Platelet Dysfunction after Trauma: Characterization and the study of function.

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Platelets: A primary mediator?
San Francisco General Hospital

- San Francisco’s *only* trauma center
- Cares for *all* patients with traumatic injury in San Francisco, regardless of ability to pay
- Serves 100,000 patients per year
- Cares for 4,900 injured patients per year
SFGH Surgical Research Lab
Trauma, and Hypoxia induce the initiation of coagulation

Tissue factor (TF) is exposed and binds to FVIIa or FVII which is subsequently converted to FVIIa

The complex between TF and FVIIa activates FIX and FX

FXa binds to FVa on the cell surface
The FXa/FVa complex converts small amounts of prothrombin into thrombin.

The small amount of thrombin generated activates FVIII, FV, FXI, and platelets locally.

FXIa converts FIX to FIXa.

Activated platelets bind FVa, FVIIIa, and FIXa.
The FVIIIa/FIXa complex activates FX on the surfaces of activated platelets.

FXa in association with FVa converts large amounts of prothrombin into thrombin creating a “thrombin burst”.

The “thrombin burst” leads to the formation of a stable fibrin clot.
Coagulopathy in the wild...
Maladaptive response to trauma. Early coagulopathy, later hypercoagulable state and loss of cytoprotectivity.
The Ratio of Blood Products Transfused Affects Mortality in Patients Receiving Massive Transfusions at a Combat Support Hospital

Matthew A. Borgman, MD, Philip C. Spinella, MD, Jeremy G. Perkins, MD, Kurt W. Grathwohl, MD, Thomas Repine, MD, Alec C. Beekley, MD, James Sebesta, MD, Donald Jenkins, MD, Charles E. Wade, PhD, and John B. Holcomb, MD

![Mortality Graph]

- Low: 1:8, Mortality: 65%
- Medium: 1:2.5, Mortality: 34%
- High: 1:1.4, Mortality: 19%

$P < 0.05$
Don’t forget platelets.
Suggestions platelets are important after injury.

- Correlation of number to outcome.
- Benefit for empiric platelet resuscitation.
- Functional data.
A Normal Platelet Count May Not Be Enough: The Impact of Admission Platelet Count on Mortality and Transfusion in Severely Injured Trauma Patients

Lisa M. Brown, MD, MAS, Mariah S. Call, BS, M. Margaret Knudson, MD, Mitchell J. Cohen, MD, and the Trauma Outcomes Group

Background: Platelets play a central role in hemostasis after trauma. However, the platelet count of most trauma patients does not fall below the normal range (100–450 × 10^9/L), and as a result, admission platelet count has not been adequately investigated as a predictor of outcome. The purpose of this study was to examine the relationship between admission platelet count and outcomes after trauma.

Methods: A retrospective cohort study of 389 massively transfused trauma patients. Regression methods and the Kruskal-Wallis test were used to test the association between admission platelet count and 24-hour mortality and units of packed red blood cells (PRBCs) transfused.

Results: For every 90 × 10^9/L increase in admission platelet count, the odds of death decreased 17% at 6 hours (p = 0.03, 95% confidence interval [CI], 0.70–0.99) and 14% at 24 hours (p = 0.02, 95% CI, 0.75–0.98). The probability of death at 24 hours decreased with increasing platelet count. For every 50 × 10^9/L increase in platelet count, patients received 0.7 fewer units of blood within the first 6 hours (p = 0.01; 95% CI, 1.3 to −0.14) and one less unit of blood within the first 24 hours (p = 0.002; 95% CI, 1.0 to −0.36). The mean number of units of PRBCs transfused within the first 6 hours and 24 hours decreased with increasing platelet count.

Conclusions: Admission platelet count was inversely correlated with 24-hour mortality and transfusion of PRBCs. A normal platelet count may be insufficient after severe trauma, and as a result, these patients may benefit from a lower platelet transfusion threshold. Future studies of platelet number and function after injury are needed.

Key Words: Platelet count, Massive transfusion, Mortality.

