Original Research Article

Quality assessment of platelet concentrates: A comparative study using three different methods

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Abstract

Background: Blood component therapy is a relatively new concept in developing countries and quality control is the most important parameter to assess the efficacy of each blood component. This study was done to assess ex-vivo quality of platelet concentrates prepared by three different methods.
Materials and methods: In our study, a total of 105 platelet concentrates were prepared by three different methods- viz., buffy coat poor-platelet concentrate (BC-PC), platelet rich plasma-platelet concentrate (PRP-PC) and apheresis-PC (PRP-PC, BC-PC and apheresis-PC: 40, 40 and 25 units respectively.) In-vitro quality of platelet concentrates were assessed by observing volume, swirling, platelet count per bag, WBC count per bag and pH changes.
Results: BC-PC and PRP-PC units were comparable in terms of swirling, volume, platelet count per unit, WBC contamination and pH. Variation in volume was more in BC-PC. Apheresis-PC units showed better swirling and platelet count.
Conclusion: It is concluded that ex-vivo quality of platelet concentrates prepared by BC-PC, PRP-PC and Apheresis-PC are acceptable.
Keywords: Buffy coat poor-platelet concentrate, platelet rich plasma-platelet concentrate, apheresis platelet concentrate.

1. Introduction

Platelets represent a crucial part of the normal primary homeostasis of living organisms by contributing to the maintenance of vascular integrity and responding quickly to a vascular injury. Until 1970, thrombocytopenic bleeding was the major cause of serious morbidity and mortality in patients undergoing intensive chemotherapy for malignancy.[1]

Existence of particles in blood smaller than leukocytes and erythrocytes were described clearly between 1865 and 1877, without understanding their origin, significance and function. In the year of 1881-1882, Giulio Bizzozzero was the first to establish their central role not only in physiological hemostasis, but also in thrombosis.[2] First case report of increased platelet count in a thrombocytopenic patient was described by Duke in the year of 1910 by giving large amount of fresh blood transfusion. Ready availability of platelet become possible when Dr. Scott Murphy and Dr. Frank Gardner (reported in

1969) provided evidence that platelet can be stored at 22±2 degree centigrade for three days and still maintain their hemostatic function.[3]

Generally two types of platelet concentrates are available for transfusion, first one is the co-product of normal blood donation i.e. random donor platelets (RDPs) and the other is single donor platelets (SDPs)/ apheresis platelet concentrate. SDPs are collected by thrombocytapheresis from voluntary donors with the help of an automated cell separator. RDPs are prepared by two methods- platelet rich plasma-platelet concentrate (PRP-PC) and buffy coat poor-platelet concentrate (BC-PC). The basic principle behind preparation of components from whole blood is that each component has its specific gravity and by applying centrifugation, each component is separated and removed, thus allowing the transfusion of desired component according to the need of the patient. The recommended shelf life of platelet concentrates in presently available platelet storage bags are 5 days at 22±2°C with continuous agitation. The platelets undergo various storage changes starting from collection, processing to storage and the underlying conditions within the patients, which may affect the therapeutic benefit to the recipients.[3]

In-vitro platelet quality can be assessed by using certain parameters viz. swirling, volume, platelet count, WBC count per bag and pH changes; and in vivo by using corrected count increment (CCI) and percentage recovery (PR) at 1 hour and 24 hours post transfusion which assesses the functional platelets in circulation. If the CCI at 1 hr and 24 hours is <7500 platelets/$\mu L/m^2$ and <4500 platelet/$\mu L/m^2$ and PR at 1 hour and 24 hours <30% and 20% respectively on two consecutive occasions, then it indicates platelet transfusion refractoriness.[4]

Blood component therapy is an apparently new concept in eastern part of India and quality control is the most important parameter to assess the benefit of each blood component specially the platelet concentrates. Thus it is required to study the ex-vivo quality of platelet concentrates prepared by different method to establish the maximum therapeutic benefits to the patients.

