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Original Research Article

Comparative evaluation of storage time on the quality of platelet concentrates prepared from buffy coat at ambient temperature

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ABSTRACT

Background: Buffy coat-platelets concentrates (BC-PC) are prepared within 6-8 hours of blood collection with 1-2 hours of hanging period which imposes time constraints and logistic problems when collecting blood from blood donation camps in wide geographic areas.
Methods: In our prospective study of one year at Dayanand Medical College and Hospital, Ludhiana we prepared 50 units of BC-PC after hanging BC for 2 hours and another 50 after storing BC O/N at ambient temperature. Platelet (plt) count, WBC contamination and other biochemical parameters in both groups of PC were analyzed on day 1 and day 5 of preparation.
Results: The mean plt counts of O/N BC-PC on day 1 was $8.36\pm1.22 \times 10^{10}$, significantly higher than that of F (fresh) BC-PC ($7.44\pm0.81 \times 10^{10}$). On day 5, F BC-PC showed $6.21\pm0.88 \times 10^{10}$ count while it was $6.87\pm0.96 \times 10^{10}$ in O/N BC-PC. WBC contamination was lower in O/N BC-PC. On day 1, in F BC-PC WBC contamination was observed as $4.44\pm2.02 \times 10^{7}$ while in O/N BC-PC it was $3.19\pm3.33 \times 10^{7}$. On day 5, in F BC-PC WBC contamination was observed as $3.77\pm0.66 \times 10^{7}$ while in O/N BC-PC it was $2.92\pm1.71 \times 10^{7}$. Results of biochemical parameters (pH, pO_2, pCO_2, glucose) were significantly higher and better in F BC-PC but both methods of preparation provided plts optimal survival conditions throughout storage period.
Conclusions: O/N BC-PC provides a better-quality product while solving logistic problems.

Keywords: Buffy coat, Fresh buffy coat platelet concentrates, Overnight buffy coat platelet concentrates, Platelet concentrates

INTRODUCTION

Blood components are traditionally separated from whole blood (WB) within 6-8 hours of blood collection.[1] It imposes time constraints on the component preparation team. Several studies have been done to prepare platelet concentrates (PC) from Buffy-coat (BC) with differences in the storage time of WB preceding the preparation of BC and the storage time of BC before preparing PC. There is an ongoing debate whether one of these types of platelet (plt) products has clear advantages over the others. Currently, it is proposed that overnight (O/N) holding practice for component manufacturing can be logistically facilitated with relative cost savings. In India BC-PCs are prepared solely as per Directorate General of Health Sciences Guidelines (DGHS).[2]

Objectives

This study has been designed to compare the quality parameters of BC-PC prepared either from fresh (F) or after overnight (O/N) storage at ambient temperature that

are ought to be measured by platelet count and white blood cell (WBC) contamination per unit and other biochemical parameters.

METHODS

In our prospective study conducted at Dayanand Medical College and Hospital, Ludhiana, Punjab, India from January 1, 2016 to December 31, 2016, after approval from institute's ethics committee 100 units of WB (450 ml) were collected through aseptic venipuncture from blood donors registered in the department of immunohematology and blood transfusion, with their informed consent into quadruple blood bags with citrate phosphate dextrose (CPD) as anticoagulant and saline-adenine-glucose-mannitol (SAGM) as additive solution (Terumo Penpol Pvt. Ltd).

Number of cases

Total number of cases included in this study was 100. These were randomly divided in two groups of 50 units each: a) 50 F BC-PC samples, b) 50 O/N BC-PC samples.

Inclusion criteria

Healthy voluntary and replacement blood donors who came to IHBT department, DMCH were included in the study after reviewing history and examination as per NBTC guidelines.[3] Specific history of drugs/medications leading to platelet dysfunction was taken and donors with positive history of these drugs were not accepted for the donation.

Exclusion criteria

Donors on antiplatelet drugs deferred for the next 2 weeks. Donors who had ingested aspirin/ NSAIDS in the last 72 hours. If the time period of whole blood donation exceeds 10 minutes for a unit of 450-500 ml. Unhealthy blood donors as per DGHS guidelines. Lipemic plasma in platelet concentrates. Visible RBC contamination in platelet concentrate. Transfusion transmitted infection reactive units.

