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Regulated upon activation, normal T cells expressed and secreted (CCL5) in platelet concentrate: Role of mode of preparation and duration of storage

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Abstract:

BACKGROUND AND OBJECTIVES: Platelet concentrates (PCs) can be prepared in several different ways, and they can be stored over few days before the use. Regulated on activation, normal T cells expressed and secreted (RANTES) levels in these concentrates may vary depending on the type of preparation and duration of storage of this component. We measured RANTES levels in platelet supernatants in different preparations and with different storage duration.

MATERIALS AND METHODS: Fifteen PCs were prepared by platelet-rich plasma (PRP) and buffy coat (BC) method each. Forty-two single donor platelets (SDPs) were prepared using cell separators Cobe Spectra, Trima Accel, and Amicus. Filtered PCs were prepared using labside and bedside filters. The supernatants were collected after 1, 18, 65, and 112 h of preparation. SDP samples were taken on the 0 day, 3rd day, and 5th day. In filtered PC, pre- and post-filtration samples were taken, and aliquots were frozen at – 56°C for the measurement of RANTES.

RESULTS: RANTES at 1 h was 1210 ± 560 pg/ml in PRP-PC, 1384 ± 463 pg/ml in BC-PC. At 112 h, 1617 ± 451 pg/ml and 1949 ± 134 pg/ml, respectively. In SDP, 0-day level was 1850 ± 278 pg/ml and >2000 pg/ml on 5th day. In prestorage, filtered PC RANTES was 1035 ± 496 pg/ml, and in the poststorage sample, it was 310 ± 508 pg/ml. With bedside filters, presample showed 1243 ± 832 pg/ml and postsample showed 556 ± 748 pg/ml.

CONCLUSION: The concentration of RANTES increased continuously from 1 h to 5 days of storage in all PCs. After 65 h, BC-PC showed higher levels of RANTES compared to PRP-PC. Filtered PRP-PCs appear to be the best in terms of low RANTES to prevent allergic reactions and cultures negative.

Keywords:

Buffy coat, platelet concentrate, regulated on activation, normal T cells expressed and secreted, single donor platelet

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Introduction

The transfusion of platelets may be responsible in many nonhemolytic transfusion reactions (FNHTR). They contain several mediators that belong to a family of proinflammatory cytokines–chemokines that are stored in the platelets.[1] Regulated on activation, normal T cells expressed and secreted (RANTES) belongs to CC chemokine family which are β-chemokine and are released and accumulated in stored platelet concentrate (PC).[2] They are mainly involved in allergic and nonhemolytic transfusion reactions. RANTES is mainly produced by CD8 lymphocytes but its secretion can also be mediated in CD4 lymphocytes after it interacts with activated monocytes. This chemokine is also stored in platelets and the amount of this chemokine tends to increase in PCs with time.[3] The

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aim of this study was to measure the level of RANTES in different types of PC at different time interval of storage. In addition, nowadays, platelets are used in regenerative medicine and sports medicine. The type of preparation of PCs and/or its storage may give products with different levels of this chemokine in the PCs and this may be an important consideration for the use in a particular clinical situation with particular levels of this chemokine.

Materials and Methods

The study was approved by the Institutional Ethics Committee. After taking informed consent, blood donor was selected as per Drugs and Cosmetics Rules, 1945 (amended from time to time).[4] Blood was collected in triple and quadruple bags from donors attending various blood donation camps and donating blood in the center. Information on their age, sex, address, and parameters of medical examination was recorded in the donor registration form which includes the consent of the donor. PCs were prepared within 6 h of blood collection.

Two types of PC were prepared namely platelet-rich plasma (PRP)-PC and buffy coat (BC)-PC using two different principles for separation. Fifteen PRP-PC were prepared from triple bags by centrifuging the bags at 20°C–22°C at light spin ×2000 g for 3 min. The bag with PRP and another satellite bag were centrifuged at 20°C–22°C at heavy spin ×5000 g for 5 min. The supernatant plasma was transferred into another empty satellite bag. Approximately 50 mL of plasma was left with the platelets. PC was left undisturbed at 20°C–22°C for 1 h, and then the platelets in plasma were resuspended by gently mixing for 10 min. Platelets were stored at 20°C–22°C under constant agitation in platelet incubator with agitator.[5]

BC method was employed for the preparation of 15 PCs using "top and bottom" bags and an automatic component extractor Optipress II (M/S Fenwal, IL). Blood was centrifuged at heavy spin for 8 min. After the preparation of fresh frozen plasma and red cell concentrates on Optipress, the primary bag with BC and plasma in satellite bag were left hanging for about 2 h at room temperature (22°C), and then centrifuged at light spin at 22°C. The supernatant plasma with platelets was slowly transferred into the empty bag using extractor.[6]

Single donor platelet (SDP) was prepared by apheresis using three different cell separators Cobe spectra (Terumo BCT, USA), Trima Accel (Terumo BCT, USA), and Amicus (Fenwal, IL, USA). Donors for plateletpheresis were selected according to the mandatory criteria and after informed consent.

