Cold-Stored Platelets

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Objectives

1. Understand the history of cold-stored platelets (CSP)
2. Understand why CSP are coming back into favor
3. Understand current recommendations for CSP

Outline

- History of cold stored platelets
- Current Practice
- Platelet Physiology
- Pros and Cons
- Logistics
- Future

History

History

- In the 1960s, platelets began to be transfused separate from whole blood
- Before 1969, all platelets were stored at 4^{o}C
- Units had to be transfused within a few hours of donation

History

- In the late 1960s/early 1970s, several studies compared CSP to room temperature platelets (RTP)
  - Small doses of platelets were labeled with radioactive chromium and transfused to healthy volunteers
  - Showed that RTP had better post-transfusion recovery and survival

History

- Suggestion was to keep a dual inventory of RTP and CSP
- Abandoned by the end of the 1970s
  - Logistical challenges
  - Most transfusions were for prophylaxis of thrombocytopenia, which RTP were better for

Today

Today

- Transfusion transmitted infections
  - Bacteria are detected in about 1 in 5,000 platelet units
  - Sepsis occurs in about 1 in 100,000 transfusions
  - Between 1 in 500,000 to 1,000,000 transfusions are fatal (2 to 4 deaths per year)

Today

- Several years ago, the FDA changed recommendations
  - Wait longer after someone donates and use a larger sample for testing, and/or
  - Use a rapid test right before releasing the platelet unit, and/or
  - Use a process that reduces the number of bacteria, i.e. pathogen reduction

Today

- Unfortunately, we continue to still have issues
  - Frequent platelet shortages
  - Wastage due to expiration (32%)
  - Access to platelets in remote and outpatient settings

Today

- Issues on the donation side
  - National donor population decreasing
  - Less young people are donating
  - More plasma centers → reduces the donor pool
  - COVID disruptions
    - Eliminated blood drives
    - Limited strategies to identify potential blood donors

Today

- Platelet transfusions are generally categorized into two groups:

Prophylactic

- Primarily chemotherapy patients
- Need maximum survival time of platelets
  - Increased intervals between transfusion
  - Less risk of alloimmunization
- 67% of transfusions
  - Decreasing due to lowered transfusion thresholds

Therapeutic

- Active bleeding
- Need platelets to activate rapidly
  - Quickly clot
- 33%
  - Increasing due to increase in traumas

Platelet Physiology

Platelet Physiology

- Platelets are small, anucleated cells that are released from megakaryocytes in the bone marrow and lungs
- 150 to 400 $\times 10^9/\mu L$
- Circulation lifespan of 7 to 10 days

Platelet Physiology

- Circulate in a quiescent state toward the edges of blood vessels
  - Ideally placed to respond rapidly to vessel damage
- Following damage to the vessel wall, platelets are captured from the circulation and adhere to the extracellular matrix
  - Become activated
  - Release their granule contents
  - Aggregate → platelet plug

Platelet Physiology

- Decline in function during storage
  - "Platelet storage lesion (PSL)"
- Manufacturing exposes them to stresses
  - Centrifugation
  - Manipulation
  - Suspension in chemical storage medium
  - Exposure to foreign surfaces (e.g. plastic of blood bags)
  - Loss of protection that is conferred by the endothelium when in the circulation
- All of these stressors can cause physiological responses that resemble platelet activation

Table I. Commonly used assays to measure the PSL in RTP.