Quality assessment

Quality of prepared BC-PC was assessed on day 1 and 5 with the following parameters: a) platelet (plt) count and white blood cell (WBC) count: done by automated cell counter LH 750 hematology analyzer (Beckman Coulter, Inc.); b) volume of the PC (ml) = weight of the full bag-weight of empty bag/specific gravity; c) Swirling: Evaluated by examining the units against light and scored as: score 0: homogen turbid and is not changed with pressure, score 1: homogen swirling only in some part of the bag and is not clear, score 2: homogenic swirling in all parts of the bag, score 3: very clear homogen swirling in all parts of the bag; d) pH, partial pressure of oxygen (pO_2), partial pressure of carbon dioxide (pCO_2) and glucose: assessed by automated analyzer (GEM Premier 3000, Instrumentation Laboratory, Werfen Group IVD, 39 Lexington, MA).

Statistical analysis

Analysis was done by the student t-test. We considered p values of 0.05 or less as significant. Results are given as means and standard deviation (SD) or median and range.

RESULTS

Volume of the bag

Of 50 F BC-PC units all met the desired quality control (QC) criteria while 96% (48/50) O/N BC-PC units met the criteria (i.e. 70- 90 ml) and 4% (2/50) had volume of less than 70 ml. Results were comparable and statistically insignificant (Table 1).

Platelet counts per unit

On day 1, 100% of F BC-PC and 98% (49/50) of O/N BC-PC had platelet count >5.5 × 10^{10}, while 2% (1/50) of O/N BC-PC had a platelet count <5.5 × 10^{10}. On day 5, 80% (40/50) of fresh BC-PC and 92% (46/50) of overnight BC-PC had a platelet count >5.5 × 10^{10}, while 20% (10/50) of fresh BC-PC and 8% (4/50) of overnight BC-PC had a platelet count <5.5 ×10^{10}. IQR and median values with outliers have been shown in the Figure 1. It was observed that O/N BC-PC showed better platelet count than F BC-PC with significant statistical difference with p value 0.00 and t value -4.414 on day 1 and 0.001, -3.558 respectively on day 5 (Table 1).

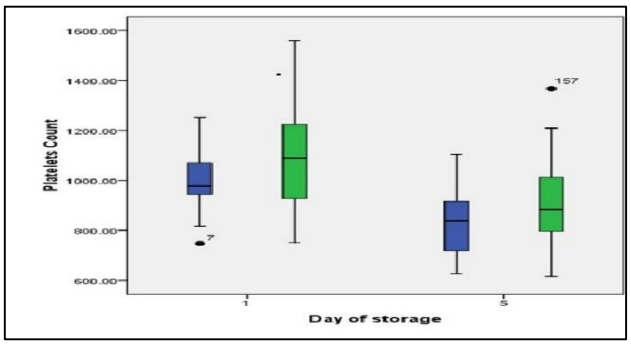

Figure 1: Platelet counts of O/N and F BC-PC on day 1 and day 5.

Entries along Y axis show platelet count per bag. Blue color representing F BC-PC and green color representing O/N BC-PC. Results are shown as median (IQR). Dots represent outlier entries of PC units on day 1 of F BC-PC and day 5 of BC-PC. Numbers around the dot represent the entry number of outlier PC unit.

Table 1: Results of F BC-PC and O/N BC-PC on day 1 and day 5.

	F BC-PC				O/N BC-PC							
	Mean value		SD		Mean		SD		t value		P value	
	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5
Platelet count per bag	7.44	6.21	0.81	0.88	8.36	6.87	1.22	0.96	-4.414	-3.558	0.000	0.001
WBC contaminate-on per bag	4.44	3.77	2.02	0.66	3.19	2.92	3.33	1.71	2.257	3.266	0.026	0.022
Volume of bag (ml)	74.80	74.80	3.77	3.77	76.20	76.20	5.21	5.21	-1.540	-1.540	0.127	0.127
pH	6.99	6.90	0.09	0.11	6.90	6.86	0.11	0.09	4.311	2.308	0.000	0.023
pO_2 (mmHg)	122.90	65.18	28.46	9.08	110.44	59.86	21.96	13.16	2.451	2.352	0.016	0.021
pCO_2 (mmHg)	93.42	72.58	16.41	9.45	105.96	64.76	14.00	16.19	-4.110	2.950	0.000	0.004
Glucose (mg/dl)	412.94	303.60	50.64	48.56	306.02	212.66	47.12	52.77	10.930	8.967	0.000	0.000
Swirling	3.00	3.00	0.00	0.00	2.88	2.88	0.33	0.33	2.585	2.585	0.011	0.011

