Evaluation of Storage Length to Blood Component Platelet Concentrate Quality in the Blood Bank, Dr. Soetomo General Hospital, Surabaya, Indonesia

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Abstract

Storage of Platelet Concentrate (PC) can cause changes in thrombocyte quality that affect thrombocyte viability and decrease hemostatic function. All biochemical, structural and functional changes to platelets during storage in the Blood Bank are known as platelet storage lesions. To analyze the effect of storage length on changes in thrombocyte morphology and function as well as the metabolic parameters of platelet concentrate bag components. This was an analytical study with time series design. Sampling was done consecutively. Samples were new PC bags which were processed and stored at room temperature with agitation during June-July 2019 in the Blood Bank of the Dr. Soetomo General Hospital, Surabaya. Examination of platelet count and MPV were performed to determine morphological changes and metabolic parameters like pH, pCO_2, and pO_2 on day 1, day 3, and day 5^{th}. Data were analyzed by using Shapiro-Wilk test, Paired t test and Wilcoxon Signed Rank Test. There were significant changes in MPV, pH, and pCO_2 values during storage (p <0.05). However, there were no significant changes in platelet count and pO_2 value. Metabolic changes in PC bag were obtained but there was no decrease in platelet count so that the PC component was still feasible to be given to the patient. Further research is needed for other parameters that can be affected by the storage process.

Keywords*: Platelet concentrate, platelet storage lesion, quality.*

Introduction

Platelets are blood cells that play an important role in hemostasis. Indications for platelet transfusion are for prophylaxis or hemostatic therapy in patients with congenital or acquired thrombocytopenia or thrombocytopathy. Over the years, there is an increase of the need for platelet transfusion due to the increasing number of patients with thrombocytopenia and platelet function disorder[1]. Platelet transfusion demand per month in 2018 at the Dr. Soetomo General Hospital were 2,442 platelet concentrate (PC) blood bags for about 325 patients, each patient needed 7-8 PC blood bags (Blood Bank Data of the Dr. Soetomo General Hospital in 2018).

Platelet transfusion is a challenging life-saving procedure in blood transfusion services because of the short shelf life of platelets. The American Association of Blood Banks (AABB) recommends storing platelets by constant agitation at room temperature (20-24 °C) for 5 days[1]. Storage at room temperature can cause platelet metabolic activity 60% lower when compared to storage at 37 °C[2].

The shelf life of PC blood components is limited because of the risk of microbial contamination and during the storage process platelets are susceptible to environmental changes that can affect the quality which is known as platelet storage lesions[3]. This change in quality can have a potentially negative impact on platelet

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viability and hemostatic function[4]. The mechanism that causes platelet storage lesion is very multifactorial and not clearly understood. Several factors including blood tapping method, component manufacturing process, storage and manipulation after blood tapping can cause platelet storage lesions[5].

Previous studies have shown that the PC blood component is adequately maintained if stored for 5 days and after that there can be a progressive loss of viability and platelet function during platelet storage[6]. Several laboratory examinations can be carried out to detect changes in quality, such as platelet count and Mean Platelet Volume (MPV) tests to detect morphological changes and to check metabolic parameters such as pH, pO_2 and pCO_2 to determine platelet viability during the storage process[4].

Platelet indices such as platelet count and MPV are used as markers to maintain PC quality control and are considered to represent changes in platelet shape caused by storage. An assessment of MPV values is used to see platelet storage lesions because MPV correlates with changes in platelet morphology during PC storage. The discoid platelet form correlates with viability in vivo after being transfused to the patient. Platelet discoid shape and the presence of swirling in stored PCs can be observed before they are transfused as quality control of platelet function present in PCs[6]. This study aimed to analyze the effect of storage duration on morphological changes (platelet count and MPV) and metabolic parameters (pH, pCO_2, and pO_2) Platelet Concentrate (PC) blood components, which were assessed at storage days 1, day 3, and day 5th.