Uncontrolled hemorrhage is a major cause of mortality in both civilian and military trauma patients. Only 2% of severely injured trauma patients arrive to the hospital in hemorrhagic shock requiring a massive transfusion, as transfusion of ≥10 units of packed red blood cells (PRBCs) over 24 hours. However, the mortality rate in this subset of trauma patients is ~40%, with half of these deaths occurring within the first 24 hours. Several studies indicate that many of these traumatic deaths caused by hemorrhage are preventable, and therefore a significant amount of research has been done to investigate resuscitation strategies and other therapeutic measures, which may lead to improved survival in these patients. Most of these studies have focused exclusively on determining the optimal ratio of fresh frozen plasma (FFP) to PRBCs needed to prevent and reverse the coagulopathy of trauma. However, one landmark study examined platelet:PRBC ratio in addition to FFP:PRBC ratio and demonstrated that patients who received an FFP:PRBC ratio ≥1:2 in addition to a platelet:PRBC ratio ≥1:2 had the greatest overall survival, compared with patients who received lower ratios. Platelets serve two critical functions of the coagulation process. Platelet adhesion and aggregation at the site of endothelial injury forms a hemostatic plug, and platelets enhance activation of coagulation proteases leading to thrombus formation. Despite these important roles, there are very few studies investigating the effects of platelet function in severely injured trauma patients and even fewer studies investigating the effect of platelet count on trauma outcomes. We investigated the effect of admission platelet count on mortality and the number of units of PRBCs transfused in a cohort of massively transfused civilian trauma patients. In addition, we examined whether platelet count is associated with injury severity and coagulopathy at admission.

METHODS

The cohort for this study included 389 massively transfused (≥10 units of PRBCs within the first 24 hours of admission) trauma patients. These patients are a subset of patients from an Institutional Review Board approved, retrospective, multicenter study that included adult trauma patients who arrived from the scene and received at least one unit of PRBCs in the emergency department (ED). The multicenter trial included patients from 16 Level I trauma centers between July 2005 and June 2006.

The primary outcome of this study was mortality. We explored mortality at two time points, i.e., 6 hours and 24 hours.
Platelet Number and Mortality

Brown L, Cohen M
J Trauma 2011
The clinical significance of platelet counts in the first 24 hours after severe injury

Lynn G. Stansbury, Aaron S. Hess, Kwaku Thompson, Betsy Kramer, Thomas M. Scalea, and John R. Hess

BACKGROUND: Admission platelet (PLT) counts are known to be associated with all-cause mortality for seriously injured patients admitted to a trauma center. The course of subsequent PLT counts, their implications, and the effects of PLT therapy are less well known.

STUDY DESIGN AND METHODS: Trauma center patients who were directly admitted from the scene of injury, received 1 or more units of unmatched red blood cells in the first hour of care, survived for at least 15 minutes, and had a PLT count measured in the first hour were analyzed for the association of their admission and subsequent PLT counts in the first 24 hours with injury severity and hemorrhagic and central nervous system (CNS) causes of in-hospital mortality.

RESULTS: Over an 8.25-year period, 1292 of 45,849 direct trauma admissions met entry criteria. Admission PLT counts averaged $228 \times 10^3 = 90 \times 10^9/L$ and decreased by $104 \times 10^9/L$ by the second hour and $1 \times 10^9/L$ each hour thereafter. The admission count was not related to time to admission. Each 1-point increase in the injury severity score was associated with a $1 \times 10^9/L$ decrease in the PLT count at all times in the first 24 hours of care. Admission PLT counts were strongly associated with hemorrhagic and CNS injury mortality and subsequent PLT counts. Effects of PLT therapy could not be ascertained.

DISCUSSION: Admission PLT counts in critically injured trauma patients are usually normal, decreasing after admission. Low PLT counts at admission and during the course of trauma care are strongly associated with mortality.

Physical injury is the major cause of death for individuals between 1 and 44 years of age, which makes injury the most common cause of loss of years of productive life. Central nervous system (CNS) injury is the primary factor in 52% of these deaths and uncontrolled hemorrhage causes another 30% of primary admission mortality when the injured are cared for in an advanced trauma system. Reducing this death toll is a major public health and medical goal.

Blood platelets (PLTs) are biologically important for the maintenance of vascular integrity and hemostasis after vascular injury. In trauma patients, the admission PLT count has been shown to be a predictor of all-cause mortality, and PLT transfusion has been associated with improved survival of massively transfused patients. The importance of subsequent PLT counts and their trends over the first 24 hours of care are less clearly described. Improved outcome after PLT therapy for both hemorrhage and brain injury is suggested in published retrospective studies.

We collected all PLT counts obtained over the first 24 hours of care from a cohort of trauma patients admitted directly from the scene of injury to a major trauma center over an 8.25-year period who met the additional criteria of having survived at least 15 minutes and received at least 1 unit of unmatched red blood cells (RBCs) in the

ABBREVIATIONS: CNS = central nervous system; ISS(i) = injury severity score(s); TRISS = Trauma Related Injury Severity Score.