Type	Assay	Purpose	Effect of PSL on RTP
Morphological	Visual inspection of swirling	Measures disk to sphere shape change in platelets. Discoid platelets when rocked gently against a light source scatter light in different directions, causing the phenomenon known as 'swirling.'	Lack of swirling [29,35]
	Platelet morphology score	Visual assessment of platelet morphology using phase-contrast microscopy. Quantified using scoring system such as Kunicki Morphology Score	Platelets lose their discoid shape [12,36,37]
Functional	Platelet aggregation in response to agonists	Measures platelets responsiveness to different agonists, e.g. thrombin, collagen, epinephrine and ADP	Ability to respond to agonists declines [38,39]
	Hypotonic Shock Response	Measures the ability of the platelet to return to its normal shape after hypotonic challenge.	Declines [40,41]
	Extent of Shape Change	Measures the amount of shape change that the platelet undergoes in response to a pre-set dose of ADP	Declines [29,42]
	CD62P/P-selectin surface expression	Monitors platelet degranulation. Flow cytometric assay for platelet activation markers released from alpha granules and subsequently expressed on the surface of platelet	Enhanced exposure [43,44]
	Annexin V binding	Flow cytometric assay using Annexin V to monitor exposure of anionic phospholipids, such as phosphatidyl serine on the platelet membrane	Enhanced exposure (also an indicator of apoptosis) [44,45]
	Soluble CD62P	Measures levels of CD62P shed from the platelet membrane by an ELISA technique	Increases during storage [29,30]
	Thrombin generation	Measures kinetics of thrombin generation in response to tissue factor stimulus. Measured by a calibrated automated thrombogram	Thrombin generation indicators suggest platelets become more procoagulant [46]
Metabolic	Lactate	Measures metabolism of platelets. Lactate is generated by glycolysis	Increases during storage while glucose is available [30,43,47]
	Glucose	Measures metabolism of platelets. Glucose is broken down to pyruvate and lactate by glycolysis	Depletes during storage [43,47]
	pH	pH meter – measures level of acidity in PC	Decreases (increased acidification secondary to glycolysis) [29]
	pO_2 and pCO_2	Measured to ensure that sufficient gas exchange is occurring during storage	While platelets are metabolically active, O_2 declines, CO_2 increases [48]
	Mitochondrial Membrane Potential	Flow cytometric assay. Key indicator of cell health – results are relatable to cells capacity to generate ATP by oxidative phosphorylation	Depolarises and thus decreases as mitochondrial function is impaired [49]
	Extracellular ATP	Measures ATP-dependent oxidation of luciferin	Decreases during storage, suggestive of a deficiency in glycolysis &/or OXPHOS [50]

Platelet Physiology

- Reduced survival of CSP in circulation is caused by…
  - Clustering of GPIbα receptors on the surface of platelets, and
  - Desialylation exposing β-N-acetylglucosamine (β-GlcNAc) moieties
- The exposed β-GlcNAc is recognized by receptors on hepatic macrophages
  - Results in CSP being rapidly cleared

Platelet Physiology

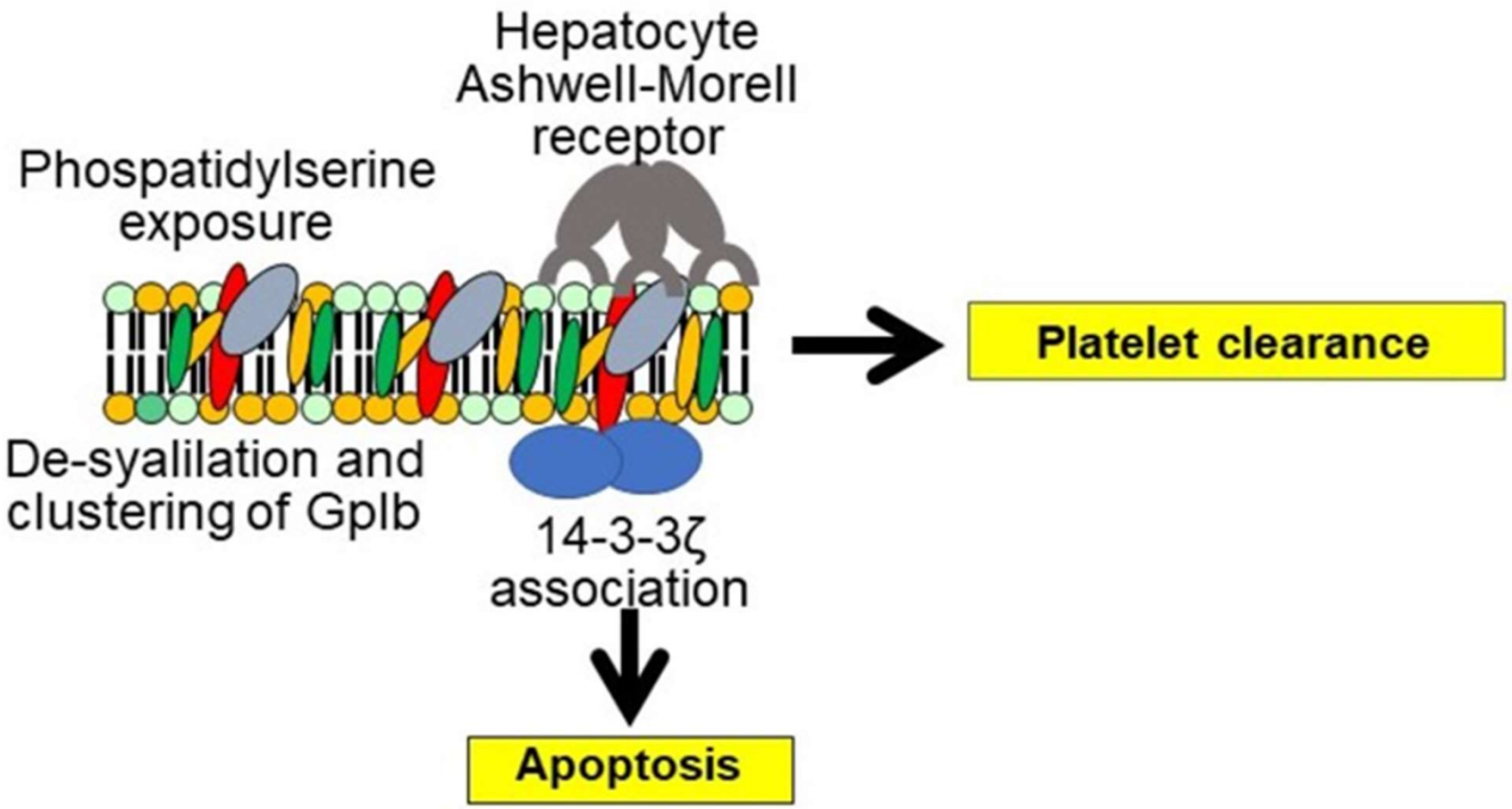


Figure 1. Mechanisms of apoptosis and clearance of human cold platelets upon transfusion

Platelet Physiology

- Significant morphological changes
  - In circulation, non-activated platelets have a thin discoid shape
  - When cold, most of the platelets lose their discord shape and become spherical
    - Loss of swirling and an increase in the mean platelet volume
  - Under the electron microscope, many "bumps" and often possess thin pseudopods extending outward
    - More clotting attachment points

Platelet Physiology

- These changes lead to…
  - Hemostatically primed state
  - Better platelet aggregation
  - Firmer clots

Pros and Cons

Pros and Cons

Cold Stored Platelets (CSP)	Room Temperature Platelets (RTP)
Not conductive to most bacterial growth due to low temperature, therefore reduced risk of transfusion-associated bacterial infection/transfusion-associated sepsis - bacterial monitoring methods not required.	Ideal environment for bacterial growth, leading to increased risk of bacterial contamination and sepsis [68], costly bacterial monitoring/ pathogen inactivation strategies required.
Reduced circulation time – half-life of around 1.3 days* [56] therefore unlikely to be suitable for prophylactic transfusions.	Increased circulation time (half-life of 3.9 days), reduces transfusion frequency for prophylaxis and thus risk of alloimmunization [16].
Dual inventory would be required – CSP for therapeutic transfusions and RTP for prophylactic transfusions, increasing complexity of supply chain	Single inventory for both therapeutic and prophylactic transfusions
Cheaper and easier to store - can be stored with red cell concentrates without agitation, no requirement for separate agitators and incubators.	Logistics of storage difficult - requires constant agitation to maintain gaseous exchange and aerobic respiration [23]. Requires use of bulky & expensive agitators at a regulated temperature of 22°C ± 2°C.
Can be transported in isothermal transport boxes with red cells and other medications to be used in pre-hospital treatment of trauma [16].	Requires separate transport boxes to other components. Logistics make it difficult to use in pre-hospital settings.
Potential to be stored to at least 14 days [69].	Shelf life 5–7 days (depending on bacterial screening protocol) due to platelet storage lesion and bacterial contamination risk.
PC in 100% plasma (current UK apheresis product) known to produce aggregates on cold storage	Minimal aggregate formation in RTP platelets
Significantly reduced accumulation of some pyrogenic cytokines whose presence correlates with the frequency and severity of Febrile Non Haemolytic Transfusion Reactions (FNHTRs) [70].	Higher levels of pro-inflammatory cytokines such as sCD40L and thus increased risk of FNHTRs [71].
Superior haemostatic function* [62].	Haemostatic function rapidly deteriorates throughout storage* [62].
Rapid clearance from the blood could reduce risk of thrombosis [72].	Develop a functional defect during storage which is not corrected until 24 hours post-transfusion* [62].
Preliminary data suggesting that CSP are superior to RTP in reversal of anti-platelet agents [73,74]	

*The evidence in these points was generated prior to introduction of platelet storage packs that allowed gaseous exchange

George C, et. al. Cold stored platelets in the management of bleeding: is it about bioenergetics? Platelets. 2023;34(1). doi:https://doi.org/10.1080/09537104.2023.2188969

Pros and Cons

- CSP
  - Better for active bleeding
    - Undergo changes that make them more likely to form a clot
    - Better aggregation time and response
    - Better clot strength
    - Better adhesion to collagen under physiologic flow
    - Better maintenance of the endothelial structure
    - Reduction of platelet storage lesion
  - Other uses
    - Extensive hemorrhage (e.g. GI bleed, obstetric)
    - Several studies have shown reduced bleeding time by in aspirin-treated healthy volunteers

Pros and Cons

- CSP
  - Reduces the growth of microorganisms → longer shelf life
    - Rural and outpatient clinics
  - Reduce the overall cost for platelets through waste reduction
    - Expiration
    - Returned to blood bank out of temperature
  - Do not require agitation
    - Eliminates cost of mechanical shakers
    - Improves logistics and handling during shipping
    - Use on trauma vehicles, e.g. ambulances, helicopters

Pros and Cons

- RTP
  - Better for thrombocytopenia prophylaxis
    - Increased platelet counts with predictable recovery
    - Improved corrected count increment

Logistics

Logistics

- Transport at 1-10°C
- Storage
  - 1-6°C
  - Came be the same refrigerator as RBCs
  - No need for agitation
  - DO NOT stack/pile platelets
  - Store label side down
    - Facilitate oxygen utilization
    - Maintain optimal morphology, physiologic function, and pH

Logistics

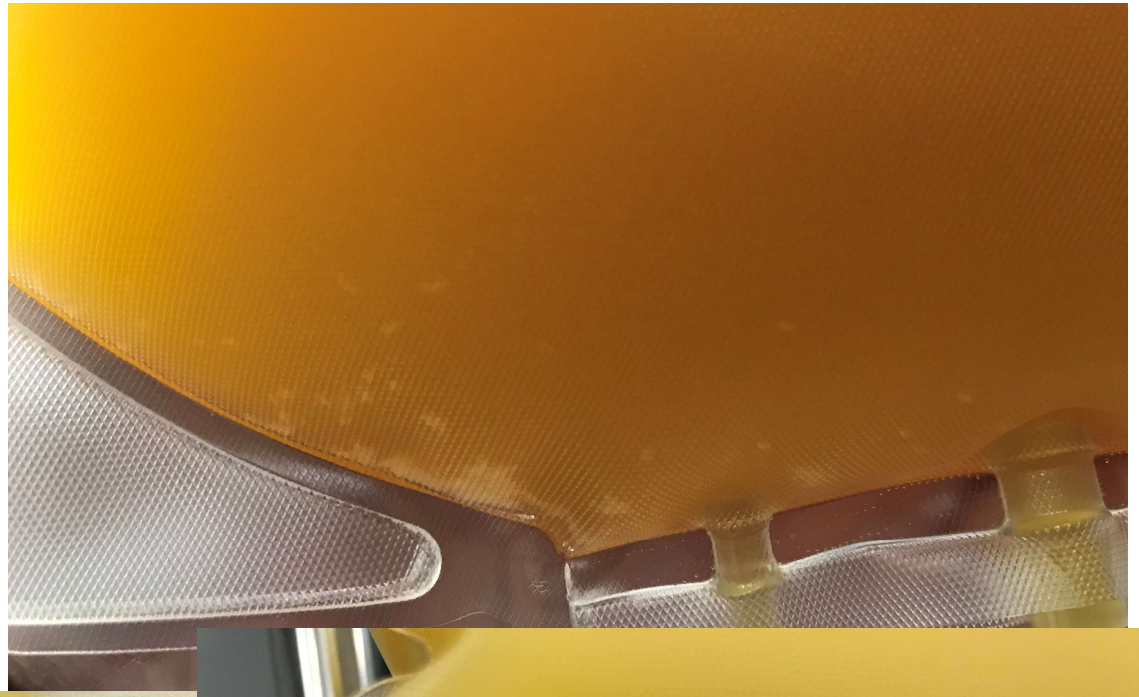
- Volume is the same as RTP
- There will be a label indicating the product is CSP

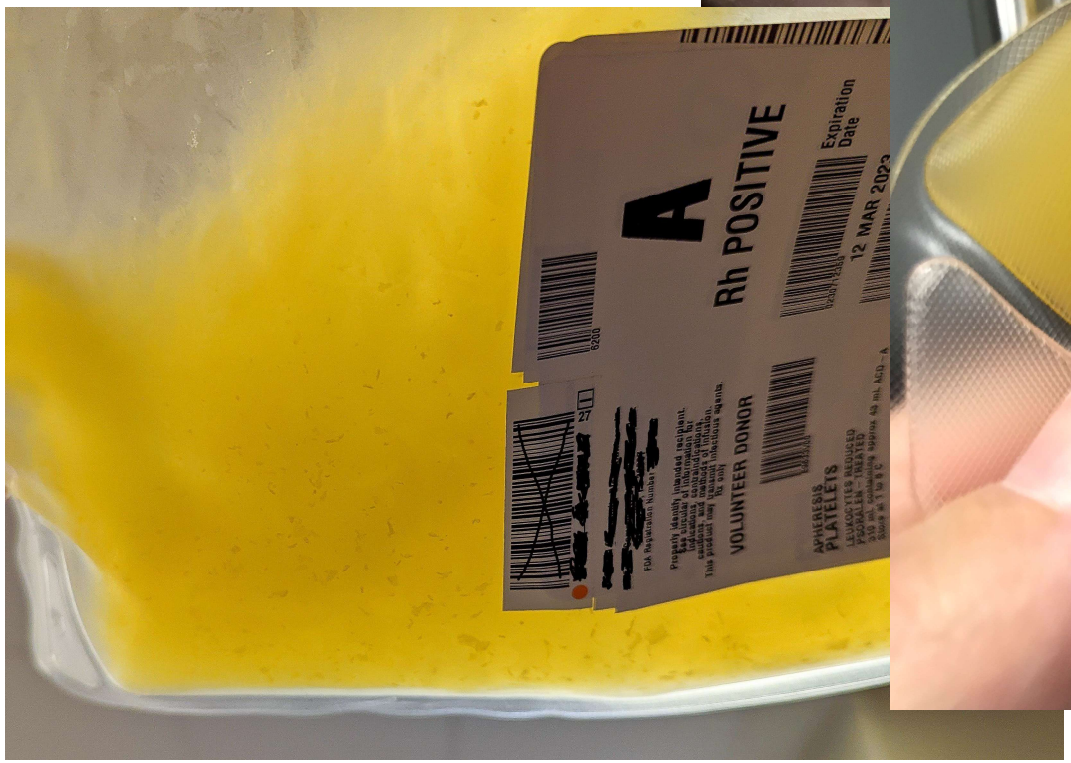
Logistics

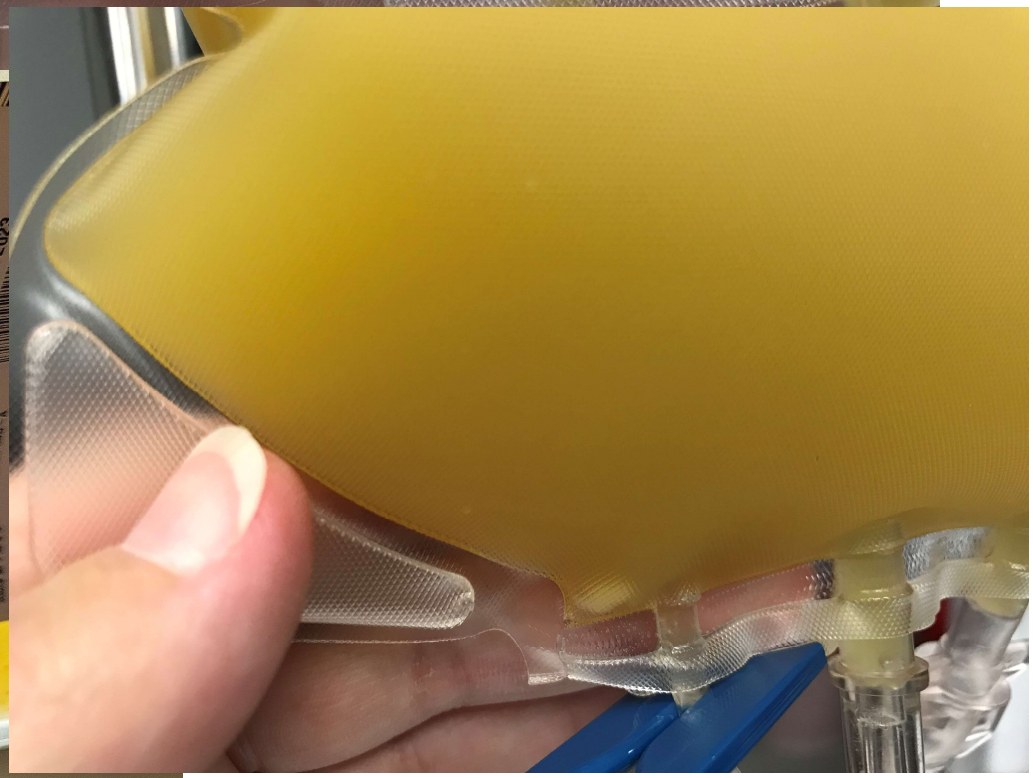
- If aggregates are present, allow product to rest at room temperature for 30 minutes, then gently rock to disperse aggregates
  - Since these are typically needed urgently, it is okay to transfuse with a small amount of aggregates

