

Analysis of Soluble Cluster of Differentiation 40 Ligand (SD40L) levels between thrombocyte apheresis and thrombocyte whole blood products in Sanglah General Hospital blood bank



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ABSTRACT

Background: Platelet transfusion leaves many problems and controversies such as short storage times, high risk of contamination, poor therapeutic response and often results in transfusion reactions. Transfusion reactions are associated with platelet storage lesion. Increased storage lesions will increase the biological response modifiers such as soluble cluster of differentiation 40 ligand (sCD40L). Soluble CD40L is associated with febrile, allergic and TRALI transfusion reactions. Given the negative impact of sCD40L, it is essential to analyze the level of sCD40L in platelets.

Methods: This type of research was an analytical observational study. Samples were 10 thrombocytes of whole blood and apheresis on the first, second and third days of storage. Two milliliters of the product was centrifuged and plasma sCD40L was examined using the BioVendor ELISA method. Data were analyzed using the SPSS version 25 software.

Results: The mean sCD40L level in thrombocyte apheresis based on storage times were 4.36 ± 1.34 ng/mL (Day-1), 6.87 ± 1.75 ng/mL (Day-2), and 7.27 ± 2.21 ng/mL (Day-3), while the mean sCD40L level in whole blood thrombocyte were 8.36 ± 3.77 ng/mL (Day-1), 9.42 ± 2.58 ng/mL (Day-2) and 11.10 ± 4.02 ng/mL (Day-3). There were no significant differences in storage times in both groups ($P > 0.05$). However, the mean sCD40L level in thrombocyte whole blood tended to be higher than the mean sCD40L level in thrombocyte apheresis. There was a significant positive correlation between storage times and sCD40L levels in the thrombocyte apheresis group ($r = 0.549$; $P < 0.05$). ANOVA test suggested a statistically significant difference between storage times in TC Apheresis ($P < 0.05$).

Conclusion: The mean sCD40L level in whole blood thrombocyte was higher than thrombocyte apheresis. There was a significant positive correlation and a statistically significant difference between storage times and sCD40L levels in thrombocyte apheresis.

Keywords: Soluble CD40L, thrombocyte apheresis, thrombocyte whole blood

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INTRODUCTION

Thrombocyte Concentrate (TC) is the second most common blood component used in the management of patients after transfusion of Packed Red Cells (PRC). Platelet components are widely used to prevent or treat bleeding in patients who have thrombocytopenia or platelet function abnormalities. In Bali, especially in Sanglah General Hospital, the average use of TC prepared through donors of whole blood reaches 770 bags per month and the average use of TC prepared from donor apheresis reaches 35 bags per month.^{1,2}

Although TC transfusions are often used, they still leave many problems and controversies. The first problem is that platelets are blood components with the shortest and most labile shelf life among other blood components so that this component is the most wasted because it can be easily damaged and expired. Platelet components only have a shelf life of five days and the average lifespan is seven days after transfusion.^{3,4}

The second problem is the response to therapy that is often not in line with expectations. Many patients receive platelet transfusion, but platelet increase is not too high even in some cases there is a post-transfusion platelet decrease. The third problem is that platelets are the blood component that is most easily contaminated with bacteria both caused by storage temperatures and conditions of blood bags with larger pores. The fourth problem is that platelets are the blood component that contributes highest to the incidence of transfusion reactions followed by PRC and plasma components.⁵

Due to the high incidence of transfusion reactions in platelets component, one of the causes of suspected etiology of transfusion reactions is high levels of certain substances in the products emerging from the platelet storage lesion, known as platelet storage lesion (PSL). During storage, platelets will undergo some changes including producing

several biochemical and molecular substances that can affect effectiveness, and it can even result in some side effects to recipients. Platelets with a shelf life of 5 days can experience increased procoagulant activity, increased lactate production, decreased pH, pO₂, glucose, and others.⁶

Some of the molecular substances produced are cytokines, chemokines, biological response modifiers (BRMs) and others. Numerous studies report that during storage, the platelet component will experience storage lesions. The longer the storage or the more exposure to stress, the more the biological response modifiers (BRMs) are produced.⁶ One of the pro-inflammatory BRMs that are produced and used as indicators to determine the severity of storage lesions is sCD40L. The higher the sCD40L level indicates the greater the degree of lesion and the greater the negative impact that arises.