2. Materials and methods

After approval from institutional ethical committee and obtaining written informed consent of the donors, this prospective observational study was conducted in Department of Immuno-Hematology and Blood Transfusion Medicine in Medical College and Hospital, a tertiary care medical college hospital in eastern India. Total 105 donors-voluntary blood donors, replacement blood donors and selected blood donors (for SDP only), were enrolled for this study *(*40 for BC-PC, 40 for PRP-PC and 25 for SDP). SDP was always collected from previously selected blood

donors. Donors ageing between 18-65 years, having weight >45 Kg (>60 Kg for SDP donors), hemoglobin >12.5 gm/dl and platelet count >150 X 10^3/microliter (for SDP donors) were included in this study. Those taking medications such as aspirin, anti-platelet drugs, NSAIDs, recent antibiotics and having transfusion transmitted infection (TTI) were excluded.

2.1 Quality assessment of platelets

The quality assessment of single donor platelets (apheresis-PC) and random donor platelets [platelet rich plasma-platelet concentrate (PRP-PC) and buffy-coat poor-platelet concentrate (BC-PC)] were done. A proposed total of 105 platelet units, (PRP-PC, BC-PC and apheresis-PC: 40, 40 and 25 units respectively) were selected randomly and tested for the following parameters:
1. Platelet concentrate volume
2. Swirling
3. Platelet count per bag
4. WBC count per bag
5. pH changes

Sample collection: 2-3 ml of sample from platelet concentrates either SDP or RDP was collected aseptically in a K_2EDTA vial.

1. Platelet concentrate volume:

The volume was determined by subtracting the weight of the empty bag from that of full bag. To convert weight to volume, the resultant weight was divided by specific gravity (1.03 for PRP-PC and 1.06 for BC-PC). Platelet concentrate volume (ml) = (Wt. of the full bag − Wt. of the empty bag)/ Specific gravity

2. Swirling

The swirling was evaluated by examining the units against light at 1 hr, 24 hr and 72 hr respectively 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: Clear homogenic swirling in all part of the bag.
- Score 3: Very clear homogen swirling in all part of the bag.

3. Platelet count per bag :

The platelet count in the bag was done either manually on a Neubauer counting chamber or automated counter.

Method for manual platelet counting: 1:20 dilution was made by adding a 50 µl sample of platelet concentrate into 950 µl of lysing fluid (1% aqueous ammonium oxalate) and kept for 10 to 15 minutes at room temperature with intermittent mixing. The Neubauer counting chamber was charged with diluted sample and kept for another 20 minutes in a moist Petri dish.

Platelet count (per ml)

= [No of cells counted/ Volume of the chamber (µ*l*)] × dilution × 1000

= [N/ 0.02] × 20 × 1000

= N × 10^6

Platelet count per bag = N × 10^6 × platelet concentrate volume

4. WBC count per bag

WBC count per bag was performed in automated cell counter or manually using a Neubauer counting chamber and WBC diluting Turk's fluid [lysing fluid] (Gentian violet and 2% glacial acetic acid).

Method for manual WBC counting: 1: 2 dilution was made by adding an equal amount of whole blood (500 µl) into lysing fluid (500 µl), which was mixed for 2 minutes and the Neubauer counting chamber was charged. Charged chamber was left for a further 2 minutes to settle the WBCs and counting was done in four large squares of the chamber under microscope (x 40 objective).

WBC count (per ml)

= [No of cells counted/ Volume of the chamber (µ*l*)] × dilution × 1000

= [N/ 0.04] × 2 × 1000

= N × 5000

WBC count per bag = N × 5000 × platelet concentrate volume

5. pH changes

Changes in pH were analyzed at 1 hr, 24 hr and 72 hr respectively by pH indicator paper (Merck Specialities Private Ltd, Mumbai; India).

Scoring was done on the basis of parameters (i.e., swirling, volume, platelet count, WBC count and pH changes) taken for quality control evaluation for platelet concentrate units. Score was given according to number of parameters fulfilled by each unit, for example, score 5 or 4 was given to those units which fulfilled 5 or 4 recommended quality control parameters etc.

3. Results and analysis

The quality parameters of 105 platelet concentrates (40 for BC-PC, 40 for PRP-PC and 25 for apheresis PC) were studied for checking the quality control of platelet concentrate prepared by different methods. Table 1 shows the platelet concentrate volume, platelet count per bag and WBC count per bag expressed in Mean±SD (SD = standard deviation) format.

