Evaluation of Red Cell Products for Transfusion

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The Goals

• Maximize RBC Efficacy
The Goals

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• Minimize RBC Toxicity
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• Maximize RBC Efficacy
• Minimize RBC Toxicity
• Assure RBC Availability
Alexis Carrell
U. of Chicago, Rockefeller Institute

Nobel Prize –1912
Vascular Anastomosis

Rous – Turner Solution

Locke's physiological salt solution, sodium citrate, and dextrose

Francis Peyton Rous
Rockefeller Institute

Rous P, Turner P. The preservation of living red blood cells in vitro. II. JAMA 1915; 64:425
Translational Medicine in the Early 20th Century
Oswald H. Robertson

Robertson OH. Transfusion with preserved red blood cells. BMJ 1918; 1: 691–5
Previously Described Refrigerated RBC Storage Lesions

- Metabolic and chemical derangements
- RBC shape change
- Rheologic changes
- Cell-Free Hemoglobin release
- Release of iron
- Loss of membrane proteins carbohydrates
- Shedding of microvesicles
- Oxidative injury to lipids and proteins
- Changes in oxygen affinity and delivery
- Increased adhesion of RBCs to endothelial cells
- Hemolysis and reduced RBC lifespan
- Accumulation of potassium and plasticizers
RBC Efficacy
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• $O_2$ Delivery
RBC Efficacy

- $O_2$ Delivery
  - $CO_2$ removal
  - NO binding
  - Cytokine binding
  - Hemostatic Function
  - Etc.
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• Surrogate Evaluation: $Cr^{51}RBC$ Recovery and Survival
RBC Toxicity

• Metabolic and Rheologic Derangement
  • Cell Free Hemoglobin
  • NO Scavenging - Vasoconstriction
  • Release of Iron/Heme
  • Etc.
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- Surrogate Evaluation:
  - Hemolysis
  - $\text{Cr}^{51}\text{RBC}$ Recovery and Survival
ADVANTAGES OF A DISODIUM-CITRATE GLUCOSE MIXTURE AS A BLOOD PRESERVATIVE*

BY
J. F. LOUTIT, B.M., M.R.C.P.
AND
P. L. MOLLISON, M.B., M.R.C.P.

In another communication (Loutit, Mollison, and Young, 1943) the advantages resulting from the use of certain citric-acid sodium-citrate-glucose mixtures as preservatives for stored blood are reported. In assessing the preservative value of these and other recommended solutions the chief criterion adopted was the survival in vivo of transfused red cells which were stored in the various solutions. By this test the citric-acid sodium-citrate-glucose mixtures were found to be definitely superior to the standard M.R.C. (1940) trisodium-citrate-glucose mixture and slightly better than the Rous-Turner (1916) solution.

Licensure of RBC in United States

- FDA’s current “gold standards”
  - 75% of cells circulate after 24h at the end of storage
  - Hemolysis < 1% at the end or storage
- No requirement for clinical studies
Chromium-51 ($^{51}$Cr) 24-hour in vivo Recovery

- 70% recovery the defined standard 1940’s to 80’s*

Chromium-51 ($^{51}\text{Cr}$) 24-hour in vivo Recovery

- 70% recovery the defined standard 1940’s to 80’s*
- 75% recovery the defined standard 1980’s to present**


**FDA workshop on red cells stored in additive solution systems; 1985 Apr 25, 1985; Bethesda, MD
Since approval of CPD (1957) FDA has requested:

- RBC ATP concentration
  - No standards set
  - Correlation with in vivo RBC recovery or hemolysis varies among labs

- Oxygen Equilibrium Binding Curve
  - No simple, reproducible method of measurement
  - Clinical relevance

- 2,3, DPG
  - No standards set

- K+, glucose, lactate
Recent FDA-suggested tests and statistical validity requirements for RBC storage systems