Soluble CD40L was first reported to be associated with the incidence of transfusion reactions in 2006. At that time, some cytokines such as interleukin-6 (IL-6), interleukin-8 (IL-8), Monocyte Chemoattractant Protein-1 (MCP-1) were found and sCD40L all increased in the supernatant TC product that had been leukoreduced and caused febrile and allergic transfusion reactions. A retrospective study found high levels of sCD40L in TC products transfused in patients who had a transfusion-related acute lung injury (TRALI) transfusion reaction compared with patients who did not experience a transfusion reaction. From the patient's plasma measurements, 8 out of 12 patients who had TRALI experienced an increase in sCD40L levels after TC transfusion. This suggests the possibility of transfusion with TC units containing high levels of sCD40L contributing to the incidence of TRALI. The role of sCD40L in inducing the incidence of transfusion reactions is not yet fully known, but it is possible that sCD40L acts as one of the mediators that can trigger hemostasis imbalances, thereby increasing the risk of transfusion reactions.

On the other hand, a strong correlation has been found between increased plasma sCD40L levels and vascular abnormalities.⁷ One study found that increased sCD40L during storage of platelet components was also known to be directly responsible for the incidence of febrile non-hemolytic transfusion reaction (FNHR) and the incidence of other acute transfusion reactions.⁸

Given the facts of many negative effects of sCD40L, it is essential to determine the levels of sCD40L in each platelet product to be transfused. Currently, there are two types of platelet components frequently used, namely TC prepared from donors from whole blood and TC apheresis.

Thrombocyte apheresis is believed to have many advantages over whole blood thrombocytes both from transfusion side effects and therapeutic effectiveness. However, the analysis of the degree of storage lesions between the two products is unknown. This study analyzed sCD40L levels between thrombocyte apheresis and thrombocytes whole blood.

METHOD

The type of research was an analytical observational study. The study population was all platelet products in Sanglah General Hospital blood bank. The research sample was platelet products that were given to patients, which were taken randomly based on storage time. The sample consisted of 10 thrombocytes whole blood on the first day of storage, 10 thrombocytes whole blood on the second day of storage and 10 thrombocytes whole blood on the third day of storage. Likewise, for thrombocyte apheresis products, 10 samples were taken in the first, second and third days of storage. The selection of storage time based on the longest storage time data when the product was given to the patient. Before the product was distributed to the wards, the sampling of the platelet bag was carried out in a sterile and closed system using an electric sealer and hand sealer. Two milliliters of the product was taken and put into a tube to be centrifuged. The plasma obtained was placed into aliquot tubes and stored at -40° until sCD40L was examined. SCD40L examination using BioVendor's enzyme link immunosorbance assay (ELISA) method. The data obtained were analyzed by the SPSS version 25 program. This study has been approved by the Ethic Committee of Faculty of Medicine of Udayana University, Bali (No.2288/UN.14.2/KEP/2017)

RESULTS

Data of the 60 samples examined for sCD40L levels in this study were obtained and are presented in [table 1](#). It can be seen from [table 1](#) that there were increased mean sCD40L levels on the first, second and third days of storage in both thrombocyte apheresis and thrombocyte whole blood products. The average sCD40L level on the second day was higher than the first day and the mean sCD40L level on the third day was higher than the second day. The highest sCD40L level was on the third day in both the thrombocyte apheresis group (7.27 ± 2.21) and thrombocyte whole blood group (11.10 ± 4.02) and the data were normally distributed ($P > 0.05$). In general, it can be seen that the mean sCD40L

levels in thrombocytes whole blood were higher than thrombocyte apheresis on the first, second and third days of storage.

As can be seen in Figure 1, the mean sCD40L level in thrombocyte apheresis on the first day of storage was still within the reference value range (the reference value sCD40L <4.7 ng / mL). The mean sCD40L level in thrombocyte apheresis were all still below 10 ng / mL on the first, second and third days of storage. In thrombocytes whole blood, the mean sCD40L level was all above the reference value starting from the first day of storage.

Table 2 shows the results of the T-Independent test analysis comparing the sCD40L levels of thrombocyte apheresis on the first day of storage and the sCD40L levels of thrombocyte whole blood on the first day of storage, which showed no significant difference (P value > 0.05). Likewise, the comparison of sCD40L levels on the second and third days of storage also showed no significant difference.

The Pearson correlation test was used to see the correlation between the duration of storage and sCD40L levels in both thrombocyte apheresis and

thrombocytes whole blood. The data obtained can be seen in Table 3.

As shown in Table 3, there was a significant positive correlation between storage time and sCD40L in the thrombocyte apheresis group ($r = 0.549$; $P < 0.05$). There was also a positive correlation between storage time and sCD40L in thrombocyte whole blood groups, yet the correlation was not statistically significant ($r = 0.315$; $P > 0.05$).

