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Analysis of Platelet Counts and Leukocyte Residues at Different Storage Times in Thrombocyte Concentrate

Tania Aryadi Pramudywardhani, Raehana Samad, Rachmawati Adiputri

Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia.
E-mail: tania.aryadi@gmail.com

ABSTRACT

Thrombocyte Concentrate (TC) is one of the blood components given as therapy to patients. Ensuring the quality of TC products is essential to know if they can be transfused to patients. This study aimed to analyze platelet counts and leukocyte residues in TC products with different storage times. Cross-sectional research with a prospective cohort design was carried out at the Blood Transfusion Unit of Dr. Wahidin Sudirohusodo Hospital, Makassar, in September 2022. The study samples were ten bags of TC products produced from whole blood using the buffy coat method. The average platelet count in TC products on the 1st, 3rd, and 5th day were $50.65x10^9$ (± 24.58); $66.24x10^9$ (± 34.83); $47.34x10^9$ per unit (± 37.75). Statistical tests showed no significant decrease between the 1st, 3rd, and 5th days. A meaningful decrease was obtained on the 3rd and 5th day ($p < 0.05$), while on the ratio of the 1st and 3rd day, there was no meaningful difference ($p > 0.05$). The average number of leukocytes is $0.0727x10^9$ per unit (± 0.0659). The average number of platelets and leukocyte residues in TC products is by PERMENKES No. 91 of 2015 standards. Thrombocyte concentrate products can be used until the 5th day of storage.

Keywords: Thrombocyte concentrate, storage times, leukocyte residues, platelets

INTRODUCTION

Thrombocyte Concentrate (TC) is one of the blood components given to patients as therapy. Other than TC, blood products that can be produced from Whole Blood (WB) are Packed Red Cells (PRC) and plasma.[1-3]

Thrombocyte concentrate produced by WB will be centrifuged. Centrifugation separates the red blood cells from the Platelet Rich Plasma (PRP) or the Buffy Coat (BC) and plasma. Platelet-rich plasma, or BC, will then be centrifuged once again to separate the platelet component. Platelets can also be obtained by apheresis, in which the blood cells are separated using an apheresis machine. The donor's blood is directly connected to the apheresis machine; the blood will be mixed with anticoagulants and separated into different components. The target component (platelets) will be collected in a separate bag, while the other components will be returned to the donor.[1,4,5]

The number of platelets in each concentrate varies in WB. There are $5.0\text{-}7.5\times10^{10}$ platelets in 200–350 mL plasma or plasma replacement solution, while for concentrates obtained through apheresis, there are 3×10^{11} platelets in 200–300 mL plasma. Thrombocyte concentrate can be stored at 20-24°C for five days to avoid bacterial contamination.[6-9]

Ensuring the quality of TC products is very important and can be done by Quality Control (QC) of the components so they meet the requirements for transfusion to patients. According to the Health Ministers Regulation of the Indonesian Republic (PERMENKES) number 91 the year 2015 about blood transfusion services standardization, the quality of blood products can be controlled in various stages, starting from donor selection, blood collection, processing, blood bag storage, blood bag recording, and distribution (Table 1). Several indicators in TC product processing that have become QC standards are the number of platelets and leukocyte residues in each unit. Leukocyte residues exceeding the standard will increase the probability of transfusion reaction. Good QC, especially during product collection, preparation, and storage, is intended to improve safety, reducing transfusion reaction risks.[4,10-12]

The metabolism of blood cells contained in TC products will continue during storage, causing platelet lysis. The Blood Transfusion Unit of Dr. Wahidin Sudirohusodo General Hospital (UTD-RSWS), Makassar established on January 21st, 2022, needs to

Table 1. Quality control according to PERMENKES number 91 year 2015

Parameter	Information	Specification	Sampling	% QC that Acceptable
Amount thrombocyte per unit final	Platelet single	> 60 x 10^9	1% of the total blood bag, minimum 10 bags per month	75%
	Platelet single -leucodepleted	> 60 x 10^9		
Amount leukocyte per unit final	Leukocyte single, from PRP	< 0.2 x 10^9	1% of the total blood bag, minimum 10 bags per month	90%
	Leukocyte single, from BC	<0.05 x 10^9		

provide facilities and infrastructure by PERMENKES number 91 of 2015 standards to ensure the quality of blood products and service. This study assessed platelet counts and residual leukocytes during different storage times as quality control indicators at UTD-RSWS.

