



THE EFFECT OF THE PROCESSING AND STORAGE OF THROMBOCYTE CONCENTRATE ON THE ACIDITY (PH) LEVELS AND THE AMOUNT OF PLATEROMBOCITES

Muhamad Syamsul Arif Setiyo Negoro¹, Tavip Indrayana¹

¹Poltekes Kemenkes Semarang

*¹e-mail : cepungmarupung@gmail.com

Abstrak

Untuk mempertahankan mutu konsentrat trombosit yang baik berdasarkan kadar pH dan jumlah trombosit, perlu disimpan pada suhu $22 \pm 2^\circ\text{C}$ selama 3 – 5 hari secara *in vitro*. Penelitian ini bertujuan untuk mengetahui pengaruh pengolahan dan umur simpan konsentrat trombosit terhadap kadar pH dan jumlah trombosit. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh pengolahan dan umur simpan konsentrat trombosit terhadap kadar pH dan jumlah trombosit. Desain penelitian adalah desain kelompok pre dan posttest quasi-experiment. Kelompok pertama adalah konsentrat trombosit yang diproses melalui whole blood dan kelompok kedua adalah konsentrat trombosit yang diproses melalui tromboferesis. Setiap kelompok disimpan selama 0 hari, 3 hari dan 5 hari, setelah itu setiap periode penyimpanan dilakukan pengecekan kadar pH dan jumlah trombosit. Hasil menunjukkan bahwa tidak terjadi penurunan pH yang bermakna pada konsentrat trombosit yang diproses dari whole blood selama masa simpan 0 hari, masa simpan 3 hari dan masa simpan 5 hari dengan nilai p 0,06, sedangkan Kadar pH konsentrat trombosit yang diproses dari tromboferesis mengalami peningkatan signifikan dengan nilai p sebesar 0,00. Terjadi peningkatan jumlah trombosit yang signifikan pada konsentrat trombosit yang diolah dari whole blood selama masa penyimpanan 0 hari, masa penyimpanan 3 hari dan masa penyimpanan 5 hari dengan nilai p 0,00, sedangkan pada konsentrat trombosit yang diproses dari tromboferesis menurun secara signifikan dengan nilai p 0,00 dan disimpulkan bahwa umur simpan dan pengolahan konsentrat trombosit mempengaruhi kadar pH dan jumlah trombosit

Kata Kunci: konsentrat trombosit, tingkat pH, jumlah trombosit

Abstract

Research background In order to maintain good quality of platelet concentrate based on pH level and platelet count, it is necessary to store it at $22 \pm 2^\circ\text{C}$ for 3 – 5 days *in vitro*. The aim of this research was to determine the effect of the processing and shelf life of thrombocyte concentrate on pH levels and platelet counts. The purpose of this research is to determine the effect of the processing and shelf life of platelet concentrate on pH levels and platelet counts. The research design was quasi-experiment pre and posttest group design. The first group is platelet concentrate which is processed through whole blood and the second group is platelet concentrate which is processed through thrombopheresis. Each group was stored for 0 days, 3 days and 5 days, after which each storage period was checked for pH levels and Platelet Count. The results showed that there was no significant decrease in the pH level of the thrombocyte concentrate which was processed from whole blood during a storage period of 0 days, a storage period of 3 days and a storage period of 5 days with a p value of 0.06, while the pH level of the thrombocyte concentrate which was processed from thrombopheresis experienced a significant increase in p value of 0.00. There was a significant increase in the number of platelets in the thrombocyte concentrate which was processed from whole blood during a storage period of 0 days, a storage period of 3 days and a storage period of 5 days with a p value of 0.00, whereas in the thrombocyte concentrate which was processed from thrombopheresis it decreased significantly with a p value of 0.00. It was concluded that the shelf life and the processing of platelet concentrates affect the pH level and the number of platelets

Key words : platelet concentrate, pH level, platelet count

1. INTRODUCTION

Blood transfusion services are an important part of health services, which until

now, for some cases, is still the only way to save lives or improve morbid conditions.