Materials and Methods

Samples were newly processed PCs stored at room temperature with agitation from June to July 2019 at the Blood Bank of the Dr. Soetomo General Hospital, Surabaya, Indonesia. PC samples had passed screening tests for infectious disease through blood transfusion, namely hepatitis B, hepatitis C, HIV, and syphilis. PC blood bag manufacturing activities were carried out in the Blood Transfusion Unit of Dr. Soetomo General Hospital. PC was prepared using the platelet rich plasma (PRP) method, 350 mL of whole blood that has been tapped from voluntary donors was put into a triple blood bag that already contained anticoagulant Citrate Phosphate Dextrose Adenine (CPDA). After settling for about 30 minutes, whole blood was centrifuged using a Rotixa 50 RS centrifuge at 375 g for 15 minutes at 22 °C to obtain PRP. After PRP was formed, the blood was centrifuged again at a speed of 1,500 g for 15 minutes, then the platelet poor plasma (PPP) supernatant was separated and platelet residue was resuspended with an average plasma volume of 60±0.9 mL. On the first day of storage, platelet samples were taken from a blood bag tube and inserted into a tube containing EDTA anticoagulants as well as syringes that already contained heparin anticoagulants. Then a platelet count and MPV were examined using a Sysmex XN 1000 hematological analyzer to determine morphological changes and examine metabolic parameters such as pH, pCO_2, and pO_2 using the BGA Gem 3500 instrument. The remaining PC blood components were stored at 22-24 °C using an agitator to be re-examined. The same method used for the first day of sampling was done for the third day and fifth day of sampling.

Result and Discussion

This study used 30 PC blood component bags. In the morphological parameters, the platelet count did not change significantly but the MPV value increased significantly between day 1 to day 3, between day 3 to day 5 and between day 1 and day 5 ($p < 0.05$) (Table 1).

Table 1. Platelet Morphology Parameters.

Samples	Storage			p Value		
	Day-1	Day-3	Day-5	1-D3	1-D5	3-D5
Platelets (x103 µL)	653.80±209.84	678.06±192.37	664.20±210.32	.103	.500	.052
MPV (fl)	7.79±0.36	7.98±0.38	8.29±0.37	.001	.000	.000

. MPV = mean platelet volume.

Obtained a significant decrease
and day 5 ($p < 0.05$) and for the
day 5 ($p < 0.05$). There were no

V	*p* Value	
	D1-D5	D3-D5
	0.000	0.000
	0.112	0.331
	.002	0.111

1.