METHODS

This study was a cross-sectional prospective cohort study. The study population was all TC products produced at the UTD-RSWS using Roto Silenta 630RS centrifugation with the PRP method. In the PRP method, WB will be centrifuged using a soft spin and separated into PRP and red cells. After separating PRP, it will be centrifuged again with a hard spin for plasma and TC. There were 10 TC products used as the study sample produced during September 2022 (according to PERMENKES number 91, the year 2015, there has to be a minimum of 4 products). The inclusion criteria were TC products produced at UTD-RSWS and examined on storage days 1, 3, and 5. The exclusion criteria were TC products with color changing through direct examination due to contamination or particulate matter, erythrocyte contamination, lipemia, and icteric.

Thrombocyte concentrate samples examined from the main bag were obtained by stripping TC products from the tube to the main pack, homogenizing the samples, and returning them to the tube. The number of platelets and leukocytes was calculated using the Sysmex XN-1000 hematology analyzer. Each result obtained was multiplied by the volume of the TC product.

Data analysis used SPSS version 25. The research data was presented descriptively in the form of frequency distribution. The difference in the number of platelets and leukocyte residues was tested with the Wilcoxon Signed Rank and Friedman tests. This study was approved by the Health Research Ethical Committee of UNHAS Medical Faculty and Dr. Wahidin Sudirohusodo General Hospital, Makassar, with number article No. 423/UN4.6.4.5.31/ PP36/2022.

RESULTS AND DISCUSSIONS

This study used 10 TC products from 10 volunteer donors with 60-65 mL product TC as samples. Table 2 shows that most of the subjects were male.

Table 2. Distribution of donor characteristics

Gender	Total	Percentage (%)
Female	4	40
Male	6	60

The average number of platelets increased between days 1 and 3 but was not statistically significant, with a p-value = 0.169 (Table 3).

Table 3. The average number of platelets on storage days 1 and 3

Day of Storage	Number of Platelets (x 10^9 per unit)		p
	Mean	SD	
Day 1	50.65	24.58	
Day 3	66.24	34.83	0.169

A significant decrease in the average number of platelets was found on day three and day 5 with p=0.017 (Table 4).

Table 4. The average number of platelets on storage days 3 and 5

Day of storage	Number of Platelets (x 10^9 per unit)		p
	Mean	SD	
Day 3	66.24	34.83	
Day 5	47.34	37.75	0.017

An insignificant decrease in the average number of platelets was found on day one and day 5 with p=0.721 (Table 5).

Table 5. The average number of platelets on storage days 1 and 5

Day of Storage	Number of Platelets (x 10^9 per unit)		p
	Mean	SD	
Day 1	50.65	24.58	
Day 5	47.34	37.75	0.721

According to data normality using the Kolmogorov–Smirnov test, platelet count was not normally distributed. The differential test used was the Friedman test, which obtained a comparison of the mean platelet counts on days 1, 3, and 5, showing no statistically significant difference (p>0.05) in the three interval groups (Table 6).

Table 6. The average number of platelets in three-time interval groups

Day of Storage	Number of Platelets (x 10^9 per unit)		P
	Mean	SD	
Day 1	50. 6521	24.57999	
Day 3	66.2357	34.83085	
Day 5	47.3425	37.75102	0.15

Based on the Friedman test for leukocyte residues on days 1, 3, and 5, there was no statistically significant decrease (p > 0.05) (Table 7).