The One-Way ANOVA test was conducted to determine the difference in sCD40L levels between storage times in both blood components. It was found that there was a significant difference in sCD40L levels between storage times in the TC Apheresis blood component only ($P = 0.003$) (Table 4). Further analysis in TC Apheresis blood component was carried out using the Post-Hoc test to determine the differences between the storage times provided. The results indicate that there was a statistically significant difference between Day-1 and Day-2 ($P = 0.005$) as well as Day-1 and Day-3 ($P = 0.001$) of storage time. However, no significant difference was found between Day-2 and Day-3 of storage time ($P = 0.627$) (Table 5).

Table 1 Average levels of sCD40L in thrombocyte apheresis and thrombocyte whole blood products based on storage time

Blood Components	Days	N (%)	Mean±SD (ng/mL)	95% CI		Normality Test (S-W)
				Lower	Upper	
Thrombocyte Apheresis	Day 1	10 (33.33%)	4.36±1.34	3.43	5.35	0.15
	Day 2	10 (33.33%)	6.87±1.75	5.61	8.12	0.19
	Day 3	10 (33.33%)	7.27±2.21	5.68	8.85	0.60
Thrombocyte whole blood	Day 1	10 (33.33%)	8.36±3.77	5.69	11.08	0.42
	Day 2	10 (33.33%)	9.42±2.58	7.57	11.26	0.76
	Day 3	10 (33.33%)	11.10±4.02	8.23	13.97	0.97

CI: confidence interval; S-W: Shapiro-Wilk test

Table 2 Differences of sCD40L levels in thrombocyte apheresis and thrombocyte whole blood based on storage time

Parameter	n	Mean±SD (ng/mL)	Mean difference (95% CI)	P-value
Day 1				
Thrombocyte apheresis	10	4.39±1.34	3.98 (1.20-6.77)	0.09
Thrombocyte whole blood	10	8.38±3.77		
Day 2				
Thrombocyte apheresis	10	6.87±1.75	2.55 (0.47-4.62)	0.19
Thrombocyte whole blood	10	9.42±2.58		
Day 3				
Thrombocyte apheresis	10	7.26±2.21	3.83 (0.78-6.87)	0.17
Thrombocyte whole blood	10	11.09±4.01		

CI: confidence interval; P-Value: Independent T-Test, statistically significant if < 0.05

Table 3 Correlation of storage time with sCD40L levels in thrombocyte apheresis and whole blood thrombocytes

Variables	N	r	P-value
Days			
Thrombocyte apheresis	30	0.549	0.002
Thrombocyte whole blood	30	0.315	0.090

Table 4 One-Way ANOVA Test of Blood Component's sCD40L between Storage Time (Days)

Variables	Days	N (%)	P-value
sCD40LTC Whole Blood	Day 1	10 (33.33)	0.237
	Day 2	10 (33.33)	
	Day 3	10 (33.33)	
sCD40L TC Apheresis	Day 1	10 (33.33)	0.003*
	Day 2	10 (33.33)	
	Day 3	10 (33.33)	

*Statistically significant if P < 0.05

Table 5 Post-Hoc Analysis of Storage Time in TC Apheresis Blood Component

TC Apheresis	Mean Difference (ng/mL)	95% CI		P-value
		Lower	Upper	
Day 1				
Day 2	-2.474	-4.133	-0.815	0.005*
Day 3	-2.872	-4.531	-1.212	0.001*
Day 2				
Day 1	2.474	0.815	4.133	0.005*
Day 3	-0.398	-2.057	1.261	0.627
Day 3				
Day 1	2.872	1.213	4.531	0.001*
Day 2	0.398	-1.261	2.057	0.627

*Statistically significant if P < 0.05; CI : Confidence Interval

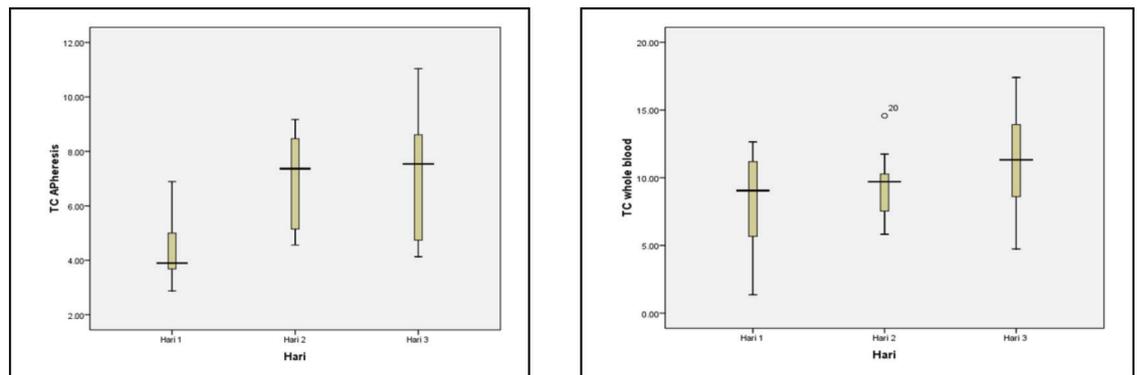


Figure 1 Average sCD40L levels in thrombocyte apheresis and thrombocytes whole blood based on storage time

