Role of Red Cells in RDCR

Philip C. Spinella, MD, FCCM

RDCR Symposium

Bergen, Norway 2013
Overview

- Role of shock in ACT/TIC
- Methods to reverse shock
- RBC functions
- RBC efficacy in reversing shock
  - Storage lesion effects on efficacy and safety
- RBC effects on coagulation
  - Storage lesion effects
- Conclusion
The Coagulopathy of Trauma: A Review of Mechanisms

John R. Hess, MD, MPH, FACP, FAAAS, Karim Brohi, MD, Richard P. Dutton, MD, MBA, Carl J. Hauser, MD, FACS, FCCM, John B. Holcomb, MD, FACS, Yoram Kluger, MD, Kevin Mackway-Jones, MD, FRCP, FRCS, FCEM, Michael J. Parr, MB, BS, FRCP, FRCA, FANZCA, FFICM, Sandro B. Rizoli, MD, PhD, FRCSC, Tetsuo Yukioka, MD, David B. Hoyt, MD, FACS, and Bertil Bouillon, MD
Prevention/Treatment of Shock

• Improve $O_2$ delivery
  – Increase Cardiac Output (Flow to microvasculature)
    • Preload/Afterload/Contractility
  – Oxygen Content
    • Hemoglobin
    • Arterial Saturation
• Reduce $O_2$ consumption
  – Sedation
  – Analgesia
  – Future use of hibernation/survival gene activation
Relationship between Blood Flow, [Hb] and O$_2$ Delivery

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

Erzurum. PNAS 2007;104:17593-17598
Methods to Improve Flow

• Don’t impair vasoregulation
  – RBC transfusion - vasoconstriction of hypoxic tissue beds

• Improve cardiac output
  – Intravascular volume
    • Preload dependent

• Careful to avoid normo/hypertension
  – Traumatic brain injury exception

• Difficult balance to achieve
Increase intravascular volume (Flow)

• Crystalloids
  – Temporary - extravascular
  – Potentially pro-inflammatory
    • Increase capillary leak

• Colloids
  – Remain intravascular > crystalloids
    – Anticoagulant effects

Cotton B, Shock. 2006
Increase intravascular volume (Flow)

- Plasma
  - Volume expander
  - May repair endothelium
    - Reduce capillary leak
    - Reduced leukocyte binding
    - Glycocalyx restoration
    - Improve hemostasis?

Pati S. et al. Transfusion 2013
Increase intravascular volume (Flow)

• Red Blood Cell Units
  – Volume expander
  – Older RBC units - limitations
    • Vasoconstriction via NO mechanisms
      – MAP vs. Flow
    • Inflammation/oxidative injury

1 Biffl WL, J Trauma, 2001
Mortality increases after massive exchange transfusion with older stored blood in canines with experimental pneumonia

Steven B. Solomon, Dong Wang, Junfeng Sun, Tamir Karias, Jing Feng, Christine C. Helms, Michael A. Solomon, Meghna Alimchandani, Martha Quezado, Mark T. Gladwin, Daniel B. Kim-Shapiro, Harvey G. Klein and Charles Natanson
Increase intravascular volume (Flow)

• Whole blood
  – Volume expander
  – If < 10 days minimal RBC storage lesion effects
  – WBC can be filtered and platelets are spared

• Risk: Exposure to platelets or plasma may not be necessary?
Increase O₂ Content

• Supplemental oxygen
  – Increase arterial saturations

• Transfuse RBC containing products
RBC Functions

• Deliver oxygen
  – Via hemoglobin
  – Altering perfusion
    • Control Vasoregulation - Perfusion of tissues
      – Transport Nitric Oxide (NO) from normoxic to hypoxic beds
RBCs are vascular control elements
control achieved by trapping or deploying NO as a function of HbSO$_2$

RBCs have context-responsive vasoactivity
↓ HbSO₂₂ provokes NO export from RBCs
SNO groups are transferred from Hb to extra-erythrocytic thiols in plasma