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TABLE 3. OR of dying when the admission PLT count is in one of six ranges

<table>
<thead>
<tr>
<th>Admission PLT count group (count x10^9/L)</th>
<th>Relative mortality unadjusted</th>
<th>Mortality adjusted for TRISS</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;400</td>
<td>0.8 (0.3-2.1)</td>
<td>1.0 (0.4-3.0)</td>
</tr>
<tr>
<td>250-400</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>150-250</td>
<td>2.4 (1.8-3.2)</td>
<td>1.9 (1.4-2.7)</td>
</tr>
<tr>
<td>100-150</td>
<td>5.5 (3.6-8.3)</td>
<td>2.7 (1.6-4.7)</td>
</tr>
<tr>
<td>50-100</td>
<td>9.9 (5.6-17.7)</td>
<td>4.2 (2.0-8.8)</td>
</tr>
<tr>
<td>0-50</td>
<td>18.7 (7.5-46.9)</td>
<td>8.2 (2.7-25.3)</td>
</tr>
</tbody>
</table>

* Data are reported as OR (95% CI). For each analysis, the group with their admission PLT count in the upper half of the normal range 250 × 10^9 to 400 × 10^9/L served as the reference group.
Benefits of platelet transfusions.

- Recent trends in hemostatic resuscitation ratios have revolutionized care of the critically injured.

- Appropriate FFP:RBC ratios are commonly a focus of therapy and research.
  - Intuitive mechanism
  - Highly evolved research techniques

- Higher platelet:RBC ratios show a similar survival advantage.
  - Less clear explanation
  - Difficult to study
  - Rare in clinical practice

Increased Plasma and Platelet to Red Blood Cell Ratios Improves Outcome in 466 Massively Transfused Civilian Trauma Patients

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Increased Platelet:RBC Ratios Are Associated With Improved Survival After Massive Transfusion

John B. Holcomb, MD, FACS, Lee A. Zarzabal, MS, Joel E. Michalek, PhD, Rosemary A. Kozar, MD, PhD, Phillip C. Spinella, MD, FCCM, Jeremy G. Perkins, MD, Nena Matijevic, PhD, Jing-Fei Dong, MD, PhD, Shibani Pati, MD, PhD, Charles E. Wade, PhD, and the Trauma Outcomes Group

The Journal of TRAUMA® Injury, Infection, and Critical Care • Volume 71, Number 2, August Supplement 3, 2011
The Prospective, Observational, Multicenter, Major Trauma Transfusion (PROMMOTT) Study

Comparative Effectiveness of a Time-Varying Treatment With Competing Risks

John B. Holcomb, MD; Deborah J. del Junco, PhD; Erin E. Fox, PhD; Charles E. Wade, PhD; Mitchell J. Cohen, MD; Martin A. Schreiber, MD; Louis H. Alarcon, MD; Yu Bai, MD, PhD; Karen J. Brasel, MD, MPH; Eileen M. Bulger, MD; Bryan A. Cotton, MD, MPH; Nena Matijevic, PhD; Peter Muskat, MD; John G. Myers, MD; Herb A. Phelan, MD, MSc; Christopher E. White, MD; Jiajie Zhang, PhD; Mohammad H. Rahbar, PhD; for the PROMMOTT Study Group

Objective: To relate in-hospital mortality to early transfusion of plasma and/or platelets and to time-varying plasma:red blood cell (RBC) and platelet:RBC ratios.

Design: Prospective cohort study documenting the timing of transfusions during active resuscitation and patient outcomes. Data were analyzed using time-dependent proportional hazards models.

Setting: Ten US level I trauma centers.

Patients: Adult trauma patients surviving for 30 minutes after admission who received a transfusion of at least 1 unit of RBCs within 6 hours of admission (n=1245, the original study group) and at least 3 total units (of RBCs, plasma, or platelets) within 24 hours (n=905, the analysis group).

Main Outcome Measure: In-hospital mortality.

Results: Plasma:RBC and platelet:RBC ratios were not constant during the first 24 hours (P<.001 for both).

In a multivariable time-dependent Cox model, increased ratios of plasma:RBCs (adjusted hazard ratio=0.31; 95% CI, 0.16-0.58) and platelets:RBCs (adjusted hazard ratio=0.55; 95% CI, 0.31-0.98) were independently associated with decreased 6-hour mortality, when hemorrhagic death predominated. In the first 6 hours, patients with ratios less than 1:2 were 3 to 4 times more likely to die than patients with ratios of 1:1 or higher. After 24 hours, plasma and platelet ratios were unassociated with mortality, when competing risks from non-hemorrhagic causes prevailed.