F BC-PC: fresh buffy coat platelet concentrates; O/N BC-PC: overnight buffy coat platelet concentrates, pO_2: partial pressure of oxygen, pCO_2: partial pressure of carbon dioxide

WBC contamination per unit

Although 4% of O/N BC-PC showed increased levels of WBC contamination but the overall WBC contamination observed in case of O/N BC-PC was lesser than F BC-PC. On comparison, O/N BC-PC had lesser mean WBC contamination than F BC-PC per bag on day 1 and day 5 of PC preparation and the difference was statistically significant on day 1 and statistically comparable on day 5 (Table 1). IQR and median values with outliers have been shown in the Figure 2.

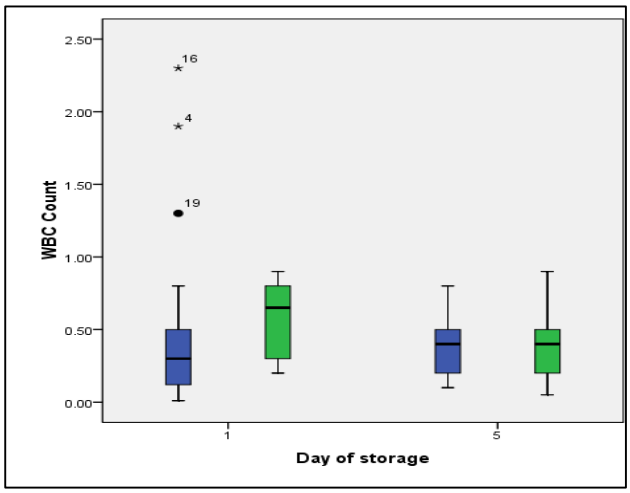

Figure 2: WBC counts of O/N and F BC-PC on day 1 and day 5.

Entries along Y axis show WBC contamination per bag. Results are shown as median (IQR). On day 5 F BC-PC represented by a horizontal line as the range/variability among the values was very small. Dots and stars represent outlier entries of PC units on day 1 of O/N BC-PC and in case of F BC-PC on day 5. Numbers around the dots represent the entry number of outlier PC unit.

Changes in pH

pH remained above 6.8 in all BC-PCs throughout storage results observed on both day 1 and day 5 was statistically significant (Table 1).

Changes in pO_2 levels

Contrary to our expectations mean value on day 5 of storage increased in case of both F BC-PC and O/N BC-PC but oxygen in the products remain well above optimum range throughout storage period providing support for the substrate metabolism. IQR and median values with outliers has been shown in the Figure 3. Statistically when both methods of preparation were compared significant difference was observed in partial pressure of oxygen (Table 1).

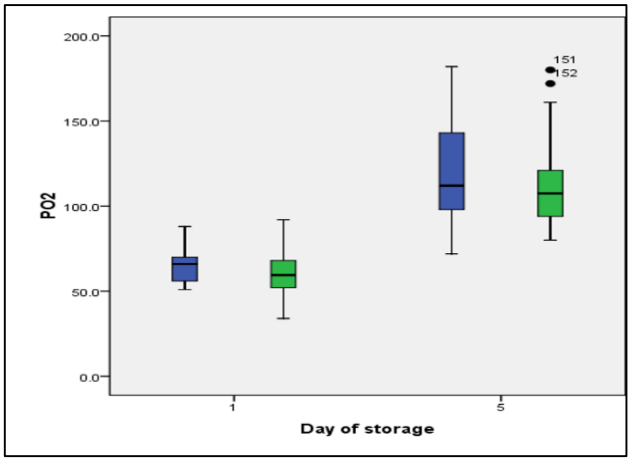

Figure 3: pO_2 of O/N and F BC-PC on day 1 and day 5.

Entries along Y axis show partial pressure of oxygen in mmHg. Results are shown as median (IQR). Dots represent outlier entries of PC units on day 1 of O/N BC-

PC. Numbers around the dots representing the entry number of outlier PC unit.

Changes in pCO_2 levels

Decreasing trend was seen in pCO_2 levels from day 1 to day 5. Results obtained were statistically significant on both days (Table 1). IQR and median values with outliers has been shown in the Figure 4.