DISCUSSION

In this study, the mean level of sCD40L tends to increase at longer storage times. Although there were no statistically significant differences between

sCD40L levels of thrombocyte apheresis and sCD40L levels of whole blood thrombocytes during storage, the mean level of sCD40L in thrombocyte

whole blood was higher than the sCD40L level in thrombocyte apheresis. This is in line with the results of research conducted by Wenzel et al. in 2011, which compared sCD40L levels and release capacities between thrombocyte apheresis and prestorage pooled TC. In this study, the mean level of sCD40L in thrombocytes whole blood on the first day of storage was $1.185 \text{ pg / mL} \pm 87 \text{ pg / mL}$ and the mean level of sCD40L in thrombocyte apheresis was $581 \text{ pg / mL} \pm 124 \text{ pg / mL}$. On the fifth day of storage, the mean level of sCD40L in thrombocytes whole blood was $4.464 \text{ pg / mL} \pm 212 \text{ pg / mL}$ and the mean level of sCD40L in thrombocyte apheresis was $2.718 \text{ pg / mL} \pm 154 \text{ pg / mL}$. The study also concluded that the accumulation of sCD40L in platelet products depended on the length of storage time but was not related to product preparation techniques.⁹ The absence of significant differences between sCD40L levels in thrombocyte apheresis and thrombocyte whole blood in this study was probably because there was no difference in the condition and storage time of the two products.

During storage, and without stimulation, platelets will be inactive or resting. There are some exposures to stress such as exposure to plastic, additives, several gases, the process of rotation or agitation, and changes in temperature that can cause the resting platelets to be activated. Activated platelets can excrete some inflammatory molecules such as chemokines, cytokines, and biological response modifiers (BRMs). Soluble CD40L is the most pro-inflammation BRMs that can be detected on the surface of the platelet component. The longer the storage, the higher the number of sCD40L produced.¹⁰ The result of this study is in accordance with the literature, which also found that sCD40L levels increased along with the length of storage time. In this study, there was no serial sCD40L examination on the same product with the consideration that patients could still use the product without disrupting service. Determination of sampling time on platelet products can also represent the conditions that occur in the field that most platelet products are transfused into patients on the first, second and third days of storage. In addition, it can represent sCD40L levels that exist in the product when it is transfused to the patient.

This study found a significant positive correlation between sCD40L levels and storage times in the thrombocyte apheresis group, while no significant positive correlation between sCD40L levels and storage times was found in the thrombocyte whole blood group. This is in line with the literature, where the longer the shelf life the more structural, biochemical and molecular changes occur. Platelets

with a shelf life of 5 days can undergo increased procoagulant activity, increased lactate production, decreased pH, pO₂, glucose and increased biological molecular substances response modifiers (BRMs) such as sCD40L.⁶

The existence of a significant positive correlation between sCD40L levels and storage times in the thrombocyte apheresis group and a non-significant positive correlation between sCD40L levels and storage times in the thrombocyte whole blood group in this study cannot be explained definitely. It could be caused by the limited number of samples, the differences in product volume, and the differences in product preparation techniques and so on. This research is a preliminary study, so further research is needed.

CONCLUSIONS AND SUGGESTIONS

The mean level of sCD40L in the thrombocyte whole blood product is higher than the mean level of sCD40L in the thrombocyte apheresis product. There was a significant positive correlation between storage times and sCD40L in the thrombocyte apheresis group ($r = 0.549$; $P < 0.05$), while there was no significant correlation between storage times and sCD40L in the thrombocyte whole blood group ($r = 0.315$; $P > 0.05$).

It is necessary to rearrange the preparation of blood products and to consider platelet storage time when giving transfusions, especially when using platelet products with a shorter shelf life. The longer the shelf life, the higher the degree of storage lesions, and the more possible side effects. There should be effort to conduct further research related to the effects that arise if transfusion is carried out with platelet products with high sCD40L content.

CONFLICT OF INTEREST

The authors declare that they don't have any competing interest regarding manuscript

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AUTHORS CONTRIBUTION

Ni Kadek Mulyantari developed the original idea, study concept and design, study protocol, and abstracted the data. I Putu Yuda Prabawa contributed to development of the protocol, statistical analysis, and prepared the manuscript.

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