Blended mix: O₂, CO₂, N₂

37°C water bath

whole blood spiked with bait thiol: N-acetyl cysteine (500 µM)
pO₂ reduced - simulating circulatory transit
Aliquots taken – fractioned for analysis
HbSO₂₂, RBCSNO (3C), plasma SNOAc (MS)

Capacity of Stored RBCs to Deliver of Oxygen

- RBCs < 7 days
  - Do increase oxygen delivery

- RBCs > 14-21 days
  - Impaired ability to deliver oxygen

- **Effect on outcomes is UNKNOWN**

Arslan E. Am J Surg 2005
Raat NJ, Crit Care Med 2005
Kiraly LN, J Trauma. 2009
Impaired Glycolysis

- ↓ 2.3 DPG
- ↑ p50
- ↓ ATP

- ↓ Ion Pump w/ Ion Leak
- ↓ RBC Deformability
- ↑ Extracellular K+

Impaired RBC Energetics

- ↓ NAD(P)H
- ↓ glutathione
- Oxidative Injury

- RBC Membrane Injury
- ↑ RBC Membrane Microparticles
- ↑ RBC Aggregation
- ↑ RBC Adhesion
- ↓ Perfusion
- ↓ O₂ Delivery

In Critically Ill, POTENTIAL ↑ Risk of MOF and Death
Effects of Stored RBCs on Extracellular Solution

↑ Free Iron (hemolysis)

↓ MAF
Immune Suppression
Infection

↑ PS Expression
Hypercoagulation
Thrombotic Complications

Bioactive lipids
↑ Inflammation
Capillary leak
Perfusion / ↓ O₂ Delivery

Free Iron (Microparticles/Hemolysis)
↓ NO Bio Availability
Altered Vasoregulation

In Critically Ill, POTENTIAL ↑ Risk MOF / Death
RBCs Trauma Patients Receive

- RBC storage solutions
  - Licensed according to survival and recovery
    - 2,3 DPG, ATP, hemolysis
  - **No direct evidence of O2 delivery**

- Preferential use of older RBCs
  - Inventory management

- Transport of older RBCs to trauma centers

- Sickest patients get the oldest RBCs
Blood Banking View on Aging
Intensivist View on Aging
Duration of red blood cell storage is associated with increased incidence of deep vein thrombosis and in hospital mortality in patients with traumatic injuries

Philip C Spinella¹,², Christopher L Carroll¹, Ilene Staff³, Ronald Gross⁴, Jacqueline Mc Quay⁴, Lauren Keibel¹, Charles E Wade² and John B Holcomb⁵

Kaplan Meier Curve of trauma associated survival over 180 days for patients transfused fresh and old RBCs. RBC: red blood cells.
• 600 trauma patients transfused > 2 units
• Compared exclusive receipt of
  – RBCs > and < 14 days
  – No Difference in RBC volume between groups
    • 6.1 vs 5.5 units
• Old vs Fresh RBC group
  – Mortality: 27% vs 20% (p=.08)
  – Adjusted OR Mortality: 1.57 (1.14-2.15)

Weinberg JA, J Trauma. 2010
Relationship between red cell storage duration and outcomes in adults receiving red cell transfusions: a systematic review

Christophe Lelubre and Jean-Louis Vincent

Abstract

Introduction: The duration of red blood cell (RBC) storage before transfusion may alter RBC function and supernatant and, therefore, influence the incidence of complications or even mortality.

Methods: A MEDLINE search from 1983 to December 2012 was performed to identify studies reporting age of transfused RBCs and mortality or morbidity in adult patients.

