What is new in Platelet processing and storage

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The hemorragic tendency of thrombocytopenic animals can be controlled temporarily by transfusions of fresh platelet concentrates. The clinical value of platelet transfusions has previously been noted. Studies in animals have shown that maximal hemostatic effects can be expected when the transfusion of platelets is followed by a sustained elevation of their level in the blood. Clinical studies comparable to those in animals have not been possible, however, because of the variations in bleeding manifestations of untreated thrombocytopenic patients and the difficulties encountered in carrying out well controlled studies during medical or surgical emergencies. In view of the widening use of transfusions of fresh platelet concentrates the need for further studies on the indications, hazards and potential usefulness of this procedure has become more apparent.

Platelet transfusions have been included in the management of patients with acute leukemia and other generalized neoplastic diseases with bone-marrow involvement. The use of total-body irradiation and potent chemotherapeutic agents in such patients has further increased the indications for platelet transfusions.

This report is concerned with observations made in a group of patients belonging to three of the categories mentioned above. The effects of single and repeated transfusions of fresh platelet concentrates on the levels of circulating platelets and on the subsequent course of bleeding is the subject of this presentation.

Methods and Materials

The patients were children with thrombocytopenia resulting from acute leukemia, metastatic neoplastic disease or bone-marrow depression after chemotherapy. Platelet transfusions had not previously been used in these patients, although the majority had received whole blood. Concentrates of human platelets were prepared by differential centrifugation, the dry-dehydrated plastic bag being used as a closed system. Separation was initiated immediately after collection of the blood, with sodium citrate as an anticoagulant, and was completed within ninety minutes. The platelet concentrates were administered to the patients not later than three hours after collection of blood. Random donors (without regard to red-cell groups) were used.

Large amounts of platelets were administered. The dose infused was equal to or larger than the amount calculated to elevate the count of circulating platelets by at least 100,000 per cubic millimeter (0.017 unit) per pound of body weight. Since counting of the platelets in the concentrates was not practical, our calculations were made on the basis of an average platelet count of 200,000 in the freshly collected blood (a total of 10,000 platelets in 1 pint of blood). If one could recover all platelets from this blood, it would be necessary to infuse platelets from half the blood volume of the patient to increase the count from 0 to 100,000. This represents giving the platelets from 0.06 pint of blood for each pound of body weight. Since the yield of platelets separated by this method was shown to be 60 to 70 per cent and may be as low as 50 per cent in routine use it was calculated that platelets from 0.25 pint of blood (or unit of plasma) would be required per pound of body weight to provide the material sufficient, although not necessarily capable of elevating the platelet count of the recipient by 100,000 platelets per cubic millimeter. Each unit of platelets was resuspended in 20
# Platelet processing and storage

## Processing
- Preparation methods
- Hold over time before separation
- Leucoreduction
- Pooling of platelets
- Automation

## Storage
- Suspension medium
- Storage containers
Platelet preparation methods

- Platelet Rich Plasma
- Buffy Coat
- Plateletpheresis
Platelet Rich Plasma Method

Rossi’s principles of Transfusion Medicine, 4th edition
Hold over time before separation

Initially limited to 4 hours before collection

Current guidelines - 6 hours period

Recent – 20 to 24 hour
PLATELET CONCENTRATES PREPARED AFTER A 20 TO 24 HOUR HOLD OF THE WHOLE BLOOD AT 22°C

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²University of Washington School of Medicine, Seattle, WA

Abstract

Background—The FDA requires that red cells must be refrigerated within 8 hours of whole blood collection. Longer storage of whole blood at 22°C before component preparation would have many advantages.

Study Design And Methods—Two methods of holding whole blood for 20 to 24 hours at room temperature were evaluated; i.e., refrigerated plates or a 23°C incubator. After extended whole blood storage, platelet concentrates were prepared from platelet-rich-plasma on day 1 post-donation, and the platelets were stored for 6 more days. On day 7 of platelet storage, blood was drawn from each subject to prepare fresh platelets. The stored and fresh platelets were radiolabeled and transfused into their donor.

