

## Effect of Storage on Platelet Concentrates

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### Abstract

**Background:** The quality of platelet concentrates (PCs) is an important issue in transfusion therapy. Under blood bank storage conditions, platelets undergo a series of physical, metabolic & physiological changes which influence their lifespan.

**Aim:** To assess effect of storage on the quality of PCs by various parameters in order to increase the lifespan

**Method:** PCs prepared from healthy blood donors were included. The platelet indices including mean platelet volume, plateletcrit and platelet count, CD41 as a measure of function, pH, glucose levels and platelet derived cytokines RANTES (by ELISA) were analyzed. Eleven PCs were included and analyzed for above parameters on Day 0 (day of collection), Day 3 and Day 5.

**Results:** The significant changes in parameters over Days 0, 3 and 5 included:

- 1) Fall in glucose levels: From a mean of 495.3 on Day 0 to 366.7 mg/dl on Day 3. The decrease in levels was statistically significant ( $p < 0.01$ )
- 2) pH fell from 7.68 (Day 0) to 7.27 on Days 3 and 7 on day 5. Two PC bags reached a pH of 6 on Day 5, which is the limit for platelet viability.
- 3) Platelet count and CD 41 antigenicity did not show significant variation ( $p > 0.5$ )
- 4) RANTES levels were markedly increased (11875pg/ml on day 5)

### Conclusion:

Measurement of platelet indices with pH can serve as quality indicator for PC. Marked increase in RANTES indicates lifespan of PCs cannot be extended beyond 5 days. Improvement in technologies in preparation, storage and filtration will enhance quality of PCs.

**Key words:** *Platelet concentrates; Quality; Storage.*

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### Introduction

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The quality of platelet concentrates (PCs) is an important issue in transfusion therapy [1]. Presently, the followed norms allow for platelet storage from 5 to a maximum of 7 days. However, under blood bank storage conditions, platelets undergo a series of physical, metabolic

and physiological changes that have been termed as platelet storage lesion "PSL". This condition depends on several factors: the platelet count, method for preparation of PCs, type of storage bag, number of leucocytes present per cell unit, cytokines release etc. Platelet indices have been used as markers for quality control of PCs, as these reflect storage induced changes in platelets [1].

It has been observed that glycogen cannot be synthesized from glucose by platelets in vitro, and 80% of the platelet glycogen is degraded during the first 24 hours of storage. During storage glucose is converted to lactate and the lactate concentration rises by approximately 2.5mmol/day. Oxygen can enter through the walls of the PC containers. The cells thus derive 85% of their ATP requirement through the tricarboxylic acid cycle (TCA) and 15% of ATP from glycolysis [2]

Apart from quality of the transfused PCs, nonhemolytic transfusion reactions (NHTR) can be a significant problem in transfusion. It has been suggested by various studies that these NHTRs are due to accumulation of cytokines during PC storage. The febrile NHTRs were observed more frequently with (non leucoreduced) platelet transfusions (2-30%) as compared to red cell transfusions (0.5%) [3]. The reason attributed to this was that PCs are stored at room temperature as compared to red cells stored at 1-6 degrees C, which has an inhibitory effect on any cellular activation. The most common pyrogenic cytokines implicated include IL-6, IL-1 and TNF $\alpha$ . In addition, the specific platelet-derived cytokines like RANTES and TGF  $\beta$ 1 are also noted to induce NHTRs [4, 5]. Thus, it can be inferred that leucoreduction may not completely eliminate the cytokine accumulation. There have been few studies on the effect of storage on the platelet membrane glycoproteins which play an important role in the functioning of the platelets.

With this background the objectives of the present study were (a). To analyze morphologic, functional and biochemical parameters to assess the effect of storage of PCs and as indicators of quality of PCs, (b). To apply the results to increase the lifespan of PCs.

## Methods

This was a prospective study. The protocol was approved by IERB. As the parameters to be assessed were on Days 0, 3 and 5, the study was started on Monday and completed on Saturday. The PCs were prepared from healthy blood donors and 11 PCs were included for the study.

### *Method of preparation*

Blood was collected in 450 ml quadruple bags with CPD- A. The PCs were then prepared by the buffy coat technique using Terumo Automated Component Extractor (TACE).

The PCs thus prepared were stored in platelet agitators at 22 degrees C for 5 days. Samples were taken aseptically on Days 0, 3 and 5.

The following parameters were measured in the platelet concentrates during the same days in the automated systems.

1. Platelet count
2. Platelet indices including MPV (Mean platelet volume) (1, 2 were analyzed by the Sysmex XR1800i analyser)
3. pH (Reagent strip method)
4. Glucose (analyzed by the glucose oxidase method)
5. Morphology by Leishman stained peripheral smear examination
6. The measurement of RANTES levels was done by ELISA method.
7. CD41 antigenicity for platelet function by flow cytometry

The above parameters were analyzed for all bags on Day 0 (day of collection), Day 3 and Day 5. four bags were released to patients on Day 3. The remaining bags were released on day 5.