Results: Fifty-five studies were identified; most were single-center (93%) and retrospective (64%), with only a few, small randomized studies (eight studies, 14.5%). The numbers of subjects included ranged from eight to 364,037. Morbidity outcomes included hospital and intensive care unit (ICU) length of stay (LOS), infections, multiple organ failure, microcirculatory alterations, cancer recurrence, thrombosis, bleeding, vasospasm after subarachnoid hemorrhage, and cognitive dysfunction. Overall, half of the studies showed no deleterious effects of aged compared to fresh blood on any endpoint. Eleven of twenty-two (50%) studies reported no increased mortality, three of nine (33%) showed no increased LOS with older RBCs and eight of twelve (66%) studies showed no increased risks of organ failure. Ten of eighteen (55%) studies showed increased infections with transfusion of older RBCs. The considerable heterogeneity among studies and numerous methodological flaws precluded a formal meta-analysis.

Conclusions: In this systematic review, we could find no definitive argument to support the superiority of fresh over older RBCs for transfusion.
Transfusion of older stored blood and risk of death: a meta-analysis

Dong Wang, Junfeng Sun, Steven B. Solomon, Harvey G. Klein, and Charles Natanson

<table>
<thead>
<tr>
<th>Study/Year (Reference)</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Straten/2011</td>
<td>1.07 (0.74, 1.55)</td>
<td>0.72</td>
</tr>
<tr>
<td>Pettila/2011</td>
<td>1.98 (1.15, 3.39)</td>
<td>0.01</td>
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<tr>
<td>Edgren/2010</td>
<td>1.09 (1.00, 1.19)</td>
<td>0.04</td>
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<tr>
<td>Eikelboom/2010</td>
<td>1.43 (0.90, 2.28)</td>
<td>0.13</td>
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<tr>
<td>Robinson/2010</td>
<td>1.22 (0.79, 1.90)</td>
<td>0.37</td>
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<tr>
<td>Weinberg/2010</td>
<td>1.21 (0.92, 1.58)</td>
<td>0.17</td>
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<tr>
<td>Karam/2010</td>
<td>1.35 (0.47, 3.89)</td>
<td>0.58</td>
</tr>
<tr>
<td>Gauvin/2010</td>
<td>1.81 (0.62, 5.32)</td>
<td>0.28</td>
</tr>
<tr>
<td>Van Buskirk/2009</td>
<td>1.23 (0.75, 2.00)</td>
<td>0.41</td>
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<tr>
<td>Spinella/2009</td>
<td>1.50 (0.73, 3.10)</td>
<td>0.27</td>
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<tr>
<td>Koch/2008</td>
<td>1.67 (1.17, 2.37)</td>
<td>0.0005</td>
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<tr>
<td>Weinberg/2008</td>
<td>1.10 (0.71, 1.69)</td>
<td>0.68</td>
</tr>
<tr>
<td>Yap/2008</td>
<td>1.38 (0.85, 2.25)</td>
<td>0.19</td>
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<tr>
<td>Weinberg/2008</td>
<td>1.16 (1.00, 1.33)</td>
<td>0.05</td>
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<tr>
<td>Leal-Noval/2008</td>
<td>0.71 (0.10, 4.93)</td>
<td>0.73</td>
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<tr>
<td>Van-De-Watering/2006</td>
<td>1.03 (0.62, 1.71)</td>
<td>0.91</td>
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<tr>
<td>Murrell/2005</td>
<td>1.54 (0.73, 3.28)</td>
<td>0.26</td>
</tr>
<tr>
<td>Fernandes-Da-Cunha/2005</td>
<td>1.18 (0.38, 3.65)</td>
<td>0.77</td>
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<tr>
<td>Heberl/2005</td>
<td>0.45 (0.10, 2.10)</td>
<td>0.31</td>
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<tr>
<td>Schulman/2002</td>
<td>0.29 (0.04, 2.32)</td>
<td>0.24</td>
</tr>
<tr>
<td>Mynster/2001</td>
<td>0.75 (0.49, 1.13)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

| All Studies            | 1.16 (1.07, 1.24) | 0.0001 |

OR (95% CI)
RBC age and severity of illness (two hit hypothesis)

1\textsuperscript{st} hit: Susceptible to develop disease/adverse event

2\textsuperscript{nd} hit: Exposure or illness that leads to clinical expression of disease

