Original Article

Are Buffy-coat Pooled Platelet Concentrates an Effective Alternative to Apheresis Platelet Concentrates? An *In vitro* Analysis at a Tertiary Care Center in Northern India

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Abstract

Background: There is a need for platelet products to have the best quality. Apheresis platelet concentrates (PCs) obtained from single-donors PCs (SD-PCs) are considered best but have issues such as feasibility and cost. Buffy-coat pooled PCs (BCP-PCs) are considered an alternative to SD-PCs. This study compares BCP-PCs and SD-PCs for *in vitro* quality parameters and their changes during storage. **Materials and Methods:** Fifteen units of BCP-PCs and 15 units of SD-PCs were prepared. In this study, a pool of five buffy coats was prepared. Fifteen units of BCP-PCs were analyzed on day 1 and day 5 of storage, while 15 SD-PCs were analyzed on day 1 while ten units on day 5. The parameters analyzed were volume, hematological parameters, pH, swirling, and sterility. **Results:** The mean platelets count of SD-PCs was found to be significantly higher as compared to BCP-PCs. White blood cells (WBCs) contamination was significantly lower in BCP-PCs as compared to SD-PCs. The mean pH and mean platelet volume of SD-PCs were significantly lower than BCP-PCs. During storage, the mean platelets count of BCP-PCs was decreased significantly while that of SD-PCs nonsignificantly. The mean WBCs count and pH decreased in both BCP-PCs and SD-PCs significantly. All units in both types of PCs were sterile. **Conclusion:** Platelet yield was significantly better in SD-PCs, while mean WBCs contamination was significantly lower in BCP-PCs. BCP-PCs may be preferred in place of SD-PCs in case of nonavailability of apheresis, difficulty in finding a willing donor, or when the cost is of consideration.

Keywords: *Buffy-coat pooled platelet concentrate, quality, single-donor platelet concentrate, transfusion*

Introduction

There is a need for a more efficacious platelets product, for example, good quality with fewer storage changes, minimal risk of transfusion-transmitted infection with a standard dose, low cost, and adequate corrected count increment after transfusion. Various platelet concentrates (PCs) are available such as random donor platelets (RDPs) derived from whole blood, prepared by platelet-rich plasma or buffy-coat method, and PCs derived from apheresis. Although there is no general dictum about the product's superiority in the modern era of transfusion medicine, apheresis PCs are preferred over other PCs because they involve lesser donor exposure with a good yield of platelets. Nevertheless, the cost of apheresis may be a limiting factor in developing countries. Along with the cost, another important variable is the availability of an apheresis facility in the blood center. Although no data are available, this facility is limited only to big cities in India. In fact, the awareness of apheresis donation in India is on increasing trend; still, the availability of this product to clinicians in emergency conditions is challenging.

Some review articles have compared buffy-coat pooled PCs (BCP-PCs) and apheresis or single-donor PCs (SD-PCs) in various quality parameters.[1,2] In the latest Drugs and Cosmetics Act amendment of the year 2020, pooled PC is listed as a separate component in which up to six units of platelets, either in the form of buffy coats or platelet units, can be pooled to make a final pooled product.[3] In Europe, BCP-PCs are quite prevalent.[4] However, there are very few studies from developing countries. If they fulfill the quality criteria, BCP-PC can be an excellent alternative to SD-PCs.

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Therefore, in this study, the BCP-PCs were compared to SD-PCs for sterility, *in vitro* quality parameters, and their changes during storage.

Materials and Methods

This study was done at Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, a leading tertiary care center in Northern India. Ethical clearance was taken from the institute's ethics committee for this study. Fifteen units of BCP-PCs and 15 units of SD-PCs were prepared. Fifteen units of BCP-PCs were analyzed on day 1 and day 5 of the storage. Fifteen units of SD-PCs were analyzed on day 1, while 10 units at day 5 (due to issue to the patients). The parameters analyzed were volume, platelets count per unit, white blood cells (WBCs) count per unit, pH, mean platelet volume (MPV), platelet distribution width (PDW), swirling, and sterility. The blood donors were selected per the Directorate General of Health Science India criteria.[5] For the preparation of BCP-PCs, 450 milliliters (ml) of whole blood was collected in quadruple top and top blood bags (Terumo BCT, USA). Five units having the same blood group were centrifuged (Cryofuge 6000i, Thermo Scientific, USA) within 4 h of collection at 3250 RPM for 9 min at 22°C. These bags were processed using a component extractor (TACE – Terumo BCT, USA), and a buffy coat of 50 ml was collected. Later, these buffy coats were hung overnight. A pooling kit (Teruflex BP – kit with Imugard III – S leukocyte filter, Terumo BCT, USA) was used for buffy-coat pooling. These five buffy coats were pooled in the pooling bag the next day. The empty bags, after pooling, were rinsed with plasma, and approximately 120 ml of plasma was added to the same pooling bag. This pooling bag was stored for an additional hour to disaggregate platelets and centrifuged at 1250 RPM for 7 min at 22°C. The PC was obtained by manually expressing the pooling bag into the platelet storage bag which was connected to the pooling bag by the platelet-leukocyte filter. This PC was then stored undisturbed for half an hour at room temperature and then finally stored in a platelet agitator. Apheresis PCs were prepared by cell separator AMICUS (Fresenius Kabi's, Germany).

The total samples analyzed were 55 (BCP-PCs, 15 on days 1 and 5 and SD-PCs, 15 on day 1 and 1 on day 5). Each time samples were taken after properly mixing the bag and adequately stripping the segments. Volume was assessed after preparation by subtracting the weight of the empty bag and dividing by the specific gravity of platelets, i.e. 1.04. WBCs count was measured by manual Nageotte chamber using the diluent Turk's fluid. Platelets count, MPV, and PDW were assessed by Sysmex–Kx-21 cell counter (Sysmex Corporation, Japan). pH was measured by the pH meter. Sterility testing was done by BacT/Alert (bioMérieux, France). Swirling was evaluated manually and graded 0, 1, 2, and 3.[6] Statistical evaluations were done by the Mann–Whitney test for comparing the SD-PCs and BCP-PCs and by the Wilcoxon's-signed rank test for comparing the changes in the parameter values between day 1 and day 5 for BCP-PCs and SD-PCs both. $P < 0.05$ was considered statistically significant. The number and the percentage of the units passing the latest national quality criteria were also analyzed.[7]

Results

The results of mean, standard deviation, and range of each parameter such as volume, platelets count per unit, WBCs count per unit, pH, MPV, and PDW are summarized in Table 1. All units were sterile. Swirling was present in all units. In BCP-PCs, Grade 3 and 2 swirling were present in 80% and 20% of PC units, respectively, at day 1, while 73% and 27% of PC units, respectively, at day 5. In SD-PCs, Grade 3 and 2 swirling were present in 93% and 7% of PC units, respectively, at day 1, while 87% and 13% of PC units, respectively, at day 5. The comparison of the units in reference to recent national quality criteria is shown in Table 2.

Discussion

The buffy-coat pooling can be done from a pool of 4–6 buffy coats with the addition of a plasma unit or platelet additive solution. Different centers have performed different preparation methods for the pooling of buffy coats. The plasma volume should be adequate to maintain pH by its buffering capacity throughout storage.[8] In other studies, the mean volume of BCP-PCs was variable.[9,10] The volume difference between our study and other studies may be due to the difference in the preparation method used for pooling the buffy coats. In our study, only 120 ml of plasma was added to a pool of five 50 ml buffy coats. In our experience, it was found that by adding lesser plasma during the pooling stage, the final volume of the product could be kept comparable to SD-PCs.

The mean platelets count of SD-PCs was found to be significantly higher as compared to BCP-PCs. For plateletpheresis, donors with platelets count >150,000/µl were selected, while BCP-PCs were prepared by whole blood donors irrespective of the platelets count. The mean platelets count of BCP-PCs varies in different studies. It depends on the platelets count of the population, processing techniques, and counting techniques. A previous study from our center demonstrated a comparatively low platelets count of the population (150,000/µl–200,000/µl).[11] Another study from India by Chatterjee *et al*. showed a higher count of BCP-PCs than our study, but the blood bags used in their study were top and bottom bags, while in our study, top and top bags were used.[12] The top and bottom technique allows better removal of a buffy coat than the top and top bags and thus higher platelets count.[10]

WBCs contamination was significantly lower in BCP-PCs as compared to SD-PCs. The reason could be the

Table 1: Comparison of buffy-coat pooled platelet concentrates and single-donor platelet concentrates

Parameters	Days	Sample number	BCP-PCs	SD-PCs	*P**
Volume (mL)		*n*=15	227±13.9 (193.4–247)	206.8±15.6 (182–230)	
Platelets count (10^{11}/unit)	1	15	2.68±0.41 (1.76–3.4)	3.25±0.65 (2.01–4.2)	0.039536
	5	BCP-PCs (*n*=15), SD-PCs (*n*=10)	2.51±0.38 (1.68–3.2)	3.12±0.58 (2.12–4.0)	0.039536
*P***			0.006	1.000	
WBCs count (10^6/unit)	1	*n*=15	0.70±0.38 (0.23–1.7)	3.37±4.86 (0.99–20.3)	0.000223
	5	BCP-PCs (*n*=15), SD-PCs (*n*=10)	0.47±0.31 (0.13–1.4)	3.17±4.7 (0.74–16.3)	0.000116
*P***			0.001	0.014	
pH	1	*n*=15	6.83±0.15 (6.54–7.0)	6.76±0.17 (6.52–7.0)	0.1146
	5	BCP-PCs (*n*=15), SD-PCs (*n*=100)	6.59±0.17 (6.31–6.8)	6.4±0.12 (6.14–6.5)	0.039536
*P***			0.006	0.012	
MPV (fL)	1	*n*=15	12.3±0.85 (10.8–13.8)	9.62±1.46 (7.4–12.7)	0.000276
	5	BCP-PCs (*n*=15), SD-PCs (*n*=10)	11.95±0.85 (10.6–13.7)	9.36±1.29 (7.2–11.5)	0.000794
*P***			0.057	1.000	
PDW (fL)	1	*n*=15	19.6±2.7 (15.2–24.2)	13.13±2.53 (9.7–17.1)	0.000174
	5	BCP-PCs (*n*=15), SD-PCs (*n*=10)	18.77±2.01 (15.1–22.7)	13.42±3.17 (8.6–18.4)	0.000712
*P***			0.132	1.000	

**P* value shows the statistical difference between BCP-PCs and SD-PCs; ***P* value shows the storage effects on counts and other parameters within BCP-PCs and SD-PCs between day 1 and day 5. All values are shown in mean±SD. The range is shown in bracket. BCP-PCs: Buffy-coat pooled platelet concentrates; SD-PCs: Single-donor platelet concentrates; SD: Standard deviation; WBCs: White blood cells; MPV: Mean platelet volume; PDW: Platelet distribution width

Table 2: Analysis of buffy-coat pooled platelet concentrates and platelet concentrates obtained from single donors by the quality criteria of National Standards for Blood Centers and Blood Transfusion Services Second Edition 2022

Parameters	BCP-PCs		SD-PCs	
	Specification	Percentage of PCs passing the criteria	Specification	Percentage of PCs passing the criteria
Platelet count/unit (at day-1)	>2×10^{11}	15 (100)	>3×10^{11}	12 (80)
pH at the end of storage (at day-5)	>6	15 (100)	>6	10 (100)
WBCs count/unit (at day-1)	<5×10^6	15 (100)	<5×10^6	13 (86.6)

PCs: Platelet concentrates; BCP-PCs: Buffy-coat pooled PCs; SD-PCs: Single-donor PCs; WBCs: White blood cells

platelet-leukocyte filter in the buffy-coat pooling kit. Amicus cell separator claims to have leukoreduced PCs without filtration.[13] Schrezenmeier and Seifried mentioned the equivalence of both products in nonallosensitized recipients.[2] However, only WBCs contamination was seen in our study, and further studies are needed to see the clinical effects of WBCs in transfused patients. The mean pH of SD-PCs was significantly lower than BCP-PCs at the end of storage. However, on day 1, the difference was not significant. Paglia *et al*. observed that glucose consumption is double in apheresis PCs compared to buffy-coat PCs.[14] In our study, 100% of the BCP-PCs units maintained pH according to criteria seven at the end of storage.

The mean MPV of BCP-PCs was significantly higher as compared to SD-PCs. Similar findings have been previously documented in a study by Albanyan *et al*.[15] However, this observation of lower MPV had been explained in a study by Albanyan *et al*. in which the author commented on the possibility of collection of smaller platelets by apheresis. In contrast, platelets in buffy-coat PCs are more significant and functional population of platelets.[15] The composition and volume of platelets are heterogeneous. Smaller platelets are regarded as older. As compared to smaller platelets, large platelets have approximately 2.1 times amino acids and twice the enzymatic activity involved in carbohydrate metabolism.[16] The higher PDW represents more anisocytosis of BCP-PCs as compared to SD-PCs.

Buffy-coat pooling involves the pooling of units and, therefore, the pooling of infectious agents. It also involves more processing techniques, so it is argued to have comparatively more bacterial contamination than apheresis PCs.[17] However, in our study, we did not find any positive unit in BCP-PCs or SD-PCs, which aligns with the results by Chatterjee *et al*., who also did not find bacterial contamination in both BCP-PCs and SD-PCs.[12] However,

Perez *et al*. reported a threefold increase in sepsis following transfusion of pooled PCs than apheresis PCs.[18]

On storage, there is a decrease in platelets count owing to several reasons.[19] In our study, the mean platelets count of BCP-PCs decreased significantly while that of SD-PCs decreased nonsignificantly. The similar finding regarding BCP-PCs had been shown in other studies.[9,15] On the contrary, a study by Albanyan *et al*. showed that the mean platelets count of SD-PCs increased over 5 days of storage, while Wagner *et al*. showed nonsignificant changes.[15,20]

The mean WBCs count decreased in both BCP-PCs and SD-PCs significantly. The reason could be the apoptosis of WBCs.[21] The mean pH of both BCP-PCs and SD-PC decreases significantly with storage. Bertolini *et al*. showed similar findings,[22] while Albanyan *et al*. showed that the pH of BCP-PCs increased with storage.[15] No reason was provided for this alkalinity of BCP-PCs with storage. It is established that the pH of platelet components decreases during storage due to glycolysis and the production of lactate.[23] Regarding SD-PCs, Albanyan *et al*. showed no difference in mean pH over 5 days of storage in their study,[15] while Wagner *et al*. found a nonsignificant decrease in mean pH.[20] The mean MPV and mean PDW changes between days 1 and 5 were not significant for both BCP-PCs and SD-PCs in our study.

On looking at the other aspects of setting a system of either BCP-PCs or SD-PCs, in our experience, we found that the cost of buffy-coat pooling is about one-third to half of the cost of apheresis. Apheresis requires expensive equipment, trained personnel, stringent licensing, and additional space. On the other hand, buffy-coat pooling requires a buffy-coat pooling kit, a sterile connecting device, and easy licensure only. Published literature mentioned the increased risk of transfusion-transmitted infection (TTI) after pooling.[24,25] However, generally, many factors influence the risk of TTI, so there should not be the multiplication of risk using more number of pooling units. Schrezenmeier and Seifried demonstrate a hypothesis of distribution effect by apheresis PCs that if an apheresis donor repeatedly donates in his window period and may have more chances of infections.[2]

At the time of the study, pooled platelets were not listed in the scope submitted to the Drug and Cosmetic Act and its amendment, so the *in vivo* analysis could not be done. In the latest Drugs and Cosmetics Act amendment of the year 2020, pooled platelets concentrate was listed as a blood component so enabling to do *in vivo* study after licensure. Another limitation was the lack of biochemical and activation parameters.

Conclusion

On comparing BCP-PCs with SD-PCs, we found that mean platelets yield was significantly better in SD-PCs, while mean WBCs contamination was significantly lower in BCP-PCs due to leukofiltration. The mean MPV and mean PDW were higher in BCP-PCs than SD-PCs, while BCP-PCs maintained better pH than SD-PCs. Swirling grades were almost similar in both methods. In our study, we did not find bacterially contaminated units in any unit of any PCs. BCP-PCs may be preferred in place of SD-PCs in case of nonavailability of apheresis, difficulty in finding a willing donor, or when the cost is considered.

Ethical statement

The study was approved by the institutional Ethics Committee of Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow (Approval No. 2012-163-MD-EXP).

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Conflicts of interest

There are no conflicts of interest.

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