Conclusions: Higher plasma and platelet ratios early in resuscitation were associated with decreased mortality in patients who received transfusions of at least 3 units of blood products during the first 24 hours after admission. Among survivors at 24 hours, the subsequent risk of death by day 30 was not associated with plasma or platelet ratios.

PROMMTTT showed benefit from early platelet transfusion despite poor and slow platelet use.
Distribution of Plasma:RBC ratios over time

Percent

Plasma:RBC ratio 1:2

Time

1 hr

30 min

2 hrs

6 hrs
Platelet function in trauma

- Platelet function is impaired after trauma
  - Flow cytometry for activation markers
  - Platelet microparticle analysis
  - Light aggregometry

Platelet function assays

- Platelet count
- Bleeding time
- Single platelet counting
- Flow cytometry
- Aggregometry
Background: Platelet function assays

- Resting platelets are non-thrombogenic
- Activation leads to aggregation and adhesion

- Light aggregometry
  - Measures occlusion of an aperture leading to decreased light transmission by platelet aggregates

- Impedance aggregometry
  - Measures increase in continuous electrical impedance between charged wires as platelets adhere to charged surface

Characterization of platelet dysfunction after trauma

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- Prospective collection of citrated whole blood from critically-injured trauma patients on arrival and throughout ICU stay
- Point-of-care measurement of platelet activation using Multiplate® multiple electrode aggregometry
- Matching to parallel standard laboratory values
- Link with resuscitation and outcomes data
Impedance aggregometry
Impedance aggregometry
Impedance aggregometry
Platelet agonists

Patient demographics

- 101 critically-injured patients
- 376 individual samples
- Normal mean platelet count
- No admission thrombocytopenia

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Age</td>
<td>41.3 ± 19.3</td>
</tr>
<tr>
<td>BMI</td>
<td>26.2 ± 5.4</td>
</tr>
<tr>
<td>Blunt injury</td>
<td>69.0%</td>
</tr>
<tr>
<td>ISS</td>
<td>23.9 ± 14.8</td>
</tr>
<tr>
<td>Base deficit</td>
<td>-5.3 ± 6.0</td>
</tr>
<tr>
<td>GCS</td>
<td>9 (5-15)</td>
</tr>
<tr>
<td>Temperature</td>
<td>35.8 ± 0.8</td>
</tr>
<tr>
<td>Prehospital IVF</td>
<td>250 (50-1000)</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>39.6 ± 5.4</td>
</tr>
<tr>
<td>Platelet count</td>
<td>274.4 ± 85.4</td>
</tr>
<tr>
<td>INR</td>
<td>1.1 (1.1-1.3)</td>
</tr>
<tr>
<td>PTT</td>
<td>27.5 (25.4-31.5)</td>
</tr>
<tr>
<td>pH</td>
<td>7.28 ± 0.17</td>
</tr>
<tr>
<td>Base deficit</td>
<td>-5.3 ± 6.0</td>
</tr>
</tbody>
</table>
Platelet function on admission

- Mean admission platelet function within normal range

<table>
<thead>
<tr>
<th>Admission values (N=78)</th>
<th>Normal range</th>
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<tbody>
<tr>
<td>ADP</td>
<td>44.6 ± 20.4</td>
</tr>
<tr>
<td>TRAP</td>
<td>86.6 ± 27.0</td>
</tr>
<tr>
<td>AA</td>
<td>44.3 ± 28.3</td>
</tr>
<tr>
<td>Collagen</td>
<td>44.7 ± 19.7</td>
</tr>
</tbody>
</table>

- Using manufacturer normal ranges:
  - 45.5% had admission platelet hypofunction to at least one measured agonist
  - 91.1% had platelet hypofunction at some time during ICU stay
Platelet function vs. platelet count

A

ADP

R²=0.514

B

TRAP

R²=0.417

C

AA

R²=0.504

D

Collagen

R²=0.427
Platelet function over time

A. ADP

B. TRAP

C. AA

D. Collagen

E. Platelet count

Platelet count
Multivariate predictors of platelet hypofunction

<table>
<thead>
<tr>
<th>Predictor</th>
<th>OR</th>
<th>P</th>
<th>95% confidence interval</th>
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</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.041</td>
<td>0.032</td>
<td>(1.003 – 1.081)</td>
</tr>
<tr>
<td>Base deficit</td>
<td>0.871</td>
<td>0.026</td>
<td>(0.772 – 0.983)</td>
</tr>
<tr>
<td>GCS</td>
<td>0.833</td>
<td>0.010</td>
<td>(0.726 - 0.957)</td>
</tr>
<tr>
<td>Platelet count</td>
<td>0.994</td>
<td>0.150</td>
<td>(0.987 – 1.002)</td>
</tr>
</tbody>
</table>
Platelet hypofunction: Outcomes