Table 7. Leukocyte residues on storage days 1, 3, and 5

Day of Storage	Residual Leukocytes (x 10^9 per unit)		P
	Mean	SD	
Day 1	0.0727	0.0659	
Day 3	0.0623	0.0594	
Day 5	0.0522	0.0627	0.199

The average leukocyte residues obtained on days 1 and 3 showed a decrease in average from 0.0727 (± 0.0659) to 0.0623 (± 0.0594) with a p-value = 0.646, on days 3 and 5 showed a decline from 0.0623 (± 0.0594) to 0.0522 (± 0.0627) with p=0.314, while on days 1 and 5 it showed a reduction in the mean from 0.0727 (± 0.0659) to 0.0522 (± 0.0627) with a value of p=0.169. The graph of the decrease in the average leukocyte residues is shown in Figure 1.

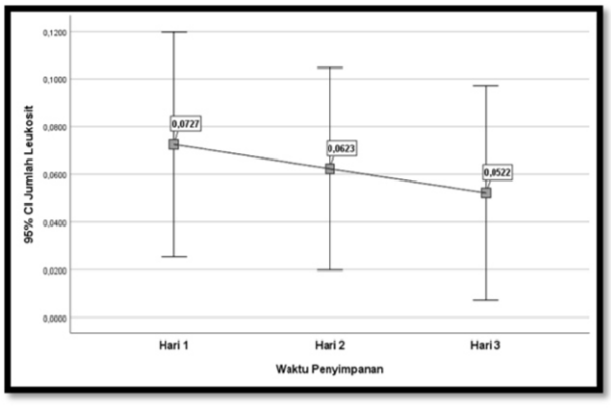

Figure 1. The average number of leukocyte residues according to time of storage

In this study, the average number of platelets on days 1, 3, and 5 were 82.92 – 20.35, 133.56–27.18, 112.08–9.60 x 109 per unit. The unknown number of donor platelets can cause variations in the average platelet count because the platelet count was not examined during donor screening. Separation of TC products done manually can affect the TC products. This can be overcome by separating the components using automatic methods. In this study, it was found that there was an increase in the average number of platelets on storage day three, followed by a decrease in the average number of platelets on day 5. The results obtained were in line with research conducted by Marpaung *et al.*, which explains the increase in the average number of platelets due to fragmentation causes an increase in platelets. The anticoagulant Citrate Phosphate Dextrose Adenine acid (CPDA-1) in the blood bag was not yet homogeneous, causing several platelets to swell like giant platelets and eventually become fragments. Fragments may cause a false increase in the platelet count. On the 5th day of storage, there was a decrease in platelets due to the shorter life span of the in vitro platelets (8-10 days), resulting in platelet lysis.[10,13,14]

During the storage period, leukocytes undergo degranulation, split, or lyse, releasing cytokines such as TNF-α, IL-1, and IL-6, which can cause transfusion reactions. Fever and symptoms of Febrile Non-Hemolytic Transfusion Reaction (FNHTR) are frequent transfusion reactions.

This study's average leukocyte residues were 0.1386–0.0068 x 10^9 per unit. This is by PERMENKES number 91 of 2015, namely leukocyte residues <0.2x10^9 per unit. The average number of leukocytes obtained in this study is in line with research conducted by Sari, who had an average number of leukocytes of 0.0024 x 10^9 per unit. The handling of TC by Roto Silenta 630RS centrifugation in separating TC products with PRP and storing TC blood bags in an agitator with a temperature of 22±2°C at UTD-RSWS Makassar met PERMENKES standards.[12]

CONCLUSIONS AND SUGGESTIONS

In this study, the average leukocyte residues on days 1, 3, and 5 did not significantly decrease, while the average platelet count on days 3 and 5 showed a significant decrease. The average number of platelets and leukocyte residues in TC products at UTD-RSWS Makassar are suitable with PERMENKES 91 year 2015 standards, meaning that TC can be used until the 5th day of storage.

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