1. In vitro studies on Day 1 and at the end of storage period
   - RBC unit volume, weight
   - RBC dose, total Hb, Hct
   - Hemolysis
   - Post-leukoreduction residual WBCs, RBC mass recovery, PLT counts
     - ATP, K, glucose, lactate, pH, PO$_2$, PCO$_2$, bicarbonate, 2,3-DPG, 2,3-DPG regeneration studies, RBC morphology, oxygen dissociation curve

2. In vivo RBC studies at the end of storage
   - In vivo radiolabeled RBC recovery studies (n = 20-24)
1. Hemolysis at the end of storage: one-sided 95% lower confidence limit for the proportion of units with hemolysis less than 1% is greater than 95%.
2. Post-leuko-reduction residual WBCs: one-sided 95% lower confidence limit for the proportion of units with residual WBC is less than $5 \times 10^6$ is greater than 95%.
3. Post-leuko-reduction RBC mass recovery: one-sided 95% lower confidence limit for the proportion of units with RBC mass recovery is greater than 85% is greater than 95%.
4. In vivo recovery of autologous transfusion of radiolabeled RBC in healthy subjects:
   - Minimum of 20 volunteers
   - At least two test laboratories independent of the sponsor
   - Sample mean in vivo recovery at 24 hours $\geq 75$
   - Sample SD $\leq 9$
   - One-sided 95% lower confidence limit for the proportion of RBC components with 24-hr RBC in vivo or recovery $\geq 75$% is $>70$%.

5. The in vitro tests are performed as a comparison between approved RBC components (control) and the RBC collection system under investigation (test). FDA recommends less than $+20\%$ difference in a paired study 95% of the time with 95% confidence to ensure that the RBCs have not experienced major alterations in shape or biochemical status. If the data show that the test results might affect product safety and effectiveness, FDA may request placing the study results in the labeling (circular of information) and performance of additional studies up to and including clinical studies.
Both RBC radiochromium recovery/survival and RBC storage show substantial intrinsic donor-to-donor variability
The Variation in the “storability” of RBCs from Different Donors

Distribution of $^{51}$Cr 24-hour in vivo RBC recovery in 27 volunteers whose blood was stored repeatedly in CPD for 3 weeks

Frequency Distribution of 24-hour RBC Recovery for RBCs

Stored for 42 days

Stored and irradiated

Stored Frozen

Dumont LJ, AuBuchon JP. Evaluation of proposed FDA criteria for the evaluation of radiolabeled red cell recovery trials. Transfusion. 2008; 48:1053–1060
The Respiratory Function Of Blood

Whole Blood O2 Dissociation Curve
Progressive leftward shift of whole blood oxygen dissociation curve with successive units of red blood cells exchanged

Improved exercise performance after exchange transfusion in subjects with sickle cell anemia

Fig. 4. Pre- and posttransfusion exercise tests, subject J.B. (see Table 1). Two tests were performed on consecutive days before transfusion (closed circles) and on a third day after exchange (open circles). The change in the relationship of heart rate versus work on retesting is insignificant compared to the change observed after transfusion.

Fig. 3. Pre- and posttransfusion exercise tests, subject R.Q. (see Table 1). Closed circles represent the pretransfusion test and open circles the posttransfusion test.
Relative Changes in \( \text{O}_2 \) Consumption* in 5 Different Species

- Combined increases in cardiac output and \( \text{O}_2 \) extraction allow maintenance of \( \text{O}_2 \) consumption until relatively low hemoglobin levels.

- At extremely low hemoglobin levels, cardiac output and \( \text{O}_2 \) consumption may decrease, indicating the exhaustion of the compensatory mechanisms.

*\( \text{VO}_2 \), % baseline

Evaluation of Red Cell Products for Transfusion

- Evaluation criteria should provide a reasonable level of assurance of RBC efficacy and safety
- Current evaluation criteria, though somewhat arbitrary, have served us well – changes should be evidence-based
- Biomarkers should reflect RBC function and clinical outcomes
- Assays should be reproducible and statistics achievable
- The ideal evaluation criteria and the appropriate statistical treatment are neither identified nor obvious (or they would have been adopted years ago)