Blood transfusions must be carried out rationally and for medical indications. To support this provision, the Blood Transfusion Unit must process whole blood into quality blood components according to predetermined standards. There are several types of products as the results of blood processing, including Whole Blood (WB), Packed Red Cell (PRC), and Thrombocyte Concentrate (TC).

The demand for TC needs is very high. One of the purposes of giving TC is to increase the number of platelets in various medical conditions, especially in thrombocytopenic patients. For this reason, an action is needed to maintain the quality of TC which can be seen from various aspects, such as from the pH level and the number of platelets in the blood bag during the processing and storage period (PMK No. 91 of 2015).

TC preparations can be obtained in several ways, the first is the method of processing Whole Blood (WB) into TC which is accommodated in a sterile blood bag system with an integrated transfer bag, and the platelet content is suspended in the plasma. The second method is through thrombopheresis, that is processing TCs using an apheresis tool (PMK No. 91 of 2015).

To maintain the quality of TC in terms of pH levels and platelet counts, the storage process is carried out using a rotator agitator at $22 \pm 2^{\circ}\text{C}$ for 3 – 5 days in vitro (Davine & Serrano, 2010).

TC is a blood component that is at risk and needs to be observed tightly because it is stored at room temperature which can affect platelet stability. If storage is not done properly, the quality of TC will decrease.

A good quality TC component should have a platelet count of $>60 \times 10^9$ thus it can pass quality control test (PMK No. 91 of 2015). The pH level of TC during storage is also maintained within normal limits. Lowering the pH will increase the breakdown of platelets. Several factors that cause changes in TC include the anticoagulant used, storage temperature, storage time and volume

thus TC must be observed during the processing and storage.

Based on this research background, the researcher is interested in conducting a study on the number of platelets in thrombocyte concentrate during a shelf life of 0 - 5 days.

2. RESEARCH METHOD

The type of research applied is a true experiment. The research design used was a pre-posttest control group design. The first group was the control group, namely platelet concentrate which was processed through whole blood processing and the second was the treatment group that is platelet concentrates which was processed through thrombopheresis. The research object is based on calculations using the Federer formula with 2 groups, therefore each group uses at least 16 TC bags.

To find out the difference in average levels of platelet counts in the thrombocyte concentrate group which was processed from whole blood and thrombocyte concentrate which was processed from thrombopheresis stored for 0 days, for 3 days and for 5 days, the Friedman test was used. Furthermore, the results of the Wilcoxon test were read to find out during the shelf life to what extent there was a significant difference in pH levels and platelet counts in the thrombocyte concentrate group which was processed from whole blood.

3. RESULT

3.1 Analysis of the average difference in pH levels.

The results of the Friedman test to find out the average difference in blood pH levels stored for 0 days, 3 days and 5 days in both the thrombocyte concentrate group which was processed from whole blood and the thrombocyte concentrate which was processed from thrombopheresis are presented in table 4.1



Group	Storage period 0 day (X ± SD)	Storage period 3 days (X ± SD)	Storage period 5 days (X ±SD)	P value
<i>trombocyte concentrate from Trombopheresis</i>	7,2 ± 0,0	7,7 ± 0,03	7,8 ± 0,03	0,00
<i>trombocyte concentrate from wholeblood</i>	7,29 ± 0,08	7,27 ± 0,18 ± 0,03	7,814 ± 0,22 ± 0,03	0,06
<i>trombocyte concentrate from Trombopheresis</i>	7,2 ± 0,0	7,7 ± 0,03	7,8 ± 0,03	0,00

Table 4.1 Differences in blood pH levels at various storage periods

Based on table 4.1, the p value is 0.06 which means that there is no significant difference in blood pH levels stored for different periods of time in the thrombocyte concentrate group which is processed from whole blood. The results of the Friedman test for the thrombocyte concentrate group processed from Thrombopheresis obtained a p value of 0.00 which means that there is a significant difference in pH levels stored for different periods of time, which are the shelf life of 0 days, 3 days and 5 days. To find out during which shelf life there is a difference in pH levels, Wilcoxon test was carried out.