<table>
<thead>
<tr>
<th></th>
<th>Platelet hypofunction (N=39)</th>
<th>Normal function (N=52)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital LOS</td>
<td>6 (2-27)</td>
<td>10 (6.5-20)</td>
<td>0.090</td>
</tr>
<tr>
<td>ICU LOS</td>
<td>3.5 (1-14)</td>
<td>3 (2-14)</td>
<td>0.436</td>
</tr>
<tr>
<td>Vent-free days</td>
<td>12 (0-26)</td>
<td>26 (7.5-27)</td>
<td>0.040</td>
</tr>
<tr>
<td>24h mortality</td>
<td>20.0%</td>
<td>2.1%</td>
<td>0.009</td>
</tr>
</tbody>
</table>
Platelet hypofunction associations with mortality
Platelet hypofunction predicts mortality

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th>P</th>
<th>Multivariate</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>ADP</td>
<td>0.958</td>
<td>0.007</td>
<td>0.974</td>
<td>0.109</td>
</tr>
<tr>
<td>TRAP</td>
<td>0.976</td>
<td>0.023</td>
<td>0.981</td>
<td>0.047</td>
</tr>
<tr>
<td>AA</td>
<td>0.969</td>
<td>0.004</td>
<td>0.964</td>
<td>0.018</td>
</tr>
<tr>
<td>Collagen</td>
<td>0.944</td>
<td>0.001</td>
<td>0.954</td>
<td>0.026</td>
</tr>
</tbody>
</table>

- Adjusted for age, GCS, base deficit, and platelet count
Platelet hypofunction predicts mortality

A. ADP
   AUC=0.662

B. TRAP
   AUC=0.579

C. AA
   AUC=0.769*

D. Collagen
   AUC=0.717*

E. Platelet count
   AUC=0.634

Area under ROC curve = 0.3381
Area under ROC curve = 0.2313
Area under ROC curve = 0.4213
Area under ROC curve = 0.2831
Area under ROC curve = 0.3665
Area under ROC curve = 0.769
Area under ROC curve = 0.717
Area under ROC curve = 0.662
Area under ROC curve = 0.579
Area under ROC curve = 0.634
Summary

- Platelet dysfunction exists after traumatic injury, and is not identifiable by standard laboratory measures.
  - 45% of critically injured patients have platelet hypofunction on arrival despite normal platelet counts
  - Older age, lower base deficit, and lower GCS are independent risk factors for platelet hypofunction

- Platelet dysfunction after trauma correlates with poor outcomes.
  - Mortality in patients with platelet hypofunction is nearly 10-fold higher than those with normal platelet function
  - Poor platelet response to AA and collagen on admission are independent predictors of mortality
Early Platelet Dysfunction: An Unrecognized Role in the Acute Coagulopathy of Trauma

Max V Wohlauer, MD, Ernest E Moore, MD, FACS, Scott Thomas, MD, FACS, Angela Sausaia, MD, PhD, Ed Evans, BA, CCP, Jeffrey Harr, MD, MPH, Christopher C Silliman, MD, PhD, Victoria Ploplis, PhD, Francis J Castellino, PhD, Mark Walsh, MD

BACKGROUND: Our aim was to determine the prevalence of platelet dysfunction using an end point of assembly into a stable thrombus after severe injury. Although the current debate on acute traumatic coagulopathy has focused on the consumption or inhibition of coagulation factors, the question of early platelet dysfunction in this setting remains unclear.

STUDY DESIGN: Prospective platelet function in assembly and stability of the thrombus was determined within 30 minutes of injury using whole blood samples from trauma patients at the point of care using thrombelastography-based platelet functional analysis.

RESULTS: There were 51 patients in the study. There were significant differences in the platelet response between trauma patients and healthy volunteers, such that there was impaired aggregation to these agonists. In trauma patients, the median ADP inhibition of platelet function was 86.1% (interquartile range [IQR] 38.6% to 97.7%) compared with 4.2% (IQR 0 to 18.2%) in healthy volunteers. After trauma, the impairment of platelet function in response to arachidonic acid was 44.9% (IQR 26.6% to 59.3%) compared with 0.5% (IQR 0 to 3.0%) in volunteers (Wilcoxon nonparametric test, p < 0.0001 for both tests).