Sample collection

Immediately after preparing PC, a volume of 5 mL sample was removed for quality control and cytokine measurement. The total volume was determined, and the pH was measured on the 5th day of storage using pH meter (Mtronics, Mumbai) which was standardized using standard buffers of pH 4.0, 7.0, and 9.0. Platelets and white blood cell (WBC) of all blood components were measured on hematology analyzer MEK 6318K (Nihon Kohden, USA). Nageotte chamber was used for counting WBC after leukoreduction. The samples of 15 PRP-PCs and BC-PCs were collected aseptically after 1, 18, 65, and 112 h of preparation and centrifuged at 3500 rpm at 22°C for 15 min. The aliquots of supernatant were frozen at −56°C for measurement RANTES concentration. Similarly, SDP samples were taken on the 0 day, 3rd day, and 5th day. Cytokine analysis was done using Pepro Tech (NJ, USA) ELISA reagents for human RANTES.

An experimental study was carried out to compare prestorage and poststorage (poststorage) filtration using leukodepletion filter for PC. Initially, the total volume was measured, and 5 mL of aliquot were taken for platelet count and WBC count. The bag was connected to prestorage filter (PALL Medical, NY, USA). The time taken for filtration was recorded and the volume of PC after filtration measured. A second sample of PC was taken after filtration. WBC and platelet count were determined. Both the aliquot of samples were centrifuged at 3000 rpm for 15 min at 22°C. The supernatants were frozen at −35°C and the RANTES level measured using ELISA.

In poststorage filtration, six patients were randomly selected and after informed consent, the blood sample was taken. The details of the patient were recorded in pro forma prepared. Among these, two patients were of dengue hemorrhagic fever. Presample of 5 mL PC to be transfused was taken. WBC and platelet count were determined. Similarly, platelet and WBC count of the patient was determined. The PC was transfused using poststorage filter Imugard III PL (Terumo Penpol, USA). The PCs transfused were of 2–4 days old. A posttransfusion sample of the patient was taken and 5 mL sample of PC after filtration was collected. After measuring WBC and platelet count, the sample was centrifuged at 3000 rpm at 22°C for 15 min and the supernatant stored at − 35°C for RANTES assays.

Results

All donors were male and in the age group of 31.2 ± 7.9 years. pH was above 6 in all PCs. Mean values of various parameters of PC are presented in Table 1. Statistical analysis using "*t*"-test revealed significantly higher WBC count (P = 0.0043) in PRP-PC compared

to BC-PC. Platelet counts in PRP-PC and BC-PC were comparable.

RANTES level was very high in all PCs. There was no sample below 600 pg/ml. The minimum value in 1 h was 797 pg/ml in BC-PC. The values increased progressively in both the types of PC with min value at 112 h, 928 pg/ml and 1589 pg/ml in PRP and BC-PCs, respectively. In BC-PC, there was no sample showing the value in the range of 1701–2000. At 112 h, 86% of units (13/15) had RANTES level >2000 pg/ml in BC-PCs and 40% units of PRP-PCs as shown in Table 2. The comparison between mean values of RANTES in PRP-PC and BC-PC shows significant high values in BC-PC compared to PRP-PC at 112 h and shows significant high values in BC-PC at 112 h compared to 1 h. It may not be out of place to mention here that though BC-PC had lower total leukocyte count, yet RANTES levels at 112 h were significantly high in larger number of donor platelets though mean and SD of RANTES levels were not significantly different in the whole group up to 65 h of collection [Tables 1 and 2].

In SDP, RANTES level was high on the 0th day which was minimum at 1408, 1160, and 786 pg/mL in Cobe, Trima, and Amicus, respectively. On 3rd day, the level was >2000 pg/ml in 100% units prepared using Trima. The mean level on 5th day could not be calculated as all samples showed the values >2000 in Cobe and Trima as shown in Table 3. In Amicus, SDP level was >1700 in 86% units.