The results of the Wilcoxon test to find out during which shelf life the pH levels experienced significant differences in the thrombocyte concentrate group which were processed through thrombopheresis are presented in table 4.2.

Table 4.2 . Differences in blood pH levels in the thrombocyte concentrate processed from the Thrombopheresis process between storage period

In table 4.2, it can be seen that the pH level in thrombocyte concentrate processed from whole blood at a shelf life of 0 days compared to a shelf life of 3 days, a shelf life of 0 days compared to a shelf life of 5 days and a shelf life of 3 days compared to a shelf life of 5

days all showed a p value of 0.00, which means that there was a significant difference in pH levels in thrombocyte concentrate processed from whole blood for various shelf lives.

3.2 Differences Analysis in the Average of Platelet Counts

The results of the Friedman test to determine the average difference in platelet count in the thrombocyte concentrate group which was processed from whole blood and thrombocyte concentrate which was processed from stored thrombopheresis for 0 days, for 3 days and for 5 days are presented in table 4.3.

Group	Storage period 0 day (X ±SD)	Storage period 3 days (X ± SD)	Storage period 5 days (X ± SD)	P value
<i>trombocyte concentrate from wholeblood</i>	1079562 ± 210797.5	1196875 ± 247657	1229250 ± 273274	0,00
<i>trombocyte concentrate from Trombopheresis</i>	634000 ± 0.00	604500 ± 30062	548000 ± 22580	0,00

From table 4.3, it can be seen that the number of platelets in the thrombocyte concentrate stored for different periods of time in the two groups obtained a p value of 0.00, which means that the length of time the thrombocyte concentrate is stored affects the level of platelet count. In the thrombocyte concentrate which was processed from whole blood, the longer the storage period increased, while in the thrombocyte concentrate group which was processed by the thrombopheresis



process, the longer the storage time, the amount of thrombocyte concentrate decreased.

Table 4.4 presents the results of the Wilcoxon test to find out during the shelf life to what extent there is a significant difference in the number of platelets in the thrombocyte concentrate group which is processed from whole blood.

Table 4.4. Differences in blood platelet count in thrombocyte concentrate processed from whole blood between storage periods(n:32)

Group	Storage period 0 day (X ± SD)	Storage period 3 days (X ± SD)	Storage period 5 days (X ± SD)	P value
trombocyte concentrate from whole blood	1079562 ± 210797.5	1196875 ± 247657	-	0.00
	1079562 ± 210797.5	-	12292 ± 27327.4	0.00
Trombopheresis	-	1196875 ± 247657	12292 ± 27327.4	0,00
	-	-	12292 ± 27327.4	0,07

Note: unit (µl)

Table 4.4 shows the results of the Wilcoxon test to find out the difference in the number of platelets in thrombocyte concentrate processed from whole blood with a shelf life of 0 days compared to a shelf life of 3 days, a shelf life of 0 days compared to a shelf life of 5 days and a shelf life of 3 days compared with the 5-day storage period, the p-value was 0.00. It means that there were significant differences in the number of platelets in various storage periods.

The results of the Wilcoxon test to find out at which shelf life there is a significant difference in platelet count levels in the thrombocyte concentrate group from Trombopheresis are presented in table 4.5.