### *Statistical analysis*

For data, comparison between groups was made using paired t test, and association between variables was assessed using Pearson's correlation coefficient.

**Table I: Changes in platelet parameters**

Parameter (Mean)	Day 0	Day 3	Day 5
PH	7.68	7.27	7
Glucose(mg/dl)	495.3	366.7	261.3
Platelet count (x103/ $\mu$ l)	366	1253	989
MPV	8.2	9.4	14
PDW	9.8	11.05	11.56
PCT	0.36	1.2	0.98
PLCR	18.9	22.13	23.4
RANTES pg/ml	486.01	11845.9	
CD41 antigenicity Peak channel value	266.7	251.3	248.5

## Results

Of the 11 PCs studied, 4 were of the blood group A, 2 were B and 5 were of the O group.

The weight of the PC bags ranged from 64 to 86 gms (mean 71.6gm) and the volume ranged from 66.4 to 88.5 ml (mean 73.6ml).

The PCs showed significant changes in various parameters over Days 0, 3 and 5 as summarized in Table I.

### Statistical analysis

The changes in numerical values were compared by the t test for 2 samples assuming equal variance.

The significant changes included:

1. Fall in glucose levels: From a mean of 495.3 on Day 0 to 366.7 mg/dl on Day 3. The decrease in level was statistically significant ( $p < 0.01$ )
2. RANTES levels showed a marked increase from Day 0 to Day 3 ( $p < 0.02$ )
3. pH fell from 7.68 on Day 0 to 7.27 on Day 3 and 7 on Day 5. Though the difference in pH was not statistically significant ( $p > 0.5$ ), 2 PC bags reached a pH of 6 on Day 5, which is the limit for platelet viability.
4. The morphological assessment by smears showed marked swelling of platelets and fragmentation on Day 3 and Day#5. This is probably reflected by the pseudo increase in platelet counts on Days 3& 5.

5. CD41 antigenicity also did not show significant variation ( $p > 0.5$ )

### Transfusion Details

#### Day 3

Four units were transfused to a non Hodgkins lymphoma patient.

The pre count was  $< 5000/\mu$ l. The post transfusion count was  $39000/\mu$ l.

The increment was consistent with the number of units transfused.

#### Day 5

Four units were transfused to a patient with Acute Myeloblastic leukemia (AML).

The pre count was  $< 5000/\mu$ l. The post count showed no significant increment at  $6000/\mu$ l. The 2 other units were transfused to another AML patient whose pre count was  $20000/\mu$ l. The post count recorded did not show any increase and was reported at  $18000/\mu$ l.

## Discussion

The studies assessing the quality of PCs and their effect on storage are few. The analytical parameters in our study can be divided as

1. Platelet count and indices
2. Biochemical variations -glucose and pH
3. Cytokines (RANTES) accumulation
4. Functional parameters

The previous studies reviewed have generally analyzed only 1 or 2 of the above parameters.

### Platelet count and indices

A detailed study on the platelet parameters like count, mean platelet volume (MPV), platelet distribution width (PDW), and platelet large cell ratio (PLCR) has been conducted at Lucknow [1]. The platelet parameters were analyzed with and without EDTA anticoagulation. The differences in parameters were calculated over Day 0 to Day 7. The mean

plateletcrit (PCT) reduced from Day 0 to Day 7 (7.25 to 6.86). The difference in indices were correlated with pH changes using regression analysis. PDW and MPV showed maximum correlation with pH, while the coefficient of determination ( $R^2$ ) was maximum for differences in PDW and PLCR. It was concluded that EDTA incubation of the PC samples added to the preservation of cells and has a great potential as quality marker of PCs.

In the present study, the count showed a remarkable increase but when the smear was checked for morphology, we noted that the Day 3 platelets were markedly swollen and showing evidence of further fragmentation, which was more enhanced on Day 5 smears. This was further proven when the PLCR showed an increase in 7 out of the 11 PCs. The MPV and PCT also increased. As done in the previous study, probably storing the PC samples in EDTA for quality checks would have overcome the morphological abnormalities and swelling we noted.

#### *Biochemical variations - glucose and pH*

As discussed earlier, the utilization of glycogen releasing lactate by glycolysis contributes to 15% of the ATP. Further conversion of the lactate and pyruvate to acetyl Co A through the TCA cycle in the mitochondria contributes to 85% of the ATP. For fresh PCs the glycogen content is 0.05mmol/L/10 [2]. In 50 ml of PC this represents a concentration of 1mmol/L in glucose equivalents. On the other hand, the initial glucose concentration is approximately 25mmol/L, five times the physiologic concentration because of the glucose added to the anticoagulant. Eighty percent of the platelet glycogen is degraded in the first 24 hours of storage. The glucose generated is further converted to lactate, which accumulates and increases by approximately 2.5mmol/L/day [2, 3]. This lactate and carbon dioxide accumulation also contributes to the fall in pH.