In a pilot study, ten PCs were used for prestorage filtration and six poststorage filters were used for the transfusion for estimating RANTES in poststorage filtered PCs. Comparison of WBC and platelet count in both the types of filtered PC is shown in Table 4. There are leukoreduction and loss of platelet after filtration.

RANTES levels were found high in poststorage filtered PC than in prestorage [Table 5].

Analysis on different days in SDP prepared using three cell separators was done using ANOVA showed Amicus system produced highest levels of this cytokine on different days of SDP storage and Cobe produced the lowest amount of this cytokine ($P < 0.005$). Platelet yield was higher with Cobe machine ($P < 0.005$).

Discussion

The WBC count in PRP-PC was $7.4 \pm 3.75 \times 10^7$ and in BC-PC was $3.9 \pm 2.2 \times 10^7$ which was significantly lower $P < 0.0005$. The WBC count is comparable with earlier study of[6] where the WBC count was $6.3 \pm 3.1 \times 10^9$/l in PRP-PC.

In SDP, the WBC contamination was in the range of $0.40–0.44 \times 10^8$/unit. Bayraktaroglu *et al*.,[7] used automated cell counter for measuring WBC contamination in apheresis PC and reported the count 142×10^6/unit which was higher than the present study as the technique

Table 1: Measurement of various parameters in platelet-rich plasma platelet concentrate, buffy coat platelet concentrate, and single donor platelet

Sample	Age (years)	Volume (ml)	pH	WBC/unit	Platelet/unit
PRP-PC	31.2±7.9	60.4±5.1	7.9±0.31	7.4±3.75×10^{7}*	6.05±1.94×10^{10}
BC-PC	31.3±7.1	72.0±6.4	7.9±0.29	3.9±2.2×10^{7}*	6.54±1.81×10^{10}§
SDP-Cobe	32.1±8.1	300±42	7.3±0.32	0.44±0.23×10^{8}	4.1±0.86×10^{11}‡,§
SDP-Trima	31.7±9.8	318±10	7.4±0.26	0.40±0.21×10^{8}	4.26±0.88×10^{11}†
SDP-Amicus	32.3±7.6	322±44	7.4±0.19	0.40±0.06×10^{8}	3.17±0.59×10^{11}†,‡

*T-test between WBC count of PRP-PC and BC-PC is highly significant (P=0.0043), †t-test between platelet count in SDP-Trima and SDP-Amicus is highly significant (P=0.00062), ‡t-test between platelet count in SDP-Cobe and SDP-Amicus is highly significant (P=0.0021), §t-test between platelet count in BC-PC and SDP-Cobe is highly significant (P=0.00001). PC = Platelet concentrate, BC = Buffy coat, SDP = Single donor platelet, WBC = White blood cell, PRP = Platelet rich plasma

Table 2: Regulated on activation, normal T cells expressed and secreted levels in 15 platelet-rich plasma platelet concentrate, and buffy coat platelet concentrate

RANTES level pg/ml	1 h, *n* (%)	18 h, *n* (%)	65 h, *n* (%)	112 h, *n* (%)
PRP-PC				
600-1700	10 (66.67)	8 (53.33)	7 (46.67)	7 (46.67)
1701-2000	2 (13.33)	1 (6.67)	8 (53.33)	2 (13.33)
>2000*	3 (20.00)	6 (40.00)	0 (0)	6 (40.0 0)
Mean±SD	1210±560	1453.41±518	1599.28±467.43	1617.3±451
BC-PC				
600-1700	10 (66.67)	7 (46.67)	8 (53.33)	2 (13.33)
1701-2000	0 (0)	0 (0)	0 (0)	0 (0)
>2000*	5 (33.33)	8 (53.33)	7 (46.67)	13 (86.66)
Mean±SD	1384.56±463.86	1500.90±426.94	1631.23±379.74	1949.27±134.38

*Significant higher number of units showed the values >2000 in BC-PC compared to PRP-PC at 112 h by χ^2 test (P=0.023). SD = Standard deviation, PC = Platelet concentrate, BC = Buffy coat, WBC = White blood cell, PRP = Platelet-rich plasma, RANTES = Regulated on activation, normal T cells expressed and secreted

Table 3: Regulated on activation, normal T cells expressed and secreted levels in 14 single donor platelet prepared using three cell separators