O_2, as well as pCO_2 and
variables on the first day

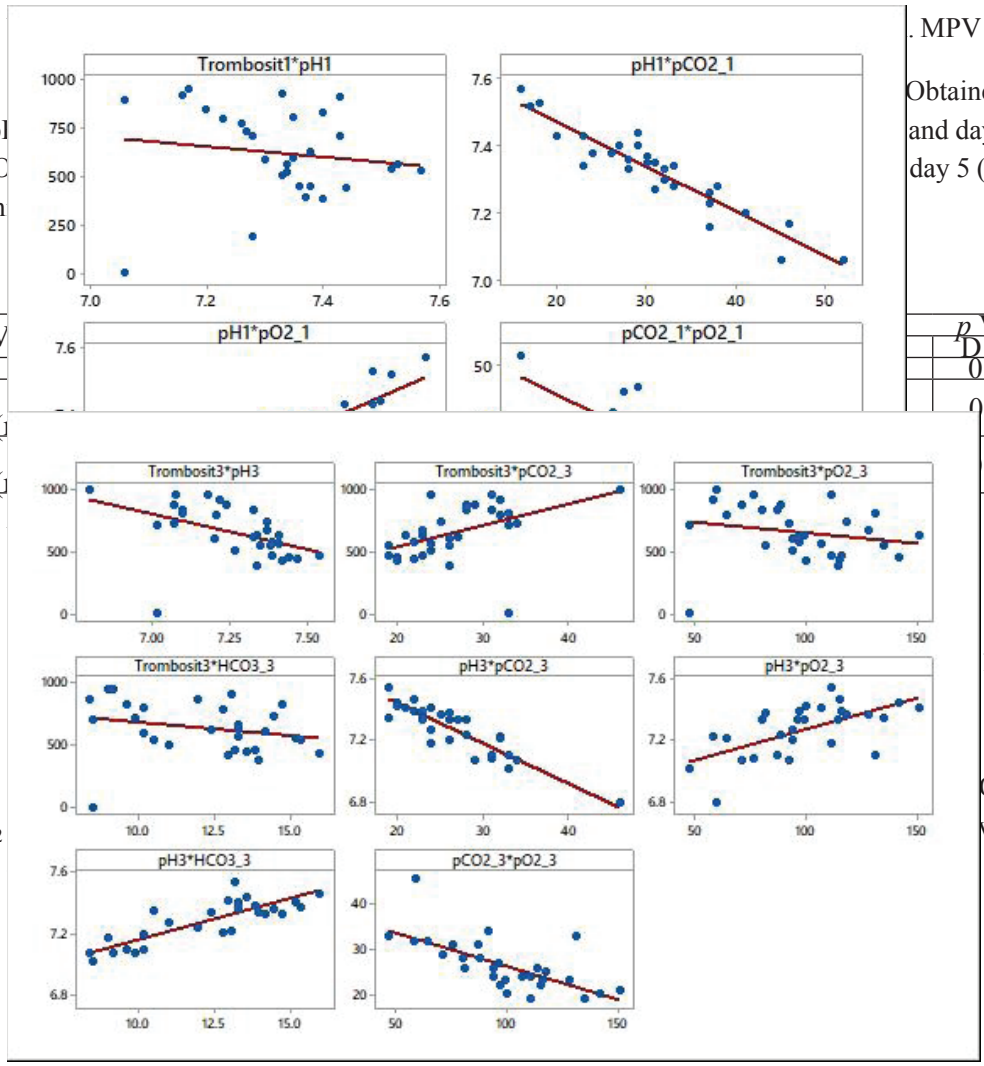

Figure 2. Relationship of morphological parameters with metabolic parameters on day 3.

It could be concluded from Figure 2 that the relationship between platelet variables and pH, platelets and pO_2, platelets and HCO_3, pH and pCO_2, and pCO_2 and pO_2 on day 3 had a negative correlation while the relationship of platelet variables and pCO_2, pH and pO_2, also pH and HCO_3 on day 3 were positively correlated.

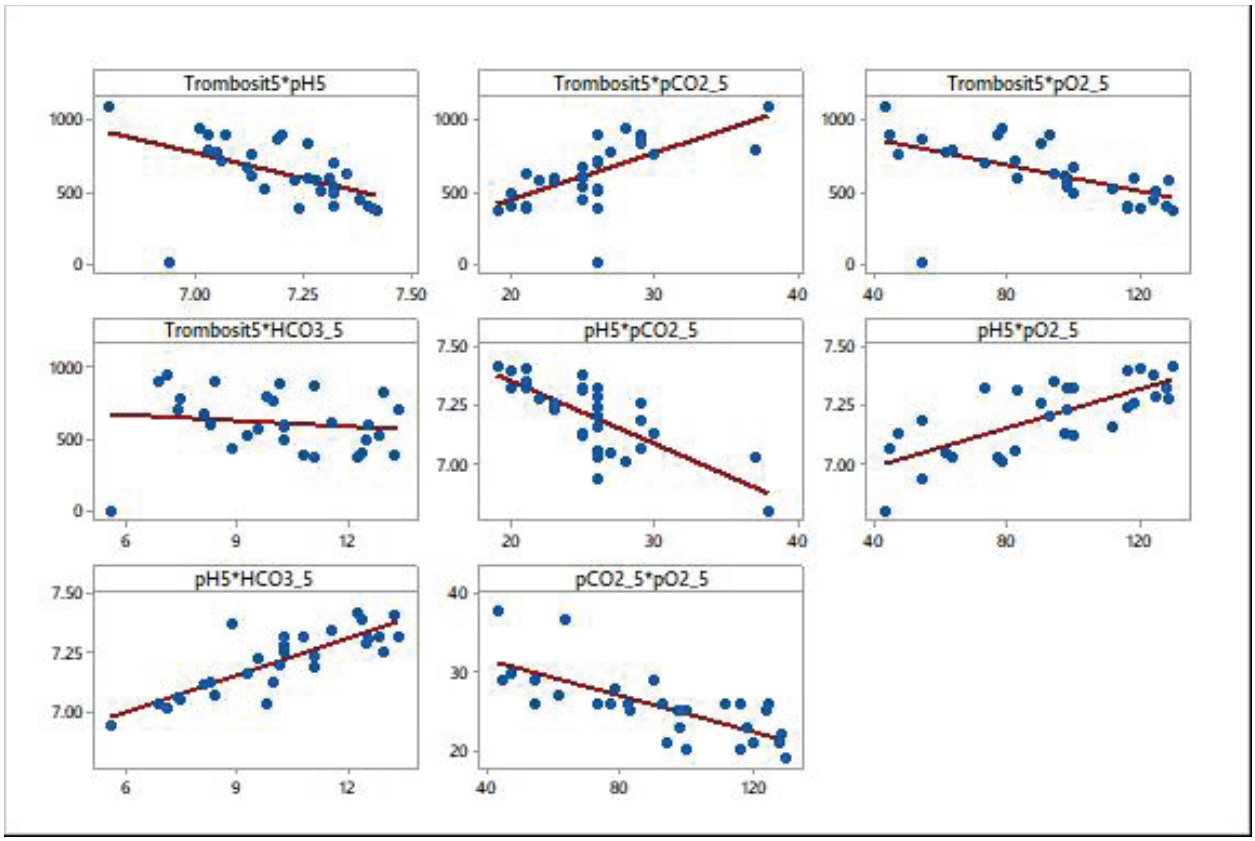

Figure 3. Relationship of morphological parameters with metabolic parameters on day 5.