In the present study, the fall in glucose levels were statistically significant. ( $p < 0.01$ ). Though the fall in pH was not statistically significant, all the bags showed a fall in pH from 0.5 to 1

units on Day 3 and up to 2 units fall on Day 5. Two of the 4 bags showed a pH of 6 on Day 5, which is the limit of platelet viability.

#### *Cytokines accumulation*

The PC transfusions are frequently associated with transfusion reactions, most commonly the NHTRs.

The common febrile NHTRs appear to have 3 possible underlying causes [5]:

(i) Infusion of passenger leucocytes into recipients alloimmunized against leucocytes/platelets. The implicated antibodies are usually HLA specific.

(ii) Infusion of pyrogenic cytokines / inflammatory mediators that accumulate in the plasma portion during storage. Cytokines implicated include IL-1 $\alpha$ , IL-6, IL-8, TNF $\alpha$ , Macrophage Inflammatory Protein 1 $\alpha$  (MIP 1 $\alpha$ ), Growth related oncogene  $\alpha$  (GRO- $\alpha$ ) and activated complement fragments. Many of these have pyrogenic activity (IL-1 $\alpha$ , IL-6, and TNF $\alpha$ ). The extracellular levels of these cytokines increase with storage and are found to be greatly decreased with prestorage filtration. Platelet derived cytokines like RANTES and TGF- $\beta$  1 are present in the plasma portion of the PCs. They are not directly pyrogenic but have stimulatory effects on other cells. TGF- $\beta$  1 stimulates the monocytes to release IL-1 $\alpha$  and TNF $\alpha$ . RANTES activates basophils and mast cells which release histamine and cause various allergic reactions [6, 7, 8, and 9].

(iii) Infusion of components contaminated with bacteria/bacterial products, with the end result being an increase in pyrogenic cytokines.

In a prospective study by Heddle et al they reported adverse reactions in 30.8% of platelet transfusions versus 6.8% of red cell transfusions [7]. They reported that the age of the PC and the absolute white blood count (WBC) were the best indicators of a reaction, speculating the biologic mediators within PC released during storage. Muelle noted an increased incidence and severity of reactions of PCs stored longer than 5 days [8]. In the study by Aye et al the feasibility of preventing cytokine accumulation by filtration to reduce WBC in

PCs prior to storage was assessed. [9] Compared to unfiltered PC, the filtered PC showed no rise by Day 3 in cytokine levels.

In the present study, we assayed the RANTES levels, which is a specific platelet-derived cytokine and noted a significant increase during storage. The exponential increase in this study and other studies strongly suggests that these cytokines are actively produced during storage of PC at 22 degrees C.

#### *Functional parameters*

The storage also induces platelet activation. As platelets are activated the expression of P-selectin (CD62P) increases and soluble glycoprotein V (GPV) is released. Studies have shown that there is a significant change in shape associated with increase in GPV [10]. The correlation of GPV changes with lactate and glucose concentrations and with CD62P and CD63 was also good. Studies have suggested that GPV might be applicable for quality monitoring of PCs, in addition to the metabolic tests. In addition, they may be helpful in analyzing potential improvements in blood component processing [10]. In the present study, CD41 expression was monitored in the PCs but no significant change in antigenicity was noted.

#### *Transfusion*

The units transfused on Day 3 showed good increment, and no increment was seen on Day 5 transfusions. However, the lack of increment in platelet count cannot be attributed to storage changes alone as there are many confounding factors influencing rise in platelet counts in vivo.

Bacterial contamination is another prime issue with stored platelet units. Though the units in our study were not sent for sterility check, 10% of the platelet units prepared from our blood bank are routinely sent for aerobic and anaerobic culture on Day 5 every month. None of the units so far yielded any positive culture. Contamination from skin of donors during collection is considered the most common cause for positive cultures.

## **Conclusion**

Many significant changes in platelets are noted during storage of PCs. In the present study the following changes were noted:

1. Fall in glucose levels.
2. Fall in pH. pH fell to 6, the limit of viability, in few bags on Day 5.
3. Significant increase in RANTES levels.

Improvement in technologies must involve

- a) Preparation methods, like use of buffy coats versus platelet rich plasma.
- b) Use of single donor platelets versus random donor platelets.
- c) Storage: advances in blood bags, anticoagulants and additives used.
- d) Filters: Use of inline pre storage filters will go a long way in improving the quality of stored platelets and reducing the associated NHTRs.

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