CONCLUSIONS: In this study, we show that platelet dysfunction is manifest after major trauma and before substantial fluid or blood administration. These data suggest a potential role for early platelet transfusion in severely injured patients at risk for postinjury coagulopathy. (J Am Coll Surg 2012;214:739–746. © 2012 by the American College of Surgeons)

Hemorrhage remains the leading cause of preventable death after trauma, and 25% of severely injured patients manifest evidence of coagulopathy on arrival to the emergency department (ED). Although the current debate on acute traumatic coagulopathy (ATC) has focused on disseminated intravascular coagulation vs an acute endogenous coagulopathy mediated by activated protein C, the question of early platelet dysfunction remains obscure. The thrombocyte is particularly suspect in the context of the cell-based model of hemostasis, which highlights the critical interaction between the platelet, endothelium, and plasma factors during hemostasis. In spite of its importance, early recognition of platelet dysfunction is challenging, as conventional plasma-based tests (e.g., aPTT, international normalized ratio [INR]) are unable to determine platelet function and are insensitive to coagulopathy unless severely deranged. Although the complete blood count with differential provides a platelet count, this quantitative test does not provide an assessment of platelet function.

Recently, point-of-care viscoelastic analyzers, including modified thrombelastography (TEG) with platelet mapping, have become available to rapidly identify and manage high-risk patients in the trauma bay. These same approaches can be used to measure platelet function at the bedside. For example, identifying ADP receptor inhibition >60% in patients on antiplatelet medications identifies those at risk for developing bleeding complications.
Figure 2. Median percent ADP receptor inhibition in trauma patients compared with healthy volunteers, including stratification according to shock (base deficit [BD]), blood transfusion (RBCs), and tissue injury (Injury Severity Score [ISS]).

Figure 3. Median percent arachidonic acid (AA) (TXA2) receptor inhibition in trauma patients compared with healthy volunteers, including stratification according to shock (base deficit [BD]), blood transfusion (RBCs), and tissue injury (Injury Severity Score [ISS]).
Summary

- Clinically significant platelet dysfunction exists and carries a grave prognosis, but remains invisible to treating clinicians

- Impedance aggregometry reliably identifies this platelet dysfunction in injured patients

- Rapid, point-of-care platelet function testing will lead to better targeted therapy and better outcomes after trauma
That’s great but what should we give?
Storage Lesion of Apheresis Platelets?
Cold platelets?

• Current blood-banking procedures call for apheresis derived platelets to be stored at 22°C for 5 days.
• The only measurements controlled for platelets are pH and sterility.
• These procedures are mainly derived from research done in the 1970’s, focusing on hemostatic functionality and circulation survival.
• Consequently, storage at 4°C had been eliminated due to elevated clearance after transfusion.
HEMOSTATIC FUNCTION OF APERHEISIS PLATELETS STORED AT 4°C AND 22°C

Kristin M. Reddick, Heather F. Piccoke, Robbie K. Montgomery, Chrisselda G. Fedyk, James K. Aden, Anand K. Ramasubramanian, and Andrew P. Cap

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ABSTRACT—Introduction: Platelet refrigeration decreases the risk of bacterial contamination and may preserve function better than standard-of-care room temperature (RT) storage. Benefits could include lower transfusion-related complications, decreased costs, improved hemostasis in acutely bleeding patients, and extended shelf life. In this study, we compared the effects of 22°C and 4°C storage on the functional and activation status of apheresis platelets. Methods: Apheresis platelets (n = 5 per group) were stored for 5 days at 22°C with agitation (RT) versus at 4°C with agitation (4°C + AG) and without (4°C). Measurements included platelet counts, blood gas analyses, aggregation responses, thromboelastography, thromboxane B2 and soluble CD40 ligand release, activation markers, and microplate formation. Results: Sample pH levels were within acceptable limits for storage products (pH 6.2–7.4). Platelet glucose metabolism (P < 0.05), aggregation response (adenosine diphosphate: RT 0; 4°C + AG 5.0 ± 0.8; 4°C 5.6 ± 0.9; P < 0.05), and clot strength (maximum amplitude: RT 58 ± 2; 4°C + AG 63 ± 2; 4°C 67 ± 2; P < 0.05) were better preserved at 4°C compared with RT storage. Refrigerated samples were more activated compared with RT (P < 0.05), although thromboxane B2 (P < 0.05) and soluble CD40 ligand release (P < 0.05) were higher at RT. Agitation did not improve the quality of 4°C-stored samples. Conclusions: Apheresis platelets stored at 4°C maintain more viable metabolic characteristics, are hemostatically more effective, and release fewer proinflammatory mediators than apheresis platelets stored at RT over 5 days. Given the superior bacteriologic safety of refrigerated products, these data suggest that cold-stored platelets may improve outcomes for acutely bleeding patients.