RANTES level (pg/ml)	Day 0, *n* (%)	Day 3, *n* (%)	Day 5, *n* (%)
Cobe			
600-1700	3 (21.43)	1 (7.14)	0 (0.00)
>1700	11 (78.57)	13 (92.86)	14 (100.00)
Mean±SD	1896.86±213.88	1975.29±92.47	>2000
Trima			
600-1700	2 (14.29)	0 (0)	0 (0)
>1700	12 (85.71)	14 (100.00)	14 (100.00)
Mean±SD	1937.71±224	>2000	>2000
Amicus			
600-1700	6 (42.86)	3 (21.43)	2 (14.29)
>1700	8 (57.14)	11 (78.57)	12 (85.71)
Mean±SD	1717.90±398.18	1884±242	1947.43±133.63

SD = Standard deviation, RANTES = Regulated on activation, normal T cells expressed and secreted

Table 4: Comparison of prestorage and bedside filtered platelet concentrate

Platelet concentrate (*n*=10)	WBC ×10^7/unit	Platelet ×10^{10}/unit
Prestorage filtered PC		
Pre	4.7±3.14*	4.8±2.43
Post	0.0014±0.0018*	3.22±1.80
Bedside filtered PC		
Pre	5.48±1.31*	7.5±2.25
Post	0.00017±0.00041*	5.60±2.48

**T*-test shows significant leukoreduction after filtration in prestorage and bedside (*P*=0.0002). WBC = White blood cell, PC = Platelet concentrate

Table 5: Cytokines in pre- and post-storage filtered platelet concentrate

Platelet concentrate (*n*=10)	RANTES pg/ml
Prestorage filtered PC	
Pre	1035.5±496*
Post	310.66±508.2*
Poststorage filtered PC	
Pre	1243.25±832.10
Post	556.65±748.81

**t*-test shows significant decrease in RANTES level after prestorage filtration (*P*=0.005). PC = Platelet concentrate, RANTES = Regulated on activation, normal T cells expressed and secreted

used was different. There is variation in other studies due to the technique used for enumeration and the different units used for interpretation. Monitoring WBC contamination is important as alloimmunization to platelet and leukocyte antigens happen most commonly by transfusion of white cell contaminated blood components.[8] Nageotte chamber is better to count WBC in leukoreduced products.

RANTES levels are very high in all PCs with increasing levels in BC-PC compared to PRP-PC. This may be due to high platelet count in BC-PC. At 112 h, 86% of units had level >2000 pg/ml. Although the count is high in both the types of PC, it is shown in that after 65 h of storage both behave differently with BC-PC showing higher values than PRP-PC.

One of the questions which arises from the present study is why BC-PC even though have lower leukocyte count compared to that of PRP-PC [Table 1] yet the rate of cytokine rise near the end of the study was significantly higher in BC-PC platelets ? RANTES is mainly produced in CD8-positive T cells[3] it is possible that though total leukocyte count may be less in BC-PC, but CD8-positive T cells might have been especially concentrated in BC-PC. However, we have not studied the leukocyte subset differences in the current study.

Bubel *et al*.[9] also demonstrated that RANTES accumulate significantly during PC storage. This study suggests that WBC reduction may reduce FNHTR, but allergic reactions do occur due to RANTES. As studied by Wakamoto *et al*.,[10] RANTES promotes chemotaxis of eosinophils, memory T cells, and basophils, induces histamine release, and stimulates activation of eosinophils mediating allergic reactions.

RANTES levels in PCs are dependent on number of contaminating leukocytes, their subsets and degree of activation of platelets and leukocytes in the concentrates. Various procedures of platelet production results in different levels of leukocyte contamination and may even cause selective enrichment of one type of leukocytes over others. However, this caveat needs to be shown by further experimentation. Platelet themselves can produce RANTES and store them. Hence, late production of RANTES on stored platelets can come either from activated leukocytes, lymphocyte monocyte interactions, or from production of procoagulant materials, and interplay of various cytokines from leukocytes as well as platelets. These interactions may activate platelets moreover contaminating small amount of endotoxin which occurs in PCs as storage time increase.[3,9-12] Can activate many types of cells in the concentrate to produce RANTES.

In India, no study demonstrates cytokine levels in apheresis PC. It is unclear whether WBC in PC is capable of producing cytokines *de novo* or the cytokines are released from degranulation of WBC or even donor WBC may still continue to produce cytokines *in vivo*. The present study investigated RANTES which is platelet derived, accumulation in plasma of apheresis PC. In terms of platelet yield, Cobe spectra proved to give the best platelet yield compared to Amicus and Trima better than Amicus because Cobe spectra have leukoreduction system device, which uses an advanced centrifugal separation procedure to create a fluidized particle bed of platelets to achieve optimal separation between leukocytes and platelets. Compared to Amicus,

the platelet yield in Cobe Spectra and Trima Accel are better as both the cell separators work on the principle of BC and has been proved statistically by ANOVA also. All three cell separators gave leukoreduction satisfactorily.