Table 1: Quality parameters of platelet concentrate prepared by different methods

Quality parameters	Platelet concentrate prepared by different methods		
	BC-PC	PRP-PC	Apheresis PC
Platelet concentrate volume (ml)	63.70±14.30	59.40±10.21	209.80±34.67
Platelet count per bag	3.1±1.1 x 10^{10}	2.1±0.9 x 10^{10}	2.3±0.58 x 10^{10}
WBC count per bag	3.2±2.1 x 10^7	1.7±1.0 x 10^7	1.7±1.1 x 10^8

There was no significant change in swirling phenomenon and pH as observed during 1 hr, 24 hrs and 72 hrs of storage by two different observers. There were significant differences in pre and post donation platelet count in 22 volunteers who had donated their platelets for apheresis (Table 2). The post donation platelet counts could not be taken in three of these apheresis donors.

Table 2: Comparative analysis of pre and post donation platelet count

	Pre donation count (/µL)	Post donation count (/µL)	P value[*]
Apheresis donors	197.65±39.62 x 10^3	170.09±39.77 x 10^3	<0.001

* Student's paired t test used, $p < 0.05$ taken as significant

Comparison between the three groups of platelet concentrates did not show any significant difference of platelet count between BC-PC & PRP-PC ($p > 0.05$). There were significant differences between mean value of BC-PC and apheresis-PC (APC), PRP-PC and apheresis-PC ($p <$ 0.05) as done by ANOVA (Analysis of Variance) followed by Tukey's post hoc test (Table 3). Similar post hoc comparison in WBC contamination between the three groups of platelet concentrates showed no significant difference (Table 3).

Table 3: Post hoc comparison of the parameters between three groups

		P value of WBC count[#]	P value of platelet count[#]		
			1 hour	24 hours	72 hours
BC-PC	PRP-PC	0.520	0.451	0.732	0.512
	APC	0.520	<0.001	<0.001	<0.001
PRP-PC	BC-PC	0.520	0.451	0.732	0.512
	APC	0.520	<0.001	<0.001	<0.001
APC	BC-PC	0.520	<0.001	<0.001	<0.001
	PRP-PC	0.520	<0.001	<0.001	<0.001

ANOVA followed by Tukey's post hoc test used, $p < 0.05$ taken as significant

Scoring was done on the basis of quality control parameters as discussed above (i.e., swirling, volume, platelet count, WBC count and pH changes) and expressed in n (%) format in table 4.

Table 4: Scoring of units

Scoring	Platelet concentrate prepared by different methods [n (%)]		
	BC-PC	PRP-PC	Apheresis PC
1	0 (0%)	0 (0%)	0 (0%)
2	0 (0%)	0 (0%)	0 (0%)
3	5 (12.5%)	5 (12.5%)	2 (8%)
4	30 (75%)	34 (85%)	17 (68%)
5	5 (12.5%)	1 (2.5%)	6 (24%)
Total	40 (100%)	40 (100%)	25 (100%)

75% (30/40) of BC-PC units have scored 4 out of 5 and 12.5% (5/40) scored 5 out of 5. 85% (34/40) of PRP-PC units have scored 4 out of 5 and 2.5% (1/40) scored 5 out of 5. Similarly, 68% (17/25) of APC units have scored 4 out of 5 and 24% (6/25) scored 5 out of 5.

4. Discussion

Functions of circulatory transfused platelets depend upon ex-vivo storage lesion and the status of the in-vivo environment of transfused individuals.[5, 6] Platelet concentrates, gently prepared and transfused within 24 to 48 hours of donation without significant storage interval, have uniformly high recovery, good survival and preserved function. This is consistent with other studies by Norol F *et al* [7] and Bock M *et al.*[8]

Higher level of platelet activation, about 30%, is noted in long time storage condition.[9] Generally 5 days storage of platelet concentrates are practiced in developed counties. In our department platelets are usually used within 24 to 72 hours of preparation. The maximum storage time of platelet concentrates in our department as observed during this study was 72 hours.

Quality assessment of platelet concentrates is an important step to evaluate in-vitro functional viability, post transfusion survival and recovery in the recipients. Various parameters are used for assessment of ex-vivo function viz. swirling, pH, volume, platelet count, WBCs contamination. Although other parameters can also be used, such as, measurements of ATP, membrane glycoprotein levels (P-selectin, GP Ib, GP IIb-IIIa) etc., these tests are complex, hazardous and lacks standardization. The *in-vivo* viability of a transfused platelet product is determined by the percentage of the transfused platelets recovered in the recipient's circulation immediately after transfusion (% recovery) and by the life span in circulation of these recovered platelets (survival).[10]

Commonly used methods for evaluation of in-vivo viability (i.e., therapeutic efficacy) of platelets are corrected count increment (CCI) and percentage recovery (PR).

In our study, platelet concentrates were prepared by three different methods-viz, BC-PC, PRP-PC and apheresis-PC. In-vitro quality of platelet concentrates were assessed by observing volume, swirling, platelet count per bag, WBC count per bag and pH changes. A total of 105 PCs (PRP-PC, BC-PC and apheresis-PC: 40, 40 and 25 units respectively) were enrolled randomly.

The platelet concentrates were stored at 20-24° C with continuous agitation at 70 oscillations/ min (Terumo Penpol, composafe, platelet agitator PA300), as recommended, until the time of issue.

Murphy *et al* [11] indicated that PCs stored at a reduced volume of 30 ml for 5 days in a polyolefin container (PL-732, Fenwal, Deerfield, IL) had reduced post transfusion percentage recoveries compared to those PCs stored at a volume of 50 ml or more. Adams *et al* [12] have suggested the PCs might be stored for 5 days with a volume as low as 30 ml without significant changes of in-vitro platelet characteristics that were believed to reflect platelet viability and hemostatic functions. Various other studies [13, 14] have shown that a volume > 40 ml maintained the pH > 6.2. Each platelet function and viability was maintained after 5 days of storage at room temperature with continuous agitation. Higher volume did not have any deleterious effect on platelet function and maintain the pH throughout the storage period by its buffering action. In the present study, mean volume of BC-PC, PRP-PC and apheresis-PC were 63.70±14.30 ml, 59.40±10.21 ml and 209.80±34.67 ml and ranged from 35-102 ml, 45-82 ml and 160-298 ml respectively. Though 75% (30/40) of BC-PC units had volume less than 70 ml, but it was certainly higher than 40 ml except in 1 unit, where it was 35 ml. The mean value of our PRP-PCs and BC-PCs are comparable to those reported by Fijnheer *et al*.[15] However, the range and standard deviation (SD) in our units were wide, thus needing more standardization and probably automation. Hirosue *et al* [16] also found no significant difference in the mean volume of PRP-PC and BC-PC.

Visual inspection of swirling correlates with platelet morphology. The presence of swirling indicates

discoid morphology and absence is indicative of spherical morphology. In our study swirling score of 3 or more were observed in all units of PRP-PC, BC-PC and apheresis-PC units. Bertolini *et al* [17] reported that fresh PCs have positive swirling in 83% of units and negative in only 2%, the rest having intermediate swirling.

During the preparation of PCs there is deterioration of platelet function manifested by abnormal shape changes, aggregation and secretory response. The main cause of deterioration of platelet function during preparation is lesions associated with preparative manipulation and storage. After 5 days of storage, Saran RK *et al* [4] did not find any difference in either type of PCs regarding morphological changes as well as in vivo survival**.** In our study the shape and morphology of the stored platelets could not be identified directly, rather, assessed indirectly by 'swirling'.

The mean platelet count of BC-PC, PRP-PC and apheresis-PC were $3.1 \pm 1.1 \times 10^{10}$/unit, $2.1 \pm 0.9 \times 10^{10}$/unit and $2.3 \pm 0.58 \times 10^{11}$/unit and ranged from $1.3\text{-}5.88 \times 10^{10}$/unit, $0.64\text{-}5.16 \times 10^{10}$/unit and $0.90\text{-}3.07 \ 10^{11}$/unit respectively. The mean platelet count of BC-PC and PRP-PC were comparable and statistically no significant differences were observed. Hirosue *et al* [16] reported higher platelet count in PRP-PC units than BC-PC. Murphy *et al* [11, 14] also found that platelet recovery was higher in patients receiving PRP-PC (60-70%) than those with BC-PC (40-60%) transfusion. No such difference was observed in the present study and the mean platelet count/unit in all three types of platelet concentrates were comparable and statistically no significant difference was observed (Table-3).