The Figure 3 showed that the relationship between platelet variables and pH, platelets and pO_2, platelets and HCO_3, pH and pCO_2, and pCO_2 and pO_2 on day 5 had a negative correlation while the relationship of platelet variables and pCO_2, pH and pO_2, also pH and pCO_2, HCO_3 on day 5 had a positive correlation.

Platelets can become active when exposed to foreign surfaces, the presence of trauma, low pH, agonists (thrombin, ADP), and shear stress. After activation, platelets lose their discoid shape and become more spherical with multiple pseudopods[7,8]. Exposure to shear stress such as centrifugation when making components[9], not only activates platelets but can also cause platelet lysis and calpain activation. This platelet lysis can cause the accumulation of cytosolic lactate dehydrogenase (LDH) and the release of granular contents. Activated calpain (protease) will cause cytoskeletal proteins such as actin, talin, and actin-binding protein to degrade and produce microvesicles which can cause a decrease in MPV value[8,10].

In contrast, the results showed that there was a significant increase in MPV values on day 1, day 3 and day 5. This was possible because the change in platelet shape due to the centrifugation process was indicated by a low MPV value on day 1 and then increased on day 3 and day 5 so that showed that platelet morphology was still viable. Increased MPV values were in the normal range of 7.4-10.4 fl[5]. This study showed that the platelet count decreased on the fifth day of storage but the decrease was not significant so it was still considered good. This could be caused by the effect of platelet storage lesions that occurred due to cell lysis caused by activation of platelets which can progressively result in accumulation of metabolic waste, a gradual decrease in the function and viability of platelets[1].

This study also showed a significant decrease in pH during storage. The American Association of Blood Banks (AABB) recommends that platelets with pH <6.2 and pH> 7.4 cannot be used for transfusion, pH of PC components in this study ranged from 7.20-7.33 so PC was considered to be in a good and tolerable condition[5]. Platelet metabolic activity continued during the storage

process. Storage temperature could affect pH, glucose consumption and lactate production. The effect of a decreased pH (<6.8) can cause the morphology of platelets to begin to change dramatically when the pH dropped below 6.0 resulting in irreversible deformation and loss of platelet viability[6,9,12].

During storage, the metabolic activity of platelets caused the consumption of O_2 and CO_2 production resulting in a downward trend in O_2 and an increase in CO_2 in the PC bag[3]. This second generation PC bag is more gas permeable, causing gas exchange to occur more easily across the walls of the PC bag and there is no buildup of CO_2 levels and a decrease in O_2 levels[5]. The results of this study showed a significant decrease in pCO_2 however pO_2 decreased not significantly. This was presumably because the PC bag used in this study had an adequate gas exchange ability.

Murphy and Gardner were the first researchers to state the importance of the influence of agitators on the quality of platelets stored at room temperature. The researchers observed that if PC was stored in a small container or not using an agitator caused the pH to drop rapidly below 6.0. Low pH levels could directly affect post-transfusion recovery. The researchers claimed that this happened because the gas exchange in the PC bag was affected by the quality of the bag used[13]. The process of agitation on PC could guarantee continuous oxygenation of platelets, O_2 could enter the bag and excess CO_2 could be removed so that the use of agitators caused an adequate gas exchange in the PC bag, preventing the buildup of lactic acid and decrease of pH[12,13,14].

Based on the results obtained in this study, PC storage for five days resulted a change in metabolic activity and cell morphology but was still within tolerable limits. PC storage in the Blood Transfusion Unit of the Dr. Soetomo General Hospital, Surabaya, Indonesia has followed the temperature and agitation requirements as recommended by AABB. This study only examined the effect of storage duration on metabolic activity and cell morphology, but there are still many other parameters that can undergo changes during the PC storage process such as platelet aggregation function, bacterial contamination, cytokines produced during storage and other materials. This was a limitation in this study.

Conclusion

In sum, PC storage for 5 days caused a decrease in pH and pCO_2 values as well as a significant increase in MPV but still within tolerable limits. Platelet count was not significantly affected therefore it can be concluded that PC samples was still in viable condition after being stored for five days. Further research is needed for other parameters that can be affected by the storage process.

Conflict of Interest : The authors declare that they have no conflict of interest.

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