This study demonstrates increased levels of RANTES in SDP. High levels of RANTES may be associated with platelet activation soon after preparation. Gutensohn *et al*.[11] demonstrated that in apheresis using Amicus, there is activation of platelets and there is interaction with monocytes and granulocytes. After the preparation of SDP with Amicus, lots of big aggregates was found, this finding was also observed by Hagberg *et al*.,[13] who showed that platelets from Amicus have increased fibrinogen receptor activation, increased the size of platelet aggregates, increased the formation of microparticles, increased degranulation, decreased von Willebrand factor receptor surface expression, and also decreased responses to *in vitro* agonist stimulation. In Amicus process, the harvested platelets are stored in a small collection belt within the centrifuge during the entire separation procedure, allowing intimate contact among themselves, and their interaction with the plastic surface of the container. In the belt, the platelets are packed and require vigorous handling to resuspend them, which may account for increased RANTES. This was observed in this study also. In Cobe spectra platelets are transferred continuously to larger platelet storage containers outside the centrifuge. According to Holme and Murphy,[14] such technical differences may be responsible for platelet activation and may affect the quality of PC. In respect of quality, it is important that there should be minimal activation of platelets.

Buffy coat platelet concentrate versus single donor platelet

Comparing the data of BC-PC and SDP RANTES are significantly high in SDP as platelet yield is higher in SDP compared to PC. The data prove that if needed and affordable, SDP is a preferable choice for transfusion due to less donor exposure to the recipient. Comparison between BC-PC and SDP by *t*-test shows a highly significant difference in platelet count, but no significant difference in terms of WBC contamination which shows that SDP is a better option for transfusion.

Filtration at present is the best leukoreduction method that exists. Our experiment proved a significant decrease in WBC after filtration. There was a decrease in platelet count and cytokine level by bedside filtration. The results of Shaiegan *et al*.,[12] showed that prestorage filtration prevents accumulation of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). Similarly, Shanwell *et al*.[15] suggested that prestorage filtration also reduced the levels of IL-1, IL-6, IL-8, and TNF-α in red blood cells during storage. In terms of RANTES, prestorage is better than poststorage as it is done immediately after the preparation of PC. As a result of this, cytokines released in PC is only through the residual WBC that passes through filter in the plasma. In poststorage filtration, the cytokines accumulate during storage of PC. Filtration of PC is carried out during transfusion. There is a time gap when patients are transfused with poststorage filters. WBCs are trapped in the filter, but cytokines already released during storage pass in the patient during transfusion. The disadvantage of poststorage filtration is that it does not remove cytokines or the WBC fragments released during storage, whereas this problem is overcome by prestorage filtration which removes WBCs from the original whole blood unit within few hours after collection. Thus the possibility of transfusion reaction is more using poststorage filters than using prestorage filtered product. However, proangiogenic[16] effects of RANTES may call for stored platelet or BC platelet to be applied locally for wound healing due to ischemic ulcers. In a similar manner, the treatment of osteoarthrosis by PC may call for low-RANTES PCs and same may be true when patients with malignancy need PCs as RANTES promotes tumor progression in general and most tumors also secrete high levels of RANTES.[17] Local PCs injections are used to treat osteoarthritis. It was found to reduce pain and improve the mobility of the joints,[18] there are some suggestions that high levels of RANTES in the joint may encourage cartilage degradation,[19] hence from that standpoint leukoreduced PCs may be a better option. In cases of hemopoietic stem cell transplantation, high levels of RANTES were found to inhibit megakaryocytic differentiation[20] of grafted hemopoietic stem cells and this may delay platelet recovery, in addition, this chemokine can also cause myeloid skewing of hemopoietic stem cells with deficient T-cell production with its attendant consequences.[21] For such condition, again fresh leukodepleted platelets must obviously be the optimum choice.

Conclusion

Platelet count is obviously maximum in SDP but more in BC-PC compared to PRP and filtered PC. The concentration of RANTES increased continuously from 1 h up to 5 days of storage in all PCs. After 65 h, BC-PC showed higher levels of RANTES compared to PRP-PC. The concentration was least in prestorage filtered PC. Comparison of different PCs shows that filtered PRP-PCs appear to be the best in terms of low RANTES to prevent allergic reactions, and BC-PC is better in terms of leukoreduction but informed choices need to be made about the type of preparation and duration of storage of different PCs depending on the indication of its usage.

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Conflicts of interest

There are no conflicts of interest.

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