KEYWORDS—Hemostasis, coagulation, aggregation, clot strength, cold storage, activation

ABBREVIATIONS—RT — room temperature; 4°C — storage at 4°C; 4°C + AG — storage at 4°C with gentle agitation; AP — apheresis platelets; ADP — adenosine diphosphate; TRAP — thrombin receptor activating peptide; CD60P — P-selectin; CD40L — CD40 ligand; sCD40L — soluble CD40 ligand; CD40 — glycoprotein lb receptor; TXB2 — thromboxane B2; RBC — red blood cell; MPV — mean platelet volume; MPC — mean platelet component; TEG — Thromboelastography; AUC — area under the curve; R — reaction time (time to initial fibrin formation); K — time to clot formation; a angle — rate of clot formation; MA — maximum amplitude (clot strength)

INTRODUCTION

Current blood-banking recommendations are that platelets be stored in incubators at 22°C, with gentle agitation for no longer than 5 days (1). This limited shelf life is necessary because of the risk of bacterial contamination that leads to life-threatening transfusion-related infections (2-4). These storage practices also result in a platelet storage lesion that is associated with a decline in platelet hemostatic function (5).

Cold storage at 4°C could prolong shelf life by diminishing the risk of bacterial sepsis, decreasing platelet metabolism, and maintaining functionally. Transportation of 4°C platelets would be convenient because the infrastructure for other refrigerated blood products, such as red blood cells (RBCs), is already in place (6). Cold platelets may be a better hemostatic product. Several in vitro studies from the 1970s showed that refrigeration of platelets results in better metabolic and functional responses such as minimal lactate accumulation, better aggregation response, and adhesion to subendothelium (7-9). In vivo human studies have shown that 4°C platelets function better than room temperature (RT) platelets in reducing the bleeding times in thrombocytopenic patients, aspirin-treated volunteers, and in aplastic thrombocytopenic patients shortly after transfusion (10-12).

Despite promising in vitro and in vivo studies in controlling acute bleeding, the practice of cold storage for transfusion was abandoned during the 1970s because of the belief that clinically effective platelets should be both hemostatically functional and survive in circulation for several days as indicated for prophylactic transfusion. It was shown by Murphy and Gardner (13) that platelets stored for up to 18 h in cold (2°C–4°C) show decreased recovery and survival upon transfusion compared with their RT (22°C–24°C) counterparts; i.e., the life span (t½) of cold- and RT-stored platelets is 1.3 and 3.9 days, respectively. Consequently, when transfused to thrombocytopenic patients, cold-stored platelets are as effective as
Platelets and/or whole blood?
Generating whole blood and reconstituted whole blood.

- WB variants (11 of each)
  - Room temperature WB
  - Cooled WB

- Modified room temperature WB
  - Modified cooled WB

- RWB (23 of each)
  - 1:1:1 (RBC:FFP:PLTS)
  - 2:1:1 (RBC:FFP:PLTS)
1:1:1 RWB

2:1:1 RWB
Laboratory methods

- Rotational thromboelastometry (ROTEM)
- Standard coagulation measures
- CBC
- Fibrinogen, factors II, V, VII, VIII, IX, X, ATIII, and protein C

Statistical analysis:
- Student’s $t$ test/ANOVA (normal)
- Wilcoxon rank sum/Kruskal Wallis (skewed)
- Fisher’s exact test for proportions (proportions)
Comparisons of resuscitation products.

- Comparison of 1:1:1 to 2:1:1 RWB variants
- Comparison of 4 types of whole blood
  - cooled, room temp, modified cooled, modified room temp
- Comparison of modified WB to 1:1:1
Comparisons of resuscitation products.

- Comparison of 1:1:1 to 2:1:1 RWB variants

- Comparison of 4 types of whole blood
  - cooled, room temp, modified cooled, modified room temp

- Comparison of modified WB to 1:1:1
## CBC

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- **ROOM TEMP**
- **COOLED**
- **MODIFIED, COOLED**
- **MODIFIED, ROOM TEMP**

* * *
### Factors

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Results

- Comparison of 1:1:1 to 2:1:1 RWB variants
- Comparison of 4 types of whole blood
- Comparison of modified WB to 1:1:1
**CBC**

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<td><strong>WBC (x10^3/µL)</strong></td>
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<td>0.01</td>
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<tr>
<td><strong>Hgb (g/dL)</strong></td>
<td>10.60</td>
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<td><strong>Hct (%)</strong></td>
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<td><strong>Plts (x10^9/L)</strong></td>
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*Modified WB