Table 4.5. Differences in the number of blood platelets in the thrombocyte concentrate processed from the Trombopheresis process between storage periods

Group	Storage period 0 day (X ± SD)	Storage period 3 days (X ± SD)	Storage period 5 days (X ± SD)	P value
trombocyte concentrate from Trombopheresis	634000 ± 0.00	604500 ± 30062	-	0.00
	634000 ± 0.00	-	548000 ± 22580	0.00
Trombopheresis	-	604500 ± 30062	548000 ± 22580	0,07
	-	-	548000 ± 22580	0,07

Note: Unit (µl)

Table 4.5 shows that the results of the Wilcoxon test to determine the difference in the number of platelets in thrombocyte concentrate processed through the Trombopheresis process at a shelf life of 0 days compared to a shelf life of 3 days and a shelf life of 0 days compared to a shelf life of 5 days obtained p value 0.00 which means there is a difference in the number of platelets. The difference in the number of platelets in the 3-day storage period compared to the 5-day storage period obtained a p value of 0.07, which means that there was no significant difference in the number of platelets.

4. RESULTS AND DISCUSSION

Based on the results related to differences in pH levels and platelet counts in thrombocyte concentrate which are processed through different methods, namely whole blood and thrombopheresis processing as shown in table 4.1, it can be said that for pH in thrombocyte concentrate processed through whole blood, it appears that the shelf life is significantly longer and the quantity has decreased. However, based on the p value in table 4.5, there is no statistically significant decrease. The decrease in the pH of thrombocyte concentrate during the storage process may have occurred due to an increase in CO₂ or acidic compounds which are the result of the metabolism of erythrocyte cells in the thrombocyte concentrate. According to Sherwood (2016), cell metabolic processes

will produce residual compounds in the form of CO₂ and acidic compounds in the form of lactic acid or acetic acid.

Platelet concentrate obtained through whole blood processing still contains some erythrocytes. According to Amaliya, et al (2022), the major content of thrombocyte concentrate is platelets, followed by some erythrocytes and leukocytes. To ensure during the storage period there is no decrease in platelet quality, according to storage procedures, thrombocyte concentrate must be stored at 20°C to 24°C (Cooper, et al, 2017). In the storage temperature range, some of the erythrocyte cells in thrombocyte concentrate did not support the quality of the erythrocyte cells and stimulated an increase in metabolism. Efforts made to maintain the quality of erythrocyte cells need to be maintained at a temperature of 2-6°C (Avila, et al, 2022). An increase in metabolism for the rest of the erythrocyte cells may increase CO₂ levels and acidic compounds so that it has the potential to cause a decrease in the pH level in thrombocyte concentrate obtained through whole blood processing.

The pH level in the thrombocyte concentrate which is processed through the thrombopheresis process during the storage process as shown in table 4.1 and table 4.5, in quantity has experienced a significant increase. An increase in pH levels may occur because the thrombocyte concentrate processed through the thrombopheresis process does not contain erythrocyte cells, thus during the storage process at 20°C to 24°C there is no increase in CO₂ and acidic compounds due to the metabolism of the remaining erythrocyte cells that are still present in the thrombocyte concentrate. The lower the CO₂ level, the higher the pH value (Victor, et al, 2018).

Thrombocyte concentrate which is processed through the thrombopheresis does not contain erythrocytes because during the process of taking blood from donors only platelets are taken, while the blood components that are not used are put back into the blood circulation. According to Maitta (2018) apheresis is a method of collecting blood by

taking the necessary components. If only platelets are taken, it is called thrombopheresis (Coffe, 2020).

The number of platelets in thrombocyte concentrate obtained through processing whole blood as shown in table 4.7 when compared to before being stored, stored for 3 days and stored for 5 days, it appears that the number has increased.

The increase in the number of platelets in the thrombocyte concentrate obtained through whole blood processing is probably due to the damaged erythrocytes in the thrombocyte concentrate due to storage at an inappropriate temperature. Damaged erythrocytes will be destroyed and fragmented. Prisce & Wilson (2013) mention that cells will experience damage or lysis and the damaged cell fragments will be scattered. According to Zang et al., (2020) cariolis is a cell that is permanently damaged and destroyed. The fragments of the erythrocyte cells are counted during the process of counting the number of platelets. Based on this phenomenon, on day 3 of storage compared to day 5 of storage, the number of damaged erythrocytes increased, thus the average number of platelets in thrombocyte concentrate obtained through processing whole blood on day 5 of storage appeared to be the highest.