- Post-op ortho (< 1% mortality)
- Simple post-op cardiac (3-5%)
- ARDS (20-40%)
- Severe Trauma (20-40%)
- Severe Sepsis (40%)

Might be increased risk of old RBC with increased critical illness
Anemia Effect on Hemostasis

- Hypocoagulation
  - Reduced viscosity
  - Platelet inhibition
    - Sheer stress induced release of NO
    - Less sequestration of NO by RBCs

- Compensation
  - Sheer stress induced release of ADP from RBCs
    - Stimulates platelet aggregation

Valeri, R. Transfusion 2007
RBCs Transfusion and Hemostasis

• Stored RBCs – Procoagulant effects
  – Reduced bleeding time, TEG-R times, less clinical bleeding
  – Increased platelet function
    • Aggregation (light transmission), P-selectin expression, TEG
      – Increased viscosity
      – RBC release of ADP
      – Platelets release of Thromboxane A2
      – NO Sequestration

Eur Heart J. 2010 Nov;31(22):2816-21
Valeri, R. Transfusion 2007
Spoerke NJ, J Trauma. 2010 Nov;69(5):1054-9;
RBC storage time effect on Hemostasis

- Reduced platelet function
  - PFA, TEG
  - Collagen stimulated single platelet disappearance
- Increased thrombin generation
  - Increased TAT formation, TEG-R time
- **Clinical effects of RBC storage age on hemostasis unknown**

Nepstad, I, Hervig T, et al. In Submission
Prevent Hemorrhagic Death Pre-hospital

• Shock and coagulopathy need to be addressed simultaneously
  – AGREE ????

• Optimal methods still not apparent
Improve $O_2$ Delivery/Hemostasis

- **Crystalloids**
  - Temporary increase in preload – $O_2$ delivery
  - Pro-inflammatory, capillary leak/edema

- **Colloids**
  - Increase preload – $O_2$ delivery
  - Anticoagulation effects with substantial amount

- **Plasma**
  - Partial Hemostasis & $O_2$ delivery, endothelial repair
  - No platelets

- **Platelets (plasma)**
  - Adequate hemostasis, endothelial repair
  - Partial $O_2$ delivery
Improve \( \text{o}_2 \) Delivery/Hemostasis

- **RBCs, 4\(^\circ\)C, <42 days**
  - **Pro**
    - Preload
    - Oxygen delivery (**impaired to reduced**)
    - ± Hemostasis
  - **Con** (older RBCs):
    - Impaired vasoregulation
    - Inflammation
    - Immune suppression
    - Infection risk
    - ± Hemostasis

- **Whole Blood, 4\(^\circ\)C, <10 days**
  - **Pro**
    - Preload
    - \( \text{O}_2 \) delivery
    - Hemostasis
    - Platelets (activated)
  - **Con**
    - WBCs (removed by filter)
    - ABO controversy
    - Platelet/plasma exposure?
Pre-hospital Resuscitative Choices?
Pre-hospital Resuscitative Choices

**Real World**

- Prioritization
  - Crystalloids
  - Colloids
  - RBCs
  - Plasma
  - Platelets
  - Whole blood

**Spinella’s Fantasy World**

- Prioritization
  - LR-Whole Blood, 4°C, <10 d
  - Platelets, 4°C > 22°C
  - Plasma
  - RBCs
  - Colloids
  - Crystalloids
Conclusions-
Role of RBCs in RDCR

• Whole Blood is potentially the optimal fluid to achieve RDCR goals
  • Oxygen delivery and hemostasis

• If not available then fresh RBCs and platelets at 4°C are potentially the second best option

• Need trials in this area to test these hypotheses in both pre-hospital and in-hospital settings

• Innovative clinical trial design will be needed
Is That Goal Out of Reach?
Thank you

Spinella_p@kids.wustl.edu
FFP repair of endothelium

• Hypothesis:
  – FFP promote vascular stability through regulation of critical junction proteins.
The glycocalyx is a ubiquitous barrier that protects the underlying endothelium.

Kozar et al.
Improved EC Adherens Junctions

VE-Cadherin (Green)

Kozar RA, Pati S. in Press
Spray-dried plasma and fresh frozen plasma modulate permeability and inflammation in vitro in vascular endothelial cells

TRANSFUSION 2013;53:80S-90S.

Fig. 2. SDP performs comparably to FFP in inhibiting EC permeability over time based on EC flux calculations of 70-kD Dextran. (A) Permeability coefficients for each group at 10% concentration. In all experiments, control represents cells treated
B. 10% Permeability w/ 10 kDa FITC dextran, t = 45 minutes

% Decrease in Transwell Permeability

Control | LR | Hextend | FFP | SDP

* N. S.
A. 30% Concentration

B. 10% Concentration

% Decrease in Endothelial Leukocyte Binding

Control  LR  Hextend  FFP  FFP-SD  SDP

N.S.

*
Pati, S. et al. Protective effects of fresh frozen plasma on vascular endothelial permeability, coagulation, and resuscitation after hemorrhagic shock are time dependent and diminish between days 0 and 5 after thaw. J Trauma 69, S55–S63 (2010).


Transfusion of Aged Packed Red Blood Cells Results in Decreased Tissue Oxygenation in Critically Injured Trauma Patients

The Journal of TRAUMA® Injury, Infection, and Critical Care • Volume 67, Number 1, July 2009

Laszlo N. Kiraly, MD, Samantha Underwood, MS, Jerome A. Differding, MS, and Martin A. Schreiber, MD

Figure 2. Tissue oxygenation versus time. Time 0 is defined as the start of transfusion.
RBCs accelerate the onset of clot formation in polytrauma and hemorrhagic shock.

METHODS:
Thirty-two Yorkshire swine were anesthetized, subjected to a complex model of polytrauma and hemorrhagic shock, and resuscitated with either fresh frozen plasma, lyophilized plasma (LP), or 1:1 ratios of fresh frozen plasma:packed RBC (PRBC) or LP:PRBC.

Activated clotting time, prothrombin time, partial thromboplastin time, and thrombelastography (TEG) were performed at 1 hour, 2 hours, 3 hours, and 4 hours after resuscitation.

RESULTS:
Animals treated with 1:1 LP:PRBC had less blood loss than the other groups (p < 0.05).

The activated clotting time was shorter in the 1:1 groups when compared with the pure plasma groups at all time points (p < 0.05).

The 1:1 groups had shorter TEG R times (time to onset of clotting) at 1 hour, 3 hours, and 4 hours compared with pure plasma groups (p < 0.05).

CONCLUSIONS:
Whole blood assays reveal that RBCs accelerate the onset of clot formation.
Spoerke NJ, J Trauma. 2010 Nov;69(5):1054-9;
Red blood cells impair hemostasis.

STUDY DESIGN AND METHODS:
• In 24 patients with chronic anemia the effect of transfusion of RBCs on coagulation studied.
• In 18 patients we evaluated whether storage time of RBCs has additional effects on hemostasis.

RESULTS:
• Correction of anemia by RBC transfusion resulted in improved fibrin formation but reduced clot strength.
• The negative effects on fibrin formation and clot strength were significantly worse when fresh RBCs were transfused compared to longer-stored RBCs.

CONCLUSIONS:
• Transfusion of RBCs was associated with impaired clot quality, with even worse effects on the initial fibrin build-up and clot quality by fresh RBCs.

Impact of red blood cell transfusion on platelet activation and aggregation in healthy volunteers: results of the TRANSFUSION study.

METHODS AND RESULTS:
In vitro transfusions (n = 45) were performed by the addition of RBCs obtained from transfusion packs to fresh whole blood provided by healthy volunteers. Residual platelet aggregation (RPA) and maximal platelet aggregation (MPA) were assessed before and after in vitro transfusion using light transmission aggregometry performed with four different agonists. Flow cytometry was used for the measurement of P-selectin expression and vasodilatator-stimulated phosphoprotein (VASP) platelet reactivity index (PRI).
To control for the effect of haemoconcentration, the same experiments were repeated after hematocrit adjustment using volunteer's platelet poor plasma.
Transfusion increased platelet aggregation as measured by RPA with ADP 5 µM (57.7 ± 25 vs. 65.7 ± 24%; P = 0.03) or Collagen 2 µg/mL (59.4 ± 28 vs. 69.7 ± 24%; P = 0.03). Platelet activation was also increased by transfusion as confirmed by an elevation of P-selectin expression. These effects were all independent of hematocrit.

CONCLUSION:
Red blood cell transfusion increases platelet activation and aggregation in vitro in healthy volunteers. This effect might be mediated through the P2Y(12) activation pathway.
Stored erythrocytes have less capacity than normal erythrocytes to support primary haemostasis.

RBC units from 17 healthy volunteers stored for 45 days. Fresh citrated blood was taken again from the same donors and platelet-rich plasma was prepared, in which RBCs were resuspended with a constant haematocrit (40%), but changing fractions of stored versus fresh autologous RBCs (0, 25, 50, 75, and 100%, respectively).

A platelet function analyser PFA-100((R)) was used.

We found that the closure time increased with increasing fractions of stored blood.

We conclude that stored RBCs have less capacity than normal RBCs to support primary haemostasis by platelet aggregation in vitro, suggesting a decreased capacity of stored RBCs to bring platelets into close contact with the wall, which may contribute to sustained bleeding seen after mass transfusion.

ABLE

• ABLE Trial
  – RCT of 2500 adult ICU patients
  – Expected > 48 hrs intubation
  – Fresh (< 7 days) vs standard (mean 21 days)
  – Primary outcome is 90 day mortality
ABLE-ARMS
(Ancillary Repository and Mechanisms)

• Collect samples over time in
  – 200 patients (100/100)
• In Fresh vs Old RBC groups
  – Inflammation and Coagulation
  – Microparticles
  – Immune function
  – Microchimerism

Funding Support:
DoD/USAMRAA, W81XWH-10-1-0023
DoD/USAMRAA, W23RYX0216N601-N602
NIH/NHLBI, 5R01HL095470-02
## Timeline of Specimen Sampling

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Day 0</th>
<th>Day 1-3</th>
<th>Day 5-7</th>
<th>Day 28</th>
<th>Day 180</th>
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<tbody>
<tr>
<td>Cytokine &amp; Coagulation</td>
<td>x</td>
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<td>x</td>
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<tr>
<td>Microparticles</td>
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<tr>
<td>Immune function</td>
<td>x</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Microchimerism</td>
<td>x</td>
<td></td>
<td></td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>
Coagulation Parameters

- Prothrombin Fragments 1+2,
- Soluble Thrombomodulin, Protein C, PAI-1
- Tissue Plasminogen Activator
- Factors V, VII, VIII,
- D-Dimer, Antithrombin III,
- Soluble Endothelial Protein C Receptor
Change in Factor V levels

Box plots showing the distribution of Factor V levels at different time points for two groups, Group 1 (blue) and Group 2 (red). The x-axis represents time points (0, 2, 6, 28), and the y-axis represents Factor V levels in percentage.
Change in Factor II levels
Change in Antithrombin III levels

![Box plot showing changes in Antithrombin III levels over time for two groups.]

- **Y-axis:** Antithrombin III
- **X-axis:** Day (0, 2, 6, 28)
- **Legend:**
  - Group 1 (Blue)
  - Group 2 (Red)
Change in Protein C levels

The graph shows the change in Protein C levels over time for two different groups (Group 1 in blue and Group 2 in red). The y-axis represents Protein C levels (%), and the x-axis represents time points (0, 2, 6, 28). The box plots indicate the distribution of Protein C levels at each time point for both groups, with the median, interquartile range, and outliers visually represented.
RBC age and Thrombin Generation

Nepstad, I, Hervig T, et al. In Submission
RBC age and Platelet function

Nepstad, I, Hervig T, et al. In Submission