WBCs in PC have a detrimental effect on the storage medium, resulting in a significant drop in pH (<6.2), increase in glucose consumption, lactic acid production and LDH release during storage. As a result, in the PCs with high concentration of leukocytes, the platelet condition up to 5 days of storage also significantly affected, as reflected by a high excretion of β-TG, loss of platelet nucleotides, decreased ability to incorporate H-adenosine [13] and poor platelet morphology.[14] In addition to these, transfused passenger leukocytes during platelet therapy may be associated with a variety of adverse reactions, including alloimmunization to leukocyte antigens, febrile non-hemolytic transfusion reaction (FNHTR), refractoriness to platelet transfusion, severe pulmonary dysfunction, transfusion associated graft versus host disease (Ta-GVHD), the transmission of cytomegalovirus (CMV) and immune modulation.[18] Platelet concentrates made from individual units of fresh whole blood may contain $0.5\text{-}2.5 \times 10^8$ WBC/unit.[19] Apheresis platelets that have been harvested using old instruments may contain up to 5×10^9

leukocytes, while apheresis platelets obtained using more recently available instruments contain 10^6 to 5×10^8 WBC/unit.[20] In this study WBC counting was done on fresh units at day zero and a total of 105 units were analyzed. The mean WBC count in BC-PC, PRP-PC and apheresis-PC units were $3.2 \pm 2.1 \times 10^7$/unit, $1.7 \pm 1.0 \times 10^7$/unit and $1.7 \pm 1.1 \times 10^{8}$/unit and ranged from $0.7\text{-}9.5 \times 10^7$/unit, $0.66\text{-}4.5 \times 10^7$/unit and $0.02\text{-}6.68 \times 10^{8}$/unit respectively.

The pH decreases during storage depends on the stabilizer in plastic platelet storage bags and storage conditions used. Increased platelet glycolysis resulting in a fall in pH to levels approaching 6.0 in PC stored in plasma is associated with substantial loss of viability.[21] The majority of fresh, un-stimulated platelets are discoid with few projections. If pH does not fall to less than 6.8, platelet volume decreases by approximately 10% during first three days. However, if pH falls below this level, there is a progressive rise in platelet volume and decrease in density suggesting swelling due to influx of extracellular fluid. The swelling begins at pH of 6.8 and reaches its maximum at a pH of 6.0, at which point platelet volume is increased almost two-fold. At the same time, there is an accelerated rate of disc-to-sphere transformation so that only swollen spheres are seen if pH reaches 5.7 to 5.9. These changes are almost entirely reversible if pH stays above 6.1, but they are not reversible if pH falls below 6.1. These morphological observations correlate well with the results of viability in-vivo and suggested in the study by Murphy S *et al*.[22]

In our study all 105 units were analyzed for pH changes. Their mean pH was 7 and no significant difference was observed among three types of platelet concentrate. Synder *et al* [23] reported that at 5 days of storage, the pH of all PC was >7.0 and platelet count was above 5.5×10^{10} per bag (except for the PL-732) with the 6 rpm vertical rotator. Bannai *et al* [24] reported that at pH 6.85, vertical agitation platelet damage was minimal up to 12 hours, but increased from 36 hours after the pH increased to 7.3 to 7.4 and these results indicate that vigorous agitation is not as injurious at low pH. However in the present study since platelets were issued within 24 to 72 hours of preparation, the pH on further storage was not assessed.

Out of total 105 units evaluated, 11.42% (12/105) had score 5 i.e., fulfilled all 5 parameters of quality control and 77.14% (81/105) units had score 4. On individual analysis, maximum number of apheresis-PC units had score 5 (24%, 6/25) followed by BC-PC units (12.5%, 5/40), but only 2.5% (1/40) of PRP-PC units fulfilled all 5 desired quality control criteria. This overall difference in score 5 and 4 was basically due to variation in platelet count in PRP-PC units.

5. Conclusion

BC-PC and PRP-PC units were comparable in terms of swirling, volume, platelet count per unit, WBC contamination and pH. Variation in volume was more in BC-PC than PRP-PC units and this suggests need for further standardization for preparation of BC-PC. Apheresis-PC units showed better swirling and platelet count than PRP-PCs and BC-PCs. All the platelet concentrates units had pH well maintained. Thus, it is concluded that ex-vivo quality of platelet concentrates prepared by BC-PC, PRP-PC and Apheresis-PC are acceptable. However further large scale study with more standardization is required to better delineate our findings.

Acknowledgements

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