In contrast to the number of platelets in thrombocyte concentrate which was processed through the thrombopheresis process as stated in table 4.7, when compared to before being stored, it was stored for 3 days and stored for 5 days it appears to have decreased significantly. According to Permenkes No. 91, the life span of platelets in in vitro storage (without any shake) will be shorter than the age of platelets in vivo.

Thrombocyte concentrate storage is recommended not to exceed 8 days. According to Permenkes No. 91, it is better to store thrombocyte concentrate for 5 days at a temperature of 20°C to 24°C to maintain platelet viability. Platelet viability in Thrombocyte concentrate is maintained by storage at 22°C for 7 days (Slichter, et al, 2012).



5. CONCLUSIONS AND SUGGESTIONS

There was no significant decrease in the pH level of thrombocyte concentrate which was processed from whole blood during a storage period of 0 days, 3 days and 5 days.

There was a significant increase in pH levels in thrombocyte concentrate which was processed from thrombopheresis during a storage period of 0 days, 3 days and 5 days with a p value of 0.00.

There was a significant increase in the number of platelets in the thrombocyte concentrate which was processed from whole blood at a storage period of 0 days, 3 days and 5 days with a p value of 0.00.

There was a significant decrease in the number of platelets in the thrombocyte concentrate which was processed from thrombopheresis at a storage period of 0 days, 3 days and 5 days with a p value of 0.00.

BIBLIOGRAPHY

- Avila OC, Sayson S.C. Bennet Bruce, 2022. Whole Blood Storage Temperature Investigation in Austere Environments. *J Spec Oper Med* 19;22(3):19-21
- Coffe C, Pouthier F, Barisien C, Slimane M, Sheytanova A, 2020. Therapeutic leukapheresis and thrombopheresis in medical emergencies, *Transfus Apher Sci* ;59(6).
- Cooper S, Lloyd S, Koch A, LinX, DobbsK, TheisenT, Zuberbuehler M, Bernhardt K, Lehmann M, Neeves K. 2021. Temperature effects on the activity, shape, and storage of platelets from 13-lined ground squirrels, *Comp Physiol* ;187(5-6):815-825
- Davine.S.V & Sherrano K. 2010. The Platelet Storage Lesion, *Clin Lab Med*; 30 : 475-487
- Kadek Lestariyani, N. and Herawati, S., 2016. Perbedaan Jumlah Trombosit konsentrat Krombosit pada Penyimpanan hari i, iii, v di Unit Donor Darah PMI Provinsi Bali/rsup Sanglah Denpasar. *E Jurnal Medika*, 6(3).
- Kementrian Kesehatan Republik Indonesia, 2015. Peraturan Menteri Kesehatan Republik Indonesia Nomor 91 Tahun 2015 Tentang Standar Pelayanan Transfusi Darah. Jakarta: Kementrian Kesehatan Republik Indonesia.
- Marpaung, E., Setiawaty, V., Ken Ritchie, N. and S. Timan, I., 2015. Function and platelet count in thrombocyte concentrate (TC) during the Storage. *Health Science Journal of Indonesia*, 6(1).
- Mudayatiningsih, S., 2019. Biokimia Darah. Jakarta: Kementrian Kesehatan RI.
- Price SA., Wilson LM., 2012, Patofisiologi: Konsep Klinis Prsoses-proses Penyakit, Edisi ke-6, Jakarta: EGC
- Samad, R., Alim Abdullah, A., A. P., K. and Arif, M., 2014. Waktu Penyimpanan Trombosit Terkait Jumlah di Konsentrat Trombosit. *Indonesian Journal of Clinