion, but....

RCCs produced using different methods (or from different donor groups) are not equivalent!

Do measured donor and manufacturing factors affecting in vitro quality predict patient outcomes?
Linking Donor – Product - Recipient

- manufacturing process (RT hold or not; timing)

Product Factors

- Donor Factors
  - Age
  - Sex
  - # donations

Recipient Factors

Patient Outcome

• 2 research questions
  1) Is there an association between exposure to female blood and in-hospital mortality among transfused male patients?
  2) To determine if the manufacturing process (whole blood filtration versus red cell filtration) is associated with in-hospital mortality in adults receiving red cell transfusions

- Demographics
- Clinical characteristics
- Procedures
- Products received
Red blood cell processing methods and in-hospital mortality: a transfusion registry cohort study

Nancy M Heddle, Donald M Arnold, Jason P Acker, Yang Liu, Rebecca J. Barty, John W. Eikelboom, Kathryn E. Webert, Cyrus C. Hsia, Sheila F. O’Brien, Richard J. Cook


Figure 3: Forest plot for in-hospital mortality
Cox regression model for in-hospital mortality for the six categories of red blood cells exposure compared with the reference category. Hazard ratios >1 are associated with a higher risk of death.

Added value of the study
To our knowledge, this study is the first to suggest that transfusion of fresh RBCs might be associated with an increased risk of in-hospital mortality depending on how the RBC product is prepared. The transfusion of fresh RBCs (stored ≤7 days) prepared by whole blood filtration was associated with a higher risk of in-hospital mortality than was transfusion with mid-age RBCs (stored 8–35 days) prepared by red cell filtration.

Findings Between April 1, 2008, and March 31, 2014, 91,065 RBC transfusions were given to 23,634 adults who were included in the analyses. When storage duration was included in the model, in-hospital mortality was significantly increased with fresh whole blood filtered units compared with the reference group of mid-age red cell filtered units (hazard ratio 2.19, 95% CI 1.09–4.42; p=0.033). Differences between other age and processing categories were not significant.
Association of blood donor age and sex with recipient survival after red blood cell transfusion

Michaël Chassé, Alan Tinmouth, Jason P Acker, Shane W English, Kumanan Wilson, Greg Knoll, Nadine Shehata, Carl van Walraven, Lauralyn McIntyre, Alan Forster, Tim Ramsay, Dean Fergusson


- 30,503 recipients who received 187,960 red blood cell transfusions from 80,755 unique donors
- for a recipient receiving a RCC from a **young donor (age 17-30)**, the risk of death was increased compared to receiving a red cell unit from any other donor age group
- receiving a RCC from a **female donor** was associated with a risk of death **6% higher** compared to receiving a unit from a male donor

**TABLE 4 — UNADJUSTED AND ADJUSTED SURVIVAL FOR DONOR AGE AND SEX, PER ADDITIONAL UNIT TRANSFUSED**

<table>
<thead>
<tr>
<th>Donor age (years)</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
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<tbody>
<tr>
<td>17 –</td>
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Adjusted for Recipient age, Recipient sex, Charlson score
Why is it difficult to show that in vitro RBC characteristic predict outcome?

- perhaps our approach is flawed....

- in vitro RBC parameters have been correlated with radiolabel survival / 24 h recovery; **but single parameters are not strongly predictive**!

- How can autologous radiolabel survival / recovery in **healthy volunteers** possibly predict outcomes in transfusion recipient outcomes?

- We need new methodologies to directly link individual product quality characteristics with patient outcomes
  - we need to account for the natural variability in the system
When we consider red cell product quality, we need to address all factors which are going to influence patient outcomes.
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