Results—Eleven subjects’ whole blood was stored using refrigerated Compocool plates and 10 using an incubator. Post-storage platelet recoveries averaged 47 ± 13% versus 53 ± 11% and survivals averaged 4.6 ± 1.7 days versus 4.7 ± 0.9 days for Compocool versus incubator storage, respectively (p=N.S.). Using all results, post-storage platelet recoveries averaged 75 ± 10% of fresh and survivals 57 ± 13% of fresh; platelet recoveries met FDA guidelines for post-storage platelet viability but not survivals.

Conclusion—Seven-day post-storage platelet viability is comparable when whole blood is stored for 22 ± 2 hour at 22°C using either refrigerated plates or an incubator to maintain temperature prior to preparing platelet concentrates.
Separation of Platelet Rich Plasma
Pooling of Platelets

- Permits storage of pooled platelets up to 5 days
- Expiry date – shortest expiry date of the pooled units
- Store: $2.2 - 5.8 \times 10^{11}$ platelets (4-6 PC at a concentration of $\leq 2.0 \times 10^6/\mu l$)
- Volume – 180-420 ml

Platelet pool and store set with in-line leukocyte reduction filter

http://www.fda.gov/BiologicsBloodVaccines/BloodProducts/ApproucedProducts
Pooled platelet product using the Acrodose plus system: Evaluation of feasibility, safety and efficacy

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\textbf{ARTICLE INFO}

Article history:
Received 30 August 2012
Received in revised form 4 May 2013
Accepted 3 September 2013

Keywords:
Platelets
Pooled
Corrected count increment

\textbf{ABSTRACT}

\textbf{Background:} Pooling of whole blood-derived platelets is not practiced in India. Currently available data shows that pooled platelets are equivalent in quality and less costly when compared with apheresis platelets.

\textbf{Aim:} To evaluate feasibility, safety and efficacy of pooled platelets using the Acrodose Plus system.

\textbf{Material and methods:} Standardization of platelet pooling procedure was done. Sterile docking device was used to maintain closed system. Pools of ABO compatible platelets (\(N = 40\)) as well as ABO incompatible (\(N = 10\)) pooled platelets were studied. ABO antibody titers were studied before and after pooling in case of ABO incompatible platelet pools. Corrected count increments (CCIs) (at 1 h and 24 h) were noted after transfusion of pooled platelets and Apheresis platelets in matched patient groups.

\textbf{Results:} Loss of platelet product due to pooling and leucodepletion was not statistically significant (\(p = 0.23\)). Leucodepletion >3 log was achieved in all pooled platelets. The total platelet content, time taken for procedure and pH were within acceptable limits. There were no issues related to platelet availability and turn around time during the study period. There was a reduction in the ABO antibody titers after pooling in case of the ABO incompatible pools. None of the pooled platelets showed evidence of bacterial contamination on testing with cBDS system at 18 h and 24 h intervals after pooling. CCI with pooled platelets was equivalent to that with apheresis platelets at 1 h (\(p = 0.36\)) and 24 h (\(p = 0.15\)).

\textbf{Conclusion:} Whole blood derived platelets can be pooled safely using closed system without altering the platelet quality in vitro as well as in vivo survival post transfusion. Pooled platelets can be a cost effective and safe alternative to thrombocytopenic patients whose transfusion requirements cannot be fulfilled with apheresis platelets alone.
Preparation of Leukocyte-Poor Platelet Concentrates from Buffy Coats
I. Special Inserts for Centrifuge Cups

Red Cross Blood Bank, Amsterdam, The Netherlands
Buffy Coat Method Top & Bottom

Plasma

SAG-Mannitol
Platelet pooling

- Pooling of platelet was associated with more consistent harvest per donation

<table>
<thead>
<tr>
<th></th>
<th>Individual BC</th>
<th>Pooled BC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average platelet content</td>
<td>54 ± 17×10^9</td>
<td>70 ± 17×10^9</td>
</tr>
<tr>
<td>Average platelet yield</td>
<td>66% ± 21%</td>
<td>75% ± 9%</td>
</tr>
</tbody>
</table>

Pooling of Buffy coat platelets
Implementation of a new platelet pooling system for platelet concentrates led to a higher corrected count increment after transfusion: a comparative observational study of platelet concentrates before and after implementation

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Received 23 April 2013; accepted for publication 13 January 2014

SUMMARY

Objectives: To study the effect of extended storage of platelet concentrates (PCs) and the implementation of a new platelet pooling system for PCs on corrected count increment (CCI) after transfusion.

Background: Due to new developments and changes in processes or procedures, one should remain alert for the effects of these changes. Besides in vitro studies and validation, in vivo studies are also important, as it has been shown that in vitro results do not always predict in vivo outcomes.

Methods/Materials: After introduction of extended storage of PCs for 5–7 days prepared from five buffy coats and plasma, transfusion monitoring for transfusions of PCs in haematological patients was set up. After 9 months, a new pooling system for PCs was implemented, Compoflect instead of Optiflute PLT, and transfusion monitoring was continued for another 8 months. The CCI was used as primary outcome.

Results: In total, 93 patients were included and transfused with PCs prepared in the Optiflute PLT system (262 transfusions) or in the Compoflect system (127 transfusions). Extended storage of PCs for 7 days had no significant effect on CCI. Although the implementation of the Compoflect system did not influence the CCI24h (13.8 ± 6.0 vs 13.0 ± 5.8; n.s.), it seemed to have a positive effect on CCI48h (7.0 ± 4.9 vs 4.7 ± 4.5; P = 0.05).

Conclusion: Although the influence of confounders could not be excluded, it seemed that implementation of the Compoflect system for PCs led to an improved CCI24h and that extended storage of PCs did not influence the CCI.

Key words: extended storage, implementation, platelet concentrates, platelet pooling system, transfusion monitoring.

Due to the new developments and changes in processes or procedures, one should remain alert for the effects of these changes. Therefore, careful implementation and monitoring are required for each change. Changes start with the literature and in vitro studies, followed by a validation, which will be guided by a so-called change control procedure in which the change, the impact and risk of the change, which departments are involved, what the expected consequences are and how to validate and implement the change are described. After implementation, a continuing quality program is needed to keep the process in control (Dumont et al., 1996). Additionally, in vivo studies or monitoring might be necessary to prove that the new developments lead to at least a similar outcome in the patient or otherwise that the additional benefits to the recipient outweigh the reduced effect in the patient (Vostal, 2006).

The question whether to perform in vivo studies is based on the concerns about loss of efficacy during collection, processing and storage through novel methods (Vostal, 2006). Additionally, it is important to perform a transfusion monitoring or post-authorization surveillance because in vitro results do not always directly correlate with in vivo results (Rinder & Smith, 2003; Diedrich et al., 2008). For transfusion monitoring, it is important to decide which outcome should be monitored. For example, for platelet transfusions, this might be mortality, bleeding or increments (Vostal, 2006; Hedle et al., 2011). As the most clinically relevant parameters rarely occur, the choice can be
Automation

First generation
- Optipress, TACEII
- Expression

Second generation
- Orbisac BC system, TACSI system
- Pooling, Balancing, Centrifugation, Expression, Leukoreduction, Sealing

Third generation
- Atreus system, Reveos system
- Balancing, Centrifugation, Expression, Sealing, Volume and platelet content determination, Procedure and process data, On-line platelet yield

Vox Sang 2014;106:1-13
## Platelet additive solution

<table>
<thead>
<tr>
<th>New Name</th>
<th>Citrate</th>
<th>Phosphate</th>
<th>Acetate</th>
<th>Magnesium</th>
<th>Potassium</th>
<th>Gluconate</th>
<th>Glucose</th>
<th>Alternative Names</th>
<th>Previous ISBT 128 Name</th>
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<tbody>
<tr>
<td>PAS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>PAS-A</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>PAS (1)</td>
</tr>
<tr>
<td>PAS-B</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PASII</td>
</tr>
<tr>
<td>PAS-C</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PASIII</td>
</tr>
<tr>
<td>PAS-D</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PAS IIIMgK (note, Composol PS should not have been called PASIIIMgK)</td>
</tr>
<tr>
<td>PAS-E</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PAS-F</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PlasmaLyte A, Isoplate</td>
<td>Not named</td>
</tr>
<tr>
<td>PAS-G</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>Not named</td>
</tr>
</tbody>
</table>

*Transfusion Medicine Reviews 2006;20:158-164*
Additive solutions differentially affect metabolic and functional parameters of platelet concentrates

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2Department of Hematology, Hanuschkrankenhaus, Vienna, Austria
3Department of Laboratory Medicine, Medical University, Vienna, Austria

Background Pathogen inactivation (PI) of platelet concentrates with extension of shelf life to 7 days requires the use of platelet additive solutions (PAS). We examined the quality of platelets resuspended in three different PAS stored for up to 7 days.

Materials and Methods Twelve triple adult dose platelet concentrates (PC) were collected using the TrimaAccel® collection system. Each highly concentrated product was divided into three equal parts, and the additive solutions (Composol®, or SSP+® or Intersol™) were added to a final concentration of 56% PAS and 44% plasma. Samples were drawn on days 1, 5 and 7 to measure pH, glucose, lactate dehydrogenase (LDH), lactate, mean platelet volume (MPV) and the aggregation response to collagen and the thrombin receptor agonist peptide-6. Further, p-selectin expression on platelets was assessed.

Results No statistically significant changes were observed for pH and MPV during 7 days of storage in all PAS containing PCs, whereas glucose decreased and LDH and lactate increased over time (P < 0.05). These changes were particularly evident in Intersol PCs on days 5 and 7 compared with Composol® PCs or SSP+® PCs (P < 0.05). Platelets from Intersol PCs exhibited the highest baseline activation of p-selectin and showed reduced collagen- and TRAP-6-induced aggregation.

Conclusion Resuspension of platelets in Intersol for 7 days results in increased platelet activation and platelet metabolism compared with SSP+® or Composol®. Further clinical studies are needed to evaluate whether the observed differences in PAS-PCs affect the recovery rate or the life span of transfused platelets.

Keywords: additive solution, aggregation capacity, platelets, storage lesion.
<table>
<thead>
<tr>
<th></th>
<th>Composol</th>
<th>PAS-II (Intersol)</th>
<th>PAS-IIIM (SSP+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>90.0</td>
<td>77.3</td>
<td>69.3</td>
</tr>
<tr>
<td>KCl</td>
<td>5.0</td>
<td>–</td>
<td>5.0</td>
</tr>
<tr>
<td>MgCl$_2$</td>
<td>1.5</td>
<td>–</td>
<td>1.5</td>
</tr>
<tr>
<td>Na$_3$ citrate</td>
<td>11.0</td>
<td>10.8</td>
<td>10.8</td>
</tr>
<tr>
<td>Na phosphate</td>
<td>–</td>
<td>28.2</td>
<td>28.2</td>
</tr>
<tr>
<td>Na acetate</td>
<td>27.0</td>
<td>32.5</td>
<td>32.5</td>
</tr>
<tr>
<td>Na gluconate</td>
<td>23.0</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
Extended storage and glucose exhaustion are associated with apoptotic changes in platelets stored in additive solution

Lacey Johnson,1,2,3 Peter Schubert,2,3 Shereen Tan,1 Dana V. Devine,2,3 and Denese C. Marks1

BACKGROUND: The storage of platelets (PLTs) in additive solution (AS) may facilitate improved PLT quality and possibly extension of the PLT shelf life. A minimum amount of plasma is required when PLTs are stored in AS, as a source of glucose. The aim of this study was to assess the effect of reducing the plasma carryover to 20% on PLT quality when stored in SSP+ for an extended period.

STUDY DESIGN AND METHODS: Using a pool-and-split design,uffy coat–derived PLTs were stored in either 30% plasma/SSP+ or 20% plasma/SSP+. In vitro analyses were carried out to Day 10. Metabolites and markers of PLT activation and apoptosis were measured using a blood gas analyzer and flow cytometry. PLT apoptotic protein expression was investigated by Western blotting.

RESULTS: Glucose exhaustion occurred in the 20% plasma group between Day 7 and Day 10. The surface expression of P-selectin and PAC-1 was comparable on Day 10 in both groups, suggesting that the PLTs were not activated. However, the exposure of phosphatidylserine and the number of phosphatidylserine-positive microparticles were significantly higher in the 20% group on Day 10. The expression of the proapoptotic proteins Bak, Bax, and cleaved caspase-3 were higher in the 20% plasma group by Day 7 of storage, compared to the 30% plasma group.

CONCLUSION: Exhaustion of glucose was associated with a proapoptotic phenotype. Results such as these should be considered before extending the PLT shelf life beyond 7 days, particularly when stored in ASs lacking glucose with low plasma carryover.

In many countries, platelet (PLT) concentrates prepared for transfusion are stored in 100% plasma. However, over the past few decades, the use of PLT additive solutions (PAS) as an alternative storage medium has been increasing. Several groups have demonstrated that storage of PLTs in PAS allows better retention of PLT quality with the potential to extend the shelf life beyond the current 5- to 7-day limit.1-3

The ability to reduce the plasma carryover in PLT components by supplementation with PAS has several benefits, including the potential to reduce the incidence of transfusion reactions, particularly allergic reactions and transfusion-related acute lung injury.4-6 The composition of PAS can be optimized to support PLT metabolism, which can reduce the PLT storage lesion, therefore improving quality and extending the shelf life.8 Further, the use of PAS allows more plasma to be recovered for clinical use or fractionation. The use of PAS is also compatible with current pathogen inactivation (PI) systems.

ABBREVIATIONS: MPV = mean PLT volume; pCO2 = partial pressure of carbon dioxide; PI = pathogen inactivation.

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Australian governments fund the Australian Red Cross Blood Service to provide blood, blood products, and services to the Australian community. This study was in part supported by a grant from Health Canada and Canadian Blood Services to DVD.

Received for publication July 15, 2015; revision received August 13, 2015; and accepted August 16, 2015.
doi:10.1111/trf.13345
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TRANSFUSION 2015;00:00-00
Advantages

- Greater plasma recovery for whole blood donation for transfusion or fractionation.

- Optimize energy metabolism or minimize activation of platelets leading to improved viability on Day 5 or allow extended storage to Day 7 or longer.

- Minimizing the adverse effects mediated by plasma.

- Facilitate bacterial detection of slow growing bacteria
Platelet Storage Containers

- Platelets are metabolically very active
- Extremely sensitive to changes in pH of medium
- Sufficient gas permeability to oxygen and carbon dioxide required
# Platelet Storage Containers

<table>
<thead>
<tr>
<th>Plasticizer</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Di ethylhexyl phthalate (DEHP)</td>
<td>Low gas permeability, storage up to 3 days</td>
</tr>
<tr>
<td>Tri-2 ethylhexyl trimelliate (TEHTM)</td>
<td>Have sufficient O$_2$ – CO$_2$ permeability, storage of platelets for at least 5 days,</td>
</tr>
<tr>
<td>Butyrl tri hexyl citrate (BTHC)</td>
<td>Equivalent to polyolefin, affected by method of sterilization</td>
</tr>
<tr>
<td>Polyolefin (PL – 732 plastic)</td>
<td>Higher permeability characteristics, storage of platelets for 7 days</td>
</tr>
<tr>
<td>Ethylene-vinyl acetate (EVA)</td>
<td></td>
</tr>
</tbody>
</table>
Conclusion

- Methods of processing and storage of platelets have changed considerably over the past decade

- Research still goes on produce perfect platelets
Thank you