RDCR Blood Products Research
Current Efforts & Future Directions for Pre-Hospital Treatments
US Army Institute of Surgical Research

LTC Andrew P. Cap, MD, PhD, FACP
Chief, Coagulation and Blood Research Program
Program Director, Clinical Research Fellowship

Bergen, Norway
June, 2013
The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.
DoD Needs

• #1 cause of preventable battlefield death = hemorrhage
  – No or limited blood/resuscitation products at point of injury
  – Prolonged evacuations
    • Profound shock
    • Coagulopathy
  – Limitations to blood supply at military treatment facilities
    • Platelets: “5 day” shelf life, declining function over storage
    • Plasma: breakage in shipping, thawed emergency plasma
    • Red cells: age at time of transfusion
    • Whole blood: TTD risk, shelf life?
  – Current operations in mature theaters – future contingencies?
    • Africa, Pacific, etc.
    • Support to massive civilian catastrophe

➤ Need FDA-approved products
New DoD Focus: Pre-Hospital (RDCR)

• Basically nothing FDA-approved and practical in the pre-hospital setting other than crystalloid/colloid
  – Most of the exsanguination mortality happens here!
  – TXA = first advance in this area (still off-label use)

• DoD efforts in Blood/Resuscitation to date focused on DCR in-hospital, but…
  – Efforts in biology of ACOT useful to whole continuum of care
  – Current blood products work aims to bridge pre-/in-hospital gap
Current Efforts

• US Army Core-Funded
  – Improved Platelet Products (including WB)
    • Longer shelf life
    • Improved safety (lower bacterial contamination risk)
    • Improved hemostatic function
  – Diagnostics and Therapeutics for Acute Coagulopathy of Trauma
    • Animal models to characterize disorder and identify targets
    • Describe platelet contribution: hemostatic dysfunction, immuno/inflammatory effects
    • Cellular microparticles
    • In vitro systems
  – Clinical studies of coagulation, platelets and transfused products in burn surgery & resuscitation
    • Massive transfusions, controlled setting, informed consent
  – REMTORN: pre-hospital diagnostics, blood products, hemostatic adjuncts
Fundamental principles

• Safety
  – TTD
  – Bacterial Contamination
  – VTE risk

• Efficacy
  – Normalization or maintenance of normal hemostatic function
  – Clot stabilization

Hemostatic function ≠ procoagulant; normal is good!
Just don’t make things worse (dilution, etc.)…
Major International Collaborations

- Norwegian Navy (w/ DMRDP & TerumoBCT): Blood Far Forward
  - Hemostatic function of Mirasol-treated, cold-stored WB
  - Mirasol & leukoreduction effects on microparticles
  - Functional studies of components from Mirasol WB
  - Mirasol WB in rat ACOT model

- French Army, US SOCOM & MRMC: Freeze-dried plasma (FDP)
Future Directions

• Clinical studies: burn surgery resuscitation
  – Cold platelets
  – Mirasol/LR/cold WB

• Pre-hospital clinical studies: REMTORN
  – WB

• Integration of improved diagnostic systems

• Stem cells
  – Collection, processing, storage
  – Role in trauma, burn

• Novel oxygen carriers, platelet-like products, hemostatic adjuncts…
Vision: FDA approved…

• Pre-hospital treatment for massive hemorrhage
  – Safe (microbiology, thrombosis, other)
  – Prevents shock, endotheliopathy, coagulopathy
  – Supports hemostasis
  – Universal products
  – Stable (“ambient” conditions for mission duration, “reasonable” shelf life)

• Improved blood products for hospital-based DCR
  – Safe
  – Improved function
  – Improved logistics (shelf life, storage conditions)
Reality Check

**Question:** What is likely to be available in the near/mid term (3-5 years)? *[DoD needs products ASAP.]*

**Answer:** existing technologies or those in clinical development

- Whole blood (LR, cold-stored)
- Cold platelets
- PRT (Mirasol for WB)
- FDP

**Maybe:** fibrinogen concentrate, PCCs (need studies with industry support for licensing in order to see broad adoption)
Why the focus on “cold” products?

Cold WB and PLT are potentially licensable now!

• Why is WB not used to treat hemorrhage?
  – “Right product, right patient”
  – Blood banks get more reimbursement for components.
  – “Must be fresh or platelets won’t work.”
  – TTD risk if “warm” and untested

• Why not refrigerate platelets?
  – More rapid clearance? Relevance to acute hemorrhage?
  – What about acute function?

If you could refrigerate platelets (or WB), you could use pre-hospital (Golden Hour box). Huge impact on inventory.
Would you store a steak at room temp?