Warm Platelets Aggregates

Cold Platelets Aggregates







Logistics

- It is normal for CSP not to swirl
- Transfuse the same as an RBC unit
- A blood warmer may be used
- Returning products is at the discretion of your laboratory director
- We will provide you product codes

Logistics

- Have indications
  - FDA: for the treatment of actively bleeding patients through day 14 of storage when conventional platelet products are unavailable, or their use is not practical
  - Management of active bleeding where immediate hemostasis is more important than prolonged platelet survival
  - Not ideal for prophylactic platelet transfusion due to increased platelet clearance and decreased survival post-transfusion

Logistics

- Educate providers and transfusion service staff on appropriate storage, issuing, and ordering
- Anticipate how it will be used
- Start with a small number of units during the first few weeks

Logistics

- Have defined patient populations
  - Trauma
  - Active bleed
  - Operating room
  - Obstetric hemorrhage
  - Massive transfusion protocol (MTP)

Logistics

- Have ordering rules
  - Specific providers
  - Service area
  - Diagnosis
- Audit
  - Get predictive data
  - Manage inventory levels accordingly

Future

Future

- CHIlled Platelet Study "CHIPS" (CHIPS) Inventory management
  - https://clinicaltrials.gov/study/NCT04834414
  - Phase 3 randomized study that will compare CSP with RTP transfusions in patients undergoing complex cardiac surgery
  - The primary objective is to establish that CSP are at the very least, non-inferior, and possibly superior to RTP in this patient population
  - Also, a storage duration trial to determine the maximum duration of storage of platelets at 4^{o}C that maintains non-inferiority (testing up to 21 days of shelf life)

Future

- Cold Stored Platelet in Hemorrhagic Shock (CriSP-HS)
  - https://clinicaltrials.gov/study/NCT04667468
  - Proposed 3-year, open label, multi-center, randomized trial designed to determine the feasibility, efficacy, and safety of urgent release CSP in patients in hemorrhagic shock
  - Patients will be randomized to receive either standard care or early infusion of urgent release CSP
  - The primary outcome for the pilot trial is feasibility, with principal secondary clinical outcome of 24-hour mortality

Future

- Cold-stored Platelet Early Intervention in TBI (CriSP-TBI)
  - https://clinicaltrials.gov/study/NCT04726410
  - A proposed 3-year, open label, single center, randomized trial designed to determine the feasibility, efficacy, and safety of urgent release CSP in patients with traumatic brain injury requiring platelet transfusion
  - Patients will be randomized to receive either standard care or early infusion of urgent release CSP
  - The primary outcome for the pilot trial is feasibility, with principal secondary clinical outcome of 6-month Extended Glasgow Outcome Scale

Future

- Recent and ongoing studies are evaluating the use of CSP in cardiac surgery patients.
  - A recent pilot study evaluated the hemostatic potential of CSP compared with RTP in adult patients undergoing complex cardiothoracic surgery
  - Although no significant differences in chest tube drainage, total blood usage, platelet function, or clinical outcomes were identified in CSP and RTP platelet recipients, the trial showed that the use of CSP in this patient population was feasible and set the stage for future clinical studies of CSP

Questions?

Outreach

- Physician available 24/7
  - Practitioners with transfusion-related questions/issues
  - Blood bank-related questions/issues
  - (515) 309-4840
- Educate the medical community to keep them up to date on transfusion-related topics
  - Presentations to medical personnel
    - Contact me: alex.smith@lifeservebloodcenter.org
  - Quarterly webinars
    - https://www.lifeservebloodcenter.org/for-hospitals/resource-guide/education
    - To request to be on the notification list please contact Rachael Muhs: rachael.muhs@lifeservebloodcenter.org

Thank you!

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