*1:1:1

* * *
### Factors

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<th>MODIFIED WB</th>
<th>1:1:1</th>
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EXTEM modified vs. 1:1:1 RWB

- **Modified WB**
- **1:1:1**

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<td>a10 (mm)</td>
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<td>MCF (mm)</td>
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Conclusions

- First characterization study
- $1:1:1 > 2:1:1$
- modified WB $> \text{non-modified}$
- modified WB $> 1:1:1$
So now that we know there is platelet dysfunction after trauma, how do we study it?
Putative Function

- Platelets must circulate without adhesion and activation while in constant contact with an undamaged endothelium, activate and stick at the site of injury form an adhesive plug until no longer needed and dissipate without further downstream or systemic damage.
Steps to Platelet Action

• Initial capture, activation and adhesion to damaged vessel wall.
• Recruitment of additional platelets to the forming plug via generation of soluble platelet agonists and $\alpha_{\text{IIb}}\beta_3$ platelet-platelet interactions
• Stabilization of platelet plug
Steps to Platelet Function

• Initial capture
  • Binding of GPIb–IX–V complex on platelet surface to VWF and binding of $\alpha_{\text{IIb}}\beta_3$ to VWF.

• Activation
  – Collagen GPVI promotes engagement of integrin $\alpha_2\beta_1$ to form platelet monolayer
  – Activation also through local release of agonists to G protein coupled receptors on platelet surface including $T_\times A_2$ and ADP.
  – This forms a local environment for thrombin activation and a dense hemostatic plug.
Steps to Platelet Function

• Activation
  – Collagen GPVI promotes engagement of integrin $\alpha_2\beta_1$ to form platelet monolayer
  – Activation also through local release of agonists to G protein coupled receptors on platelet surface including $\mathrm{TxA}_2$ and ADP.
  – This forms a local environment for thrombin activation and a dense hemostatic plug.
  – $\alpha_{\text{IIb}}\beta_3$ binds fibrinogen and plasma protein
  – This is different in different types of injury and different vessels!
Intracellular Signaling

• PLC activation produces IP$_3$ to raise CA$^{++}$ leading to $\alpha_{IIb}\beta_3$
Potential Mechanisms

Actual platelet dysfunction via binding, activation, platelet-platelet interactions, G protein receptor signaling (including but not limited to G-G interactions, RGS proteins), adhesion ligand receptors (numerous), the local environment, changing flow and shear, and the remainder of the coagulant milieu.
How should this be studied?
Hierarchical organization in the hemostatic response and its relationship to the platelet-signaling network

Timothy J. Stalker, Elizabeth A. Traxler, Jie Wu, Kenneth M. Wannemacher, Samantha L. Cermignano, Roman Voronov, Scott L. Diamond, and Lawrence F. Brass

Departments of Medicine and Pharmacology and the Department of Chemical and Biomolecular Engineering, University of Pennsylvania, Philadelphia, PA

Key Points

- Hemostatic plugs develop a regional architecture defined by the extent of platelet activation and packing density.
- The regional composition of a hemostatic plug is due to the interaction of local conditions with the platelet-signaling network.

Achieving hemostasis following vascular injury requires the rapid accumulation of platelets and fibrin. Here we used a combination of confocal intravital imaging, genetically engineered mice, and antiplatelet agents to determine how variations in the extent of platelet activation following vascular injury arise from the integration of different elements of the platelet-signaling network. Two forms of penetrating injury were used to evoke the hemostatic response. Both produced a hierarchically organized structure in which a core of fully activated platelets was overlaid with an unstable shell of less-activated platelets. This structure emerged as hemostasis was achieved and persisted for at least 60 minutes following injury, its organization at least partly reflecting agonist concentration gradients. Thrombin activity and fibrin formation were found primarily in the innermost core. As proposed previously, greater packing density in the core facilitated contact-dependent signaling and limited entry of plasma-borne molecules visualized with fluorophores coupled to dextran and albumin. Blocking contact-dependent signaling or inhibiting thrombin reduced the size of the core, while the shell was heavily influenced by adenosine 5’-diphosphate and regulators of G12-mediated signaling. Thus, the hemostatic response is shown to produce a hierarchical structure arising, in part, from distinct elements of the platelet-signaling network. (Blood. 2013;121(10):1875-1885)
Take home points...

• There is platelet dysfunction after trauma.
• Platelet transfusions early show a large mortality benefit.
• Platelet transfusions late are associated with organ failure (ARDS etc)
• There is considerable variability in platelet function.
• It is hard to define function and even more difficult to know which mechanisms to study.
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  - Xiaoming Yao

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- Byron Miyazawa
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