Really?

What do you think is going to happen?
Platelets: the First Responders

http://quizlet.com/18495470/heme-path-pics-1-flash-cards

Dr Catherine Pear’s Lab
Oxford University Dept. of Biochemistry


Nature Reviews | Immunology
Cold Platelet Evaluation

- Apheresis units collected on Trima
- Storage at 4C or RT, w/ or w/o agitation
- Duration: up to 14 days
- Focus on tests of hemostatic function
  - Recovery and survival of 4C platelets already known (Murphy, NEJM, 1969.) to be adequate for acute hemostasis
  - Cold platelets are safer: less bacterial contamination
4°C platelets: higher activation vs. RT

A

\[
\text{Graph A: CD62P \% Total vs. Days (RT, 4C, 4C+AG)}
\]

B

\[
\text{Graph B: Lactadherin \% Total vs. Days (RT, 4C, 4C+AG)}
\]

C

\[
\text{Graph C: PAC1 \% Total vs. Days (RT, 4C, 4C+AG)}
\]

D

\[
\text{Graph D: CD154 \% Total vs. Days (RT, 4C, 4C+AG)}
\]
Spontaneous aggregation occurs at 4° C but does not affect platelet function.
4°C platelets: superior clot strength & stability (TEG)
4°C platelets: better aggregation response to agonists vs. RT

A

\[
\begin{align*}
\text{ADP (AUC)} \\
\text{RT} & \quad 20 \\
4C & \quad 15 \\
4C+AG & \quad 10 \\
\end{align*}
\]

B

\[
\begin{align*}
\text{Collagen (AUC)} \\
\text{RT} & \quad 20 \\
4C & \quad 15 \\
4C+AG & \quad 10 \\
\end{align*}
\]

C

\[
\begin{align*}
\text{TRAP (AUC)} \\
\text{RT} & \quad 80 \\
4C & \quad 60 \\
4C+AG & \quad 40 \\
\end{align*}
\]
No significant increase in microparticles
RT platelets release more sCD40L and Thromboxane B₂.

A

B
Platelet/VWF interaction under flow

Day 4 vwf Calcein

FIU

seconds

vWf 100ug 4C_Calcein
vWf 100ug 22C_Calcein
vWf 100ug pH_4C_Calcein
vWf 100ug pH_22C_Calcein
Platelet/Collagen interaction under flow

Day 4

- AphPRP_AphPFP_4C_Calcein
- AphPRP_AphPFP_22C_Calcein
- AphPRP_AphPFP_pH_4C_Calcein
- AphPRP_AphPFP_pH_22C_Calcein
The elastic and viscous strength are similar for:
- fresh and 5 day stored 4C PRP
- 5 day RT PRP and 10 day 4C PRP

**Dynamic Mechanical Modeling**

- $G'$ - Elastic/Storage modulus
- $G''$ - Loss modulus (viscous strength)
# 4° C storage preserves metabolic parameters

<table>
<thead>
<tr>
<th>Condition</th>
<th>Day</th>
<th>Na</th>
<th>K</th>
<th>Cl</th>
<th>Glu</th>
<th>pH</th>
<th>pCO2</th>
<th>pO2</th>
<th>HCO3</th>
<th>Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>1</td>
<td>137.5±1</td>
<td>3.24±.18</td>
<td>97.6±.89</td>
<td>321.5±8.1</td>
<td>7.24±.07</td>
<td>38.5±4.7</td>
<td>91.8±9.7</td>
<td>18.04±1.6</td>
<td>1.77±.55</td>
</tr>
<tr>
<td>RT+AG</td>
<td>3</td>
<td>139.5±3.7</td>
<td>3.34±.23</td>
<td>101.3±4.9</td>
<td>294±22.1</td>
<td>7.45±.16</td>
<td>13.8±1.5</td>
<td>113±17.8</td>
<td>10.06±2.9</td>
<td>6.85±.77</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>140.25±1.3</td>
<td>3.45±.31</td>
<td>104.8±1.8</td>
<td>238.3±42.5</td>
<td>7.28±.08</td>
<td>13.1±.25</td>
<td>132±41.2</td>
<td>6.27±.91</td>
<td>12.87±2.1</td>
</tr>
<tr>
<td>4C</td>
<td>3</td>
<td>137.4±1.7</td>
<td>3.52±.19</td>
<td>99.2±2.8</td>
<td>310.2±14.2</td>
<td>7.45±.21</td>
<td>24.1±4.2</td>
<td>120.4±32.7</td>
<td>14.0±1.7</td>
<td>4.51±.55</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>136.75±.96</td>
<td>3.60±.25</td>
<td>99.7±1.2</td>
<td>307.5±25.0</td>
<td>7.44±.13</td>
<td>19.5±4.4</td>
<td>139.8±26.8</td>
<td>12.78±.81</td>
<td>5.67±.29</td>
</tr>
<tr>
<td>4C+AG</td>
<td>3</td>
<td>137.8±1.6</td>
<td>3.52±.25</td>
<td>99±2.2</td>
<td>312.4±28.1</td>
<td>7.48±.25</td>
<td>22.6±5.6</td>
<td>116.6±31.0</td>
<td>14.0±2.7</td>
<td>4.83±.65</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>136.5±1.3</td>
<td>3.55±.27</td>
<td>98.8±1.7</td>
<td>303.5±24.6</td>
<td>7.44±.15</td>
<td>18.7±4.7</td>
<td>128±29.3</td>
<td>12.38±.98</td>
<td>6.05±.36</td>
</tr>
</tbody>
</table>
Don’t take my word for it...

In vitro function and phagocytosis of galactosylated platelet concentrates after long-term refrigeration.
Babic AM, Josefsson EC, Bergmeier W, Wagner DD, Kaufman RM, Silberstein LE, Stossel TP, Hartwig JH, Hoffmeister KM.

Source
Department of Pathology, Division of Translational Medicine, Brigham and Women's Hospital, Boston, Massachusetts 02115, USA.

CONCLUSION:
It is shown that refrigerated human PLTs retain in vitro function better than RT PLTs during storage and demonstrate that galactosylation prevents recognition of stored refrigerated PLTs by macrophages in vitro.
There’s still more…

Influence of storage temperature on the responsiveness of human platelets to agonists.
Choi JW, Pai SH.

**Source**
Department of Clinical Pathology, College of Medicine, Inha University, Jung-gu, Inchon, Korea

**CONCLUSION:**
In summary, refrigerated storage of human blood improves the stability of platelet responsiveness to agonists. *Storage at RT causes platelet non-responsiveness to epinephrine and disturbs the release reaction of endogenous ADP.*

**Haematologica.** 2001 May;86(5):530-6.
Effect of cold-storage in the accumulation of bioreactive substances in platelet concentrates treated with second messenger effects.
Ferrer F, Rivera J, Lozano ML, Corral J, García VV.

**Source**
Hematology and Medical Oncology Unit, School of Medicine Los Arcos, Ronda de Garay s/n, 30003 Murcia, Spain.
vvg@um.es

Refrigeration: lower levels of IL-6, IL-8, TGF-B, C3a, C4a
Inhibition of cytokine accumulation and bacterial growth during storage of platelet concentrates at 4 degrees C with retention of in vitro functional activity.
Currie LM, Harper JR, Allan H, Connor J.
Source
LifeCell Corporation, The Woodlands, Texas, USA.

CONCLUSION:
The storage of PCs at refrigerated temperatures inhibits the accumulation of white cell-produced cytokines in the PCs, an effect that could alleviate cytokine-associated febrile transfusion reactions. The 4 degrees C storage was also bacteriostatic, which indicates that the storage of PCs at that temperature increases safety by decreasing the potential for sepsis. Thus, the ability to store PCs at 4 degrees C may allow extension of the storage limit beyond 5 days.
Becker Study 1973


89 thrombocytopenic patients
**Reduction in clinical bleeding?**

84% response rate for 4C-stored vs. 39% for 22C-stored

---

**TABLE 1. Response of Bleeding Time of Thrombocytopenic Patients to Transfusion of Platelet Preparations**

<table>
<thead>
<tr>
<th>Platelet Preparations</th>
<th>Number of Patients</th>
<th>Number Improved</th>
<th>Number Not Improved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>8</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>24 hr 22 C</td>
<td>11</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>24 hr 4 C</td>
<td>9</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>48 hr 22 C</td>
<td>14</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>48 hr 4 C</td>
<td>7</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>72 hr 22 C</td>
<td>7</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>72 hr 4 C</td>
<td>12</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

---

![Graph showing mean response (%) of the template bleeding time (per cent correction toward normal) induced by stored autologous platelets upon being transfused to six normal subjects who had ingested aspirin.](image-url)
So, compared to “cold” platelets...

- RT platelets don’t work as well as they could
- RT platelets are much more likely to cause an infection
- RT platelets have an effective shelf life of 3 days vs. ??
- RT platelets are MORE EXPENSIVE

And, we apply a transfusion strategy designed for cancer patients (now being questioned*) for all bleeding patients…

FOR NO GOOD REASON!

A word on prophylactic transfusion...

- N=197 prophylactic, N=199 therapeutic
- Therapeutic strategy reduces transfusion by 33.5%
- No increased risk of serious hemorrhage in autoSCT group
- Small increase Gr4 (CNS) bleeding in patients with AML

Prophylaxis for AML patients; for others: therapeutic transfusions only…

Therapeutic platelet transfusion versus routine prophylactic transfusion in patients with haematological malignancies: an open-label, multicentre, randomised study

Hannes Wandt, Kerstin Schaefer-Eckart, Knut Wendelin, Bettina Pilz, Martin Wilhelm, Markus Thalheimer, Ulrich Mahlknecht, Anthony Ho, Markus Schaic, Michael Kramer, Martin Kaufmann, Lothar Leimer, Rainer Schwertfeger, Roland Conradi, Gottfried Dößen, Anne Klenner, Mathias Hänel, Regina Herbst, Christian Junghanss, Gerhard Ehninger, for the Study Alliance Leukemia
Would you believe?

• “Cold” platelets are still on the books, BUT no one uses them!

Code of Federal Regulations [Title 21, Volume 7] [Revised as of April 1, 2012] [CITE: 21CFR640]

TITLE 21--FOOD AND DRUGS
CHAPTER I--FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
SUBCHAPTER F--BIOLOGICS PART 640 ADDITIONAL STANDARDS FOR HUMAN BLOOD AND BLOOD PRODUCTS
Subpart C--Platelets

Sec. 640.24 Processing. (a) Separation of plasma and platelets and resuspension of the platelets must be in a closed system. Platelets must not be pooled during processing unless the platelets are pooled as specified in the directions for use for the blood collecting, processing, and storage system approved for such use by the Director, Center for Biologics Evaluation and Research.

(d) The volume of original plasma used for resuspension of the platelets shall be determined by the maintenance of a pH of not less than 6.2 during the storage period. The pH shall be measured on a sample of platelets which has been stored for the maximum dating period at the selected storage temperature. One of the following storage temperatures shall be used continuously:

(1) 20 to 24 deg. C.
(2) 1 to 6 deg. C.


WB Platelet Aggregation (ADP)

- Time: $p \leq 0.001$
- Temp: $p \leq 0.001$
- PRT: $p = 0.603$

Agonist: ADP
WB Platelet Aggregation (Collagen)

Agonist: COLLAGEN
- Time: p ≤ 0.001
- Temp: p ≤ 0.001
- PRT: p = 0.489

- Con 04
- Con 22
- TX 04
- TX 22
WB Platelet Aggregation (TRAP)

- Time: $p \leq 0.001$
- Temp: $p \leq 0.001$
- PRT: $p \leq 0.009$

Agonist: TRAP-6

AUC

STUDY DAY

Agonist: TRAP-6
- Time: $p \leq 0.001$
- Temp: $p \leq 0.001$
- PRT: $p \leq 0.009$
WB platelet adhesion and aggregation under shear

Cone-and-Plate Aggregometry Measured by Percent Surface Coverage (%SC)

Temp: p<0.05
TX: NS
**TEG parameters for WB at 4C in normal range to 21 days**

Table: Average TEG values over the 21 day observation period

<table>
<thead>
<tr>
<th></th>
<th>DAY 0</th>
<th>DAY 1</th>
<th>DAY 4</th>
<th>DAY 7</th>
<th>DAY 10</th>
<th>DAY 14</th>
<th>DAY 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEMP</td>
<td>AVE ± SD</td>
<td>AVE ± SD</td>
<td>AVE ± SD</td>
<td>AVE ± SD</td>
<td>AVE ± SD</td>
<td>AVE ± SD</td>
<td>AVE ± SD</td>
</tr>
<tr>
<td>R</td>
<td>9.1 ± 1.1</td>
<td>7.9 ± 0.9</td>
<td>7.4 ± 1.3</td>
<td>8.2 ± 0.7</td>
<td>8.0 ± 0.6</td>
<td>8.1 ± 0.8</td>
<td>8.6 ± 0.5</td>
</tr>
<tr>
<td>(1-11 min)</td>
<td>9.2 ± 1.5</td>
<td>9.2 ± 1.6</td>
<td>10.6 ± 1.8</td>
<td>10.8 ± 1.8</td>
<td>10.3 ± 1.4</td>
<td>10.4 ± 2.3*</td>
<td>10.2 ± 2.4*</td>
</tr>
<tr>
<td>K</td>
<td>2.4 ± 0.3</td>
<td>2.5 ± 0.5</td>
<td>2.1 ± 0.5</td>
<td>2.5 ± 0.4</td>
<td>2.8 ± 1.0</td>
<td>2.9 ± 0.8</td>
<td>3.6 ± 0.8</td>
</tr>
<tr>
<td>(-2-6 min)</td>
<td>2.3 ± 0.6</td>
<td>2.8 ± 1.1</td>
<td>2.8 ± 0.8</td>
<td>2.4 ± 0.5</td>
<td>4.0 ± 3.1</td>
<td>7.5 ± 4.8*</td>
<td>9.4 ± 1.2*</td>
</tr>
<tr>
<td>a</td>
<td>58 ± 4</td>
<td>58 ± 6</td>
<td>62 ± 6</td>
<td>58 ± 4</td>
<td>55 ± 8</td>
<td>55 ± 7</td>
<td>49 ± 6</td>
</tr>
<tr>
<td>(36-81°)</td>
<td>59 ± 7</td>
<td>56 ± 8</td>
<td>55 ± 8</td>
<td>52 ± 14</td>
<td>48 ± 14</td>
<td>42 ± 10*</td>
<td>38 ± 9*</td>
</tr>
<tr>
<td>MA</td>
<td>60 ± 3</td>
<td>57 ± 4</td>
<td>60 ± 4</td>
<td>57 ± 3</td>
<td>54 ± 5</td>
<td>54 ± 5</td>
<td>48 ± 5</td>
</tr>
<tr>
<td>(39-81mm)</td>
<td>60 ± 7</td>
<td>57 ± 8</td>
<td>53 ± 8</td>
<td>41 ± 17</td>
<td>40 ± 18</td>
<td>28 ± 12*</td>
<td>20 ± 6*</td>
</tr>
</tbody>
</table>

Note: bolded measurements are outside of the normal range.

*p ≤ 0.05
Warm Fresh Whole Blood Is Independently Associated With Improved Survival for Patients With Combat-Related Traumatic Injuries

Philip C. Spinella, MD, Jeremy G. Perkins, MD, Kurt W. Grathwohl, MD, Alec C. Beekley, MD, and John B. Holcomb, MD


Fig. 1. Kaplan-Meier curve of 30-day survival according to study group.
# Manno Study Results

<table>
<thead>
<tr>
<th></th>
<th>Warm FWB</th>
<th>Cold WB</th>
<th>Recon Blood</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hr blood loss (ml/kg)</td>
<td>50.9 (±9)</td>
<td>44.8 (±6)</td>
<td>74.2 (±9)</td>
<td>0.03∞</td>
</tr>
<tr>
<td>24 hr blood loss (ml/kg)</td>
<td>52.3 (±11)</td>
<td>51.7 (±7.4)</td>
<td>96.2 (±11)</td>
<td>0.001§</td>
</tr>
<tr>
<td>&lt; 2 yrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTT (30 min)</td>
<td>38.2 (±1.1)</td>
<td>39.7(±3.4)</td>
<td>43.3 (±1.8)</td>
<td>0.06</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>202 (±5.4)</td>
<td>195 (±5.6)</td>
<td>184 (±4.8)</td>
<td>0.07</td>
</tr>
<tr>
<td>PLT aggregation (30 min)</td>
<td></td>
<td></td>
<td>most reduced</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ADP, epinephrine, collagen</td>
<td></td>
</tr>
</tbody>
</table>

∞ cold vs recon
§ warm and cold vs recon


Double-blind RCT, 161 children, cardiac surgery
Warm FWB ≤ 6hrs
Cold WB ≤ 48hrs
Cold platelets and WB

**In vitro:**
- Better hemostatic function than RT storage

**Clinical experience:**
- Standard of care until 1970s, still used until 1980s in many US centers
- Vietnam: cold WB was primary resuscitative blood product
  - Type O, low anti-A, anti-B was universal product
- Pediatric cardiac surgery: Manno, et al. (WB stored 48 hrs)

**Way forward:**
- Clinical studies in current era, especially pre-hospital, trauma, burn; *longer storage*
Achievable vision for improved hemostatic resuscitation?

Medic at POI:
- FDP (EA-IND status, need FDA approval)
- TXA (give anywhere in evac chain w/in 3 hrs if not at POI)
- WFWB (when facing delayed evacuation)
- Fibrinogen??

MEDEVAC:
- Cold WB (doable now)

DCR in-hospital:
- Cold WB
Or
- Components: RBC, FFP/FDP, cold PLT (doable now)