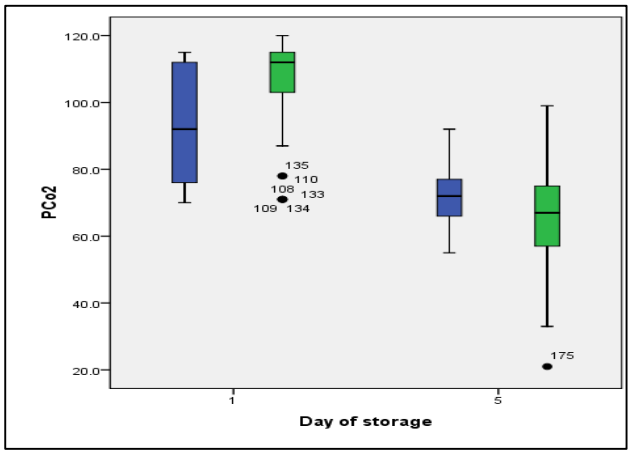

Figure4: pCO_2 levels of O/N and F BC-PC on day 1 and day 5.

Entries along Y axis show partial pressure of carbon dioxide in mmHg. Results are shown as median (IQR). Dots represent outlier entries of PC units on day 1 and day 5 of O/N BC-PC (green color). Numbers around the dots representing the entry number of outlier PC unit

Glucose levels

Glucose levels were well maintained throughout storage period. IQR and median values with outliers has been shown in the Figure 5. Statistically significant results were obtained on day 1 as well as day 5 of storage (Table 1).

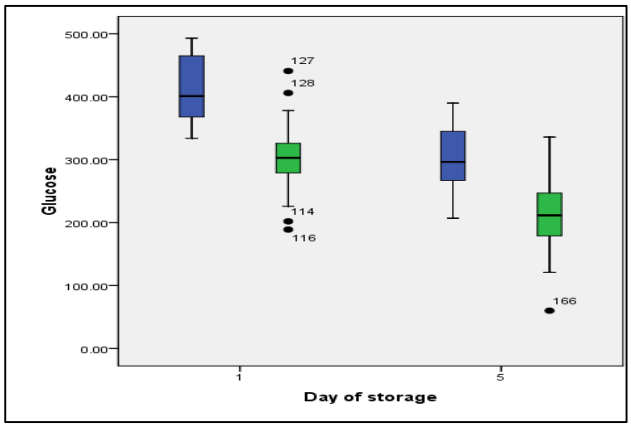

Figure 5: Glucose levels of O/N and F BC-PC on day 1 and day 5.

Entries along Y axis show glucose levels in mg/dl. Results are shown as median (IQR). Dots represent outlier entries of PC units on day 1 and day 5 of O/N BC-PC. Numbers around the dots representing the entry number of outlier PC unit.

Swirling

Swirling with score 3 on day 1 and day 5 was observed in 100% (50/50) of F BC-PC and 88% (44/50) of O/N BC-PC while score 2 on day 1 was observed in 12% (6/50) of O/N BC-PC and none of F BC-PC. Swirling was present in both BC-PC products throughout the period of storage. Statistically significant difference was observed inferring that the F BC-PC show better swirling than O/N BC-PC.

DISCUSSION

When subjected to extended resting period, platelet aggregation and activation, entrapment among concentrated WBCs would reduce which will eventually lead to more platelet release into the plasma layer in soft spin of platelet production in case of O/N BC-PC. The ability of transfused platelets to circulate and function is dependent on both the effect of the ex-vivo storage lesions that undermines platelet functionality and the status of the in-vivo milieu of the transfused individual.[4,5]

Boeri et al also reported that the platelet yields increased to 73±4 percent ($p<0.001$) and 74±9 percent ($p<0.001$) after 3-hour and 12-hour hold of BCs, respectively (n=15 per type of PC). Although sample size used by Boeri and co-workers was small but the results are comparable to the present study.[6]

Dijkstra-Tiekstra et al observed in their study that the O/N PC showed higher platelet count (450-470 × 10^9/PC versus 290-320 × 10^9/PC) compared to that of F BC-PC but the difference between their study and the present study is that Dijkstra-Tiekstra prepared PCs after pooling of BC using four different methods of pooling. Another difference between study of Dijkstra-Tiekstra and present study is that they cooled down their whole blood under butandiol after collection.[7] Dijkstra-Tiekstra in a later study compared PCs based on the method of preparation and divided them into three groups; PCs prepared from fresh whole blood (fresh/fresh), PCs prepared from O/N BCs from fresh whole blood (fresh/stored), and PCs prepared from O/N stored whole blood(stored/fresh).[8] It was seen that fresh/fresh PCs had the lowest platelet counts while stored/fresh PCs had the highest PLT counts, fresh/stored 68 PCs showed a better platelet count than fresh/fresh PCs though it was lesser than stored/fresh category. Difference between their study and the present study was that it was a multicentre study and rapid cooling of WB was done after collection. All PCs were prepared using four to six BCs and 100% plasma and all were leukoreduced according to the centers' standard methods. In the present study although single BC-PC were included and no separate filters were used for

leukoreduction but the results of platelet counts obtained by them are in concordance with our study.

Philip et al also reported a higher platelet count in O/N BC-PC ($6.32\pm1.18 \times 10^{10}$ versus $5.7\pm1.57 \times 10^{10}$) which is in concordance with present study.[9] Baroti et al showed comparable results after comparing platelet yield from O/N buffy coats to that of fresh blood or overnight-stored blood which is not similar to the results obtained by our study. O/N exposure to ambient temperature might have led to WBCs lysis, and also may have helped WBCs to settle down in the stored BC resulting in lesser WBC count in O/N BC-PC.[10] Philip et al, Racz Baroti et al and Perez-pujol et al concluded leukocyte contamination of the PCs was lower, when they were produced from O/N stored blood or O/N stored BC ($p<0.0001$ compared to PCs) as compared to fresh blood and statistically significant results seen which is in concordance with the present study.[9-11] Boeri et al showed WBC contamination to be on the higher side in O/N BC-PC which is contrary to our results.

Lesser WBC content of the product could decrease the chances of HLA allo-immunization and platelet refractoriness in multi-transfused patients and thus providing us with a better-quality product.

Metabolic variables like pH, glucose showed best results for F BC-PCs compared to O/N BC-PCs which might be explained by the lower amount of platelet in the storage bag, resulting in lower total metabolism in the bag. Platelets producing lactic acid as a by-product might be the reason for the reduced amount of glucose and lower pH levels in O/N BC-PC. On day 1 of storage, pO_2 levels were lesser in O/N BC-PC but pCO_2 levels were more than F BC-PC. As the oxygen consumption is relatively constant per platelet, the presence of a high total platelet bag content might have stressed the oxygen permeability of the storage container resulting in the lower pO_2 levels in O/N BC-PCs. Dijkstra-Tiekstra et al found that the overnight PC has higher PCO_2, and lactate concentration and lower pH, pO_2, glucose concentration, CD62P expression (until day 5).[7] Philip et al when compared metabolic parameters between F BC-PC and O/N BC-PC and found pO_2 and pCO_2 values of both the products were almost similar.[9] Böck et al presented a paper comparing biochemical and functional properties of both BC-PCs and apheresis derived PCs and saw an increase in pO_2 and fall in pCO_2 levels from day 0 to day 7 in case of BC-PCs.[12] Keegan et al also observed an increase in the pO_2 levels and decrease in pCO_2 levels from day 1 to day 5 in BC-PCs which is in contrary to our study in terms of pO_2 levels but similar for pCO_2.[13]

The plasma volume used to suspend platelets is to maintain buffering capacity while minimizing the risk of volume overload in the recipient. Murphy et al and Adams et al suggested that the PCs may be stored for 5 days with a volume as low as 30 ml without significant changes in vitro platelet characteristics.[14,15] In the present study, although 4% (2/50) of BC-PC units had volume less than 70 ml, but certainly higher than 40 ml, and various studies have shown that a volume >40 ml maintained the pH >6.2.[16]

Swirling correlates with platelet morphology. The presence of swirling indicates discoid morphology and absence is indicative of spherical morphology. Boeri et al and Dijkstra-Tiekstra et al reported swirling effect did not differ much between fresh and overnight BC-PC.[6,7] In our study, swirling was present in all PC components throughout the period of storage indicating that quality of both fresh and overnight BC-PC remains acceptable.

Smaller sample size of our study might limit its application on a larger scale.

CONCLUSION

Stored BC-PC contained higher platelet count which would ensure better quality product. Platelets can be prepared from stored BC, during business hours of following day. This would provide opportunity for better supervision. This would benefit blood banks where the preparation of components is delayed due to longer transportation time either from satellite blood banks or blood donation camps, which is a common problem in India. Also, fewer platelet concentrates are discarded due to low platelet counts, as this method recovers comparatively more platelets. Less number of units requires to be transfused to the patients, since the quality per unit is better, which eventually leads to patients' exposure to fewer donors.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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