Optimizing Platelet Transfusion for Clinical Practice. Do Platelets's Age Matter for Therapeutic Platelets Transfusion?

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Abstract

Background: Platelet concentrates (PCs) with the implementation of bacterial testing have a storage period of 7 days. It was reported that platelet function decreased gradually in vitro until the 5th or 7th day of storage. Pooled PCs (PPCs) are mainly used in Europe and Canada and there is the possibility to use many PPCs with 7 days storage for transfusion in case of bleeding or massive transfusion.

Aim of Study: The aim of this study is to test in vitro both storage conditions and function of platelet-rich plasma (PRP-PCs) during storage for 7 days to assess the function of the PCs for the possibility to extend the use of stored PCs up to 7 days and its use for therapeutic and massive platelets transfusion.

Patients and Methods: In this study 18 PRP-PCs, were evaluated for chemical analysis by measuring pH, LDH production, and glucose consumption levels and platelet aggregation testing using adenosine diphosphate (ADP), collagen (COLL), and ristocetin (RIST) and epinephrine (EPI) on days 1, 3, 5, and 7 of storage of PCs.

Results: The platelets yield was >5.58 x 1010 which indicates accurate platelet separation and storage. The metabolic parameters indicated stable storage conditions throughout the 7-day period. The chemical analysis showed that there is stable pH during all days of storage age above 6.4. Aggregation responses of the PCs decreased gradually and significantly ($p < 0.001$) starting from 1st day when compared with 3rd, 5th and 7th day. On the 7th day the platelets lost their response to EPI and ADP, while still maintaining a normal response to RIST and weak to COLL.

Conclusion: There is a loss of some function on the 6-7th day of platelet storage which may indicate that it is suitable for prophylactic more than therapeutic transfusion. In order to provide more clinical benefit to the patients with thrombocytopenic bleeding, the platelets should be less than 7 days of storage.

Key Words: Platelets transfusion — Platelets concentrate — Platelets function.

Introduction

PLATELETS for transfusion are stored at room temperature between 20 and 24°C under agitation and were limited to five days post donation due to the risk of bacterial contamination as bacteria thrive in these conditions [1]. The new development of new plastics and thinner plastic bags which allowed increased oxygen transport with preservation of aerobic metabolism has permitted the further extension of storage for 5 days [2] and more recently to 7 days [3]. Recently, FDA allowed extending expiration beyond day 5 and up to day 7 which requires the use of safety measures and storage containers cleared or approved by the FDA for 7-day storage [4].

Platelet preparation and storage for transfusion may cause platelet activation, which contributes to decreased ability of stored platelet to function and survive in vivo after transfusion compared with that seen with freshly prepared platelets which are called platelets storage lesions (PSL) which are characterized by morphological changes and impaired platelet function. The collection method and storage medium may influence the magnitude of the storage lesion [5,6].

Platelets aggregometry is used both in diagnosis and in evaluating platelet function. The response is monitored in the function of different agonists, such as collagen, ADP, arachidonic acid, epinephrine, or different combinations. Usually, the median aggregation response gradually decreases in function of the storage time and it is influenced by the platelet additive solution used [7].

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Platelets transfusions are used to treat patients with active hemorrhage or to prevent hemorrhage in patients with thrombocytopenia [8]. The transfusion of fresh platelets can lead to a better transfusion outcome than transfusion of old platelets as in-vitro characteristics of fresh platelets are considered preferable to the characteristics of stored platelets for 5 days [9,10].

In this study, we investigated the storage condition of routinely used blood bags in our hospital and tested the quality of stored PCs by testing the yield of platelets under appropriate conditions and the aggregation response during 7 days of storage of PRP-PCs for selection of the best platelets for transfusion in case of bleeding as the guidelines do not differentiate between platelets required for prophylactic or therapeutic intervention during the storage for 7 days.

Material and Methods

This study was carried out in the blood bank of King Fahad Armed Forces Hospital, Jeddah, Saudi Arabia, on the PCs from 18 donors who had not taken ASA in the last 15 days and with platelets count >150 000/ul. Whole blood (450mL) was collected into a triple-bag system (Terumo corporation Tokyo, Japan), in which the primary pack contains 63mL of CPD with a satellite bag containing 100m1 of SAGM red cell preservative. Whole blood was centrifuged at 2170g x 3min using Cryofuge 8500 machine of the Heraeus firm with centrifugation of the platelet-rich plasma at 3450g x 6min. The platelets were stored in a Forma platelet incubator at 22 degrees °C with gentle horizontal agitation (70 rpm) (Helmer, PAS 40 Model, Noblesville USA) [ill.

Specimens were obtained aseptically from units stored on days 1,3,5,7 for assessment of, platelets dose, PH, lactate production, glucose consumption, and invitro platelets function. No air was allowed in the collection syringes and was sealed immediately, to minimize any pH effect that may be incurred by loss of CO2 from plasma to surrounding air. pH, and PCO2, were measured in the Radiometer ABL at 37°C and converted to value at 22°C while glucose and LDH were measured by the Dimension chemistry analyzer.

For calculation of total platelet content, the gram weight was obtained by weighing with a sensitive balance. The cell count was obtained using an automatic cell counter (Colter LH USA) after diluting 1/5. The resulting platelet count was multiplied by 5 so that the platelet count in mm^3 was determined.

On each of the D1, D3, D5, and D7. The aggregation response to adenosine ADP, COLL., RIST. and EPN wa studied using a BCT coagulation analyzer (Dade Behring, USA) and Dade Cluster Platelet aggregation reagents. The concentration of the stimulant used after reconstitution with lml of distilled water was as follows Cluster ADP reagent 200uM, Cluster Epinephrine reagent 100uM, and Cluster collagen reagent 2.0mg/ml. Ristocetin regent from Chrono-log company 2mg/ml. 500u1 of platelets and 500u1 of plasma from the product obtained during collection, were used for each sample.

Statistical analysis:

All the data were analyzed using SPSS (version 15) software for windows. The data was grouped according to the days of storage. Paired samples statistics (t-test) and Annova test were used to compare the various parameters and platelets aggregation studies in different groups.

Results

Eighteen PRP-PCs units, all from separate donors, were used in this study. The results of the analysis of the PCs in relation to their storage condition during days 1,3,5, and 7 of storage are shown in Table (1), Figs. (1,2).

Table (1): Statistical analysis of chemical and functional characters of PRP-PCs using one way ANOVA test and t-test.

| Parameters	Days 1 M±SD	Days 3 M±SD	Days 5 M±SD	Days 7 M±SD	p-value (anova test)	p-value D1-D3	p-value (D1-D5)	p-value (D1-D7)
pH (N>6.4 during storage)	7.25±0.04	7 .18±0.08	7.06±0.13	7.02±0.15	p>0.05	p>0.05	p>0.05	p>0.05
Glucose (mg/di)	360±14.5	399±14.7	348±15.5	327±12.4	p>0.05	p>0.05	p>0.05	p>0.05
LDH (mmo1/1)	1.2±0.02	2.1±0.08	1.9±01.02	2.9±01.1	p>0.05	p>0.05	p>0.05	p>0.05
Plat. count/1010	5 .71±1 .8	6.9±1.1	5.58±0.6	5.42±0.6	p>0.05	p>0.05	p>0.05	p>0.05
Tot/ul	156±57.4	153±52.7	151±27 .6	158±27.6	p>0.05	p>0.05	p>0.05	p>0.05
ADP%	93.2±10.5	68.7±9.6	8.7±4.6	0.0±4.6	p<0.001	p<0.001	p<0.001	p<0.001
EPN%	91.3±10.5	62.7.7±6.9	4.5±6.5	0.0±6.5	p<0.001	p<0.001	p<0.001	p<0.001
COLL%	89.6±8.4	74.2±3.3	11.7±3.6	5.7±3.6	p<0.001	p<0.001	p<0.001	p<0.001
RIST%	89.4±5.6	73.6±10.7	63.7±2.6	53.7±2.6	p<0.001	p>0.05	p<0.001	p<0.001
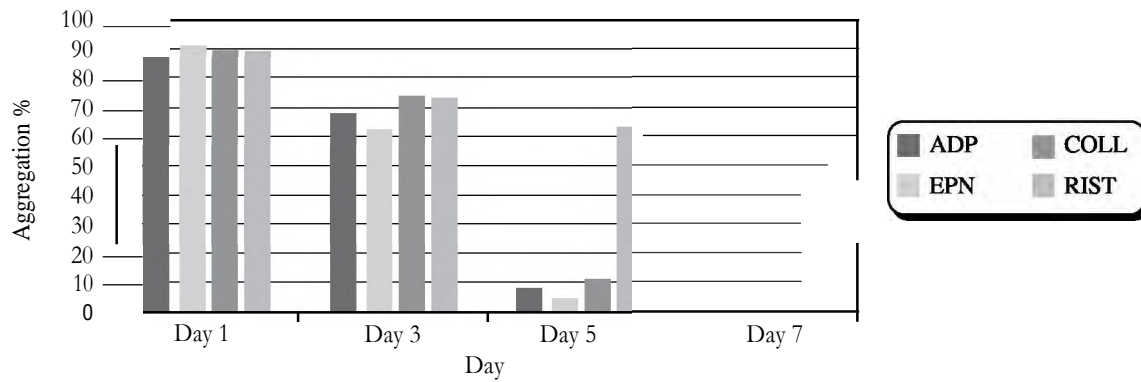
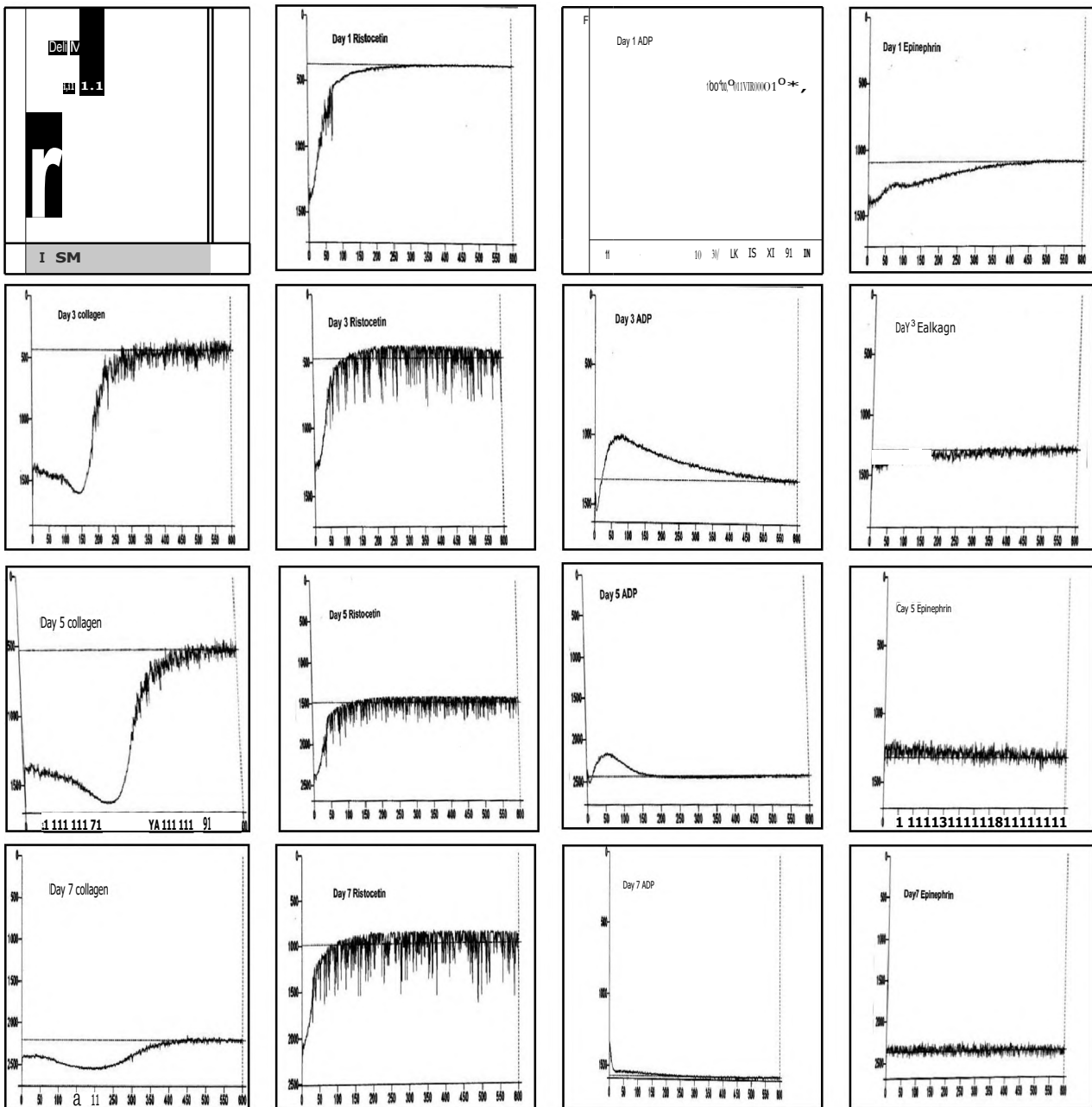Fig. (1): Platelets aggregation during the 7 days of storage.

Fig. (2): The effect of different agonists on PC during storage days 1,3,5, and 7. The collagen showed a progressive increase in the lag period with a progressive decrease in aggregation %, The ristocetin showed a stable response during storage, The ADP showed a progressive decrease in aggregation which was lost at day 7, The Epinephrin showed a progressive decrease in aggregation which lost on day 5 and 7.

Hematology parameters:

Platelets counts values were >5.58 x 1010 and were generally stable during storage which indicates no loss of platelets during storage.

Chemical analysis:

Table (1) shows the metabolic parameters and quantitative values of pH, Glucose, LDH and Platelets count during days 1,3,5, and 7 of storage and showing stable PH >6.4 at the end of shelf life and there was no difference (p>0.05) between the 1st, 3rd, 5th and 7th day, which indicate stable storage conditions throughout the 7-day period. Aerobic metabolism shows continuous glucose consumption and lactate production which indicate functioning platelets although statistically there was a nonsignificant difference in glucose and LDH value between the 1st, 3rd, 5th, and 7th day of storage.

PLT aggregation by light transmission aggregometry:

Table (1), Figs. (1,2) show the effect of different agonists on PCs during storage on days 1,3,5, and 7. The ADP, COLL, and EPI showed a significant decrease in the aggregation % on days 3,5, and 7 of storage when compared with the first day (p<0.001). Day 1 and 3 show normal platelet function of more than 50% although day 3 is less than day1 and this was statistically significant (p<0.001). Day 5 platelets showed a marked decrease in the ADP, COLL, and EPI when compared with day 1 (p<0.001) so it is still functioning with a weak response. On day 7 there is a loss response to EPI and ADP and a weak to COLL while there is still a good response to RIST. The collagen showed a progressive decrease in aggregation % from day 1 to day 7 with a progressive increase in the lag period which indicates a progressive loss of function with the progression of time. RIST showed the non-significant difference between the first and 3 days (p>0.05) but there was a statically significant decrease in aggregation % when Days 5 and 7 compared with the first day (p<0.001) but on all days it was more than 50%.

Discussion

PCs are well-established therapeutic products in transfusion medicine. This study described supplementing the data already available concerning the viability of platelets stored at 22°C with information relevant to their aggregation response to different agonists and their use for 5-7days [12,13].

In 2017, Canadian Blood Services applied bacterial detection prior release of PCs, and this improved inventory management by extending the platelet shelf life from five to seven days [14], but these guidelines did not take into consideration other factors such as cytokines-related transfusion reactions, also platelets undergo a series of biochemical and morphological changes leading to the formation of the PSL, with reduced mitochondrial

function, release of soluble mediator, shedding of microparticles, and increased cell surface receptor expression and this reduces consequently hemostatic function and in vivo survival, in addition, the guidelines do not differentiate between PCs for prophylactic or therapeutic transfusion as patients with bleeding need immediate acting platelets with good function [15,16].

The aim of this study was to show if the PCs of different age have the same full function to be used to arrest bleeding in case of therapeutic transfusion and it showed that PRP-PCs stored at 22°C has optimum platelet metabolism with a good concentration of platelets and optimum pH but its function decreased from 3rd day of storage but still maintain its function till 5th day while there is loss of some function in the 7th day which may indicate that PCs at day 7 is suitable for prophylactic more than therapeutic transfusion as there are some reports that platelets restore its function in circulation and of course it takes time.

This result is in agreement with Prudent [17] who reported that PCs with optimum donor qualification, volume, the concentration of platelets, residual cell contaminations, pH, and temperature, should guarantee that the platelets are still functional for transfusion even after 7 days of storage, also with Fiedler et al. [18] who reported that glucose values steadily declined and, conversely, lactate concentrations increased equally in the course of storage of PCs, Consistently, pH values moderately decreased and the **LDH** concentrations only slightly increased also they reported that there is a progressive loss of platelets aggregation, and on days 4 and 7 became evident. Aggregation responses to ADP and collagen were heterogeneous, with marked losses in collagen responsiveness on day 4 in some concentrates. Our result is also in agreement with Sperling et al., [19] who studied flowcytometric measurement of agonist-induced platelet aggregation during storage in buffy-coat platelet concentrates and apheresis platelet units and showed PLT-aggregation capacity decreased from day 1 to day 7 for almost all product—agonist combinations. Other studies have also shown reduced PLT aggregation capacity in during storage as measured by platelet aggregation testing using adenosine diphosphate (ADP) and collagen and flowcytometric platelet activation analysis using CD41 and CD62 [20,21]. Our result is also similar to Kocazeybek et al. [22] who reported that aggregation responses of the platelets decreased significantly in the course of time throughout the storage process, but our study showed a higher % of aggregation with ristocetin and this can be explained by the fact that ristocetin, in concentrations of 1.0-1.5mg/ml, aggregates normal platelets in citrated **PRP** and forms complexes with vWF and induce the aggregation of platelets by binding of vWF to the platelet GPIb receptor while the release reaction plays only a minor role so it is less sensitive

to platelets storage lesion [23]. Our study is also in agreement with Akay et al. [24] who reported that ADP and collagen-induced platelet aggregation responses decreased significantly on the 3rd and 5th days compared to 1st day and flow cytometric analysis revealed minor changes in CD41 expression after ADP on the 3rd day compared to 1st day and on the 5th day compared to 3rd day. Also, our results are in agreement with Aubron et al. [25] who reported that the freshest PLTs (less than 3 days) were associated with a better CCI, although there was no impact on bleeding events.

This study against the in vivo study of Mac Lennan et al. [26] who reported no evidence that 6-or7-day PLTs are inferior to 2- to 5-day PLTs, as measured by using clinical parameters with mean corrected platelet count increments, bleeding events, the proportion of patients with successful transfusions, or interval to next transfusion in stable hematology patients. Our study is also against a study by Serrano K et al. [27] who reported the difference between 5 and 7 days of storage regarding the number of platelets in the storage bag, glucose, lactate, pH, CD 62P, morphology, and swirl score was smaller than the observed standard deviation which indicates that platelets stored for 6-7 days show equivalent efficacy to platelets stored for 5 days or less. This allowed extending the platelet shelf life from five to seven days. This difference from our study can be explained as they used CD62P, morphology and swirl score as a marker of platelet activation and responsiveness and did not use platelets aggregation study which is more sensitive for platelet storage lesions. Our result is also against Miyaji et al. [20] who reported that the decrease in PLT aggregation after storage can improve in the body after transfusion, and transfused PLTs have similar aggregation ability compared to the PLTs derived from the patient but in his study no data about the age of transfused platelets.

Conclusion and recommendation:

From this study, we conclude that in order to provide more clinical benefit to the patientsas PSLincreases by the time of Platelet storage, and it is not certain that this lesion will be recovered completely after transfusion , we can divide PCs into fresh PCs with 5 days and old PCs with 6-7 days. The fresh PCS should be used for thrombocytopenia with bleeding or massive transfusion while old PCs are preferred for prophylactic platelets transfusion. It is advised in general for patients with thrombocytopenic bleeding, that the more recent PCs should be transfused. Larger studies are needed to evaluate platelets stored for up to 7 days for transfusions in trauma and surgery patients.

Conflict of interest:

The authors have disclosed no conflicts of interest.

Statement of Ethics:

Ethical principles, donor informed consent, and anonymization were obeyed.

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تحسين نقل الصفائح الدموية للممارسة السريرية.
هل عمر الصفائح الدموية مهم فى نقل الصفائح الدموية العلاجية؟

استخدام الصفائح الدمويه المكدسه مع إجراء اختبار إكتشاف البكتيريا لها زاد من مدة صلاحيتها من ٥ إلى ٧ أيام ولكن ليس من المعروف هل تستمر الصفائح فى أداء وظيفتها بنفس الكفاءه حتى اليوم السابع حيث أنه من المعروف أنه تنخفض وظيفتها تدريجياً ومعظم الدراسات تمت حتى اليوم الخامس. الهدف هو دراسة التغيرات الكيميائيه ووظائف الصفائح اثناء التخزين لمدة ٧ أيام حيث تمت دراسة ١٨ وحدة صفائح دمويه للتغيرات الكيميائيه ووظائف الصفائح فى يوم ١ و٣ و٥ و٧. وجد فى هذه الدراسه ان التحاليل الكيميائيه تنخفض بالتدريج ولكن بدرجه غير هامه وكذلك تنخفض وظائف الصفاح وتفقد بعضها ولكنها تستمر فى الاستجابه حتى اليوم السابع. تدل هذه الدراسه فى إمكانية استخدام الصفائح الدمويه كعامل وفائى للنزيف فى أمراض نقص الصفائح ولكن فى امراض نزيف الدم مع نقص الصفائح يفضل استخدام الصفائح المخزنه لمدة ٥ أيام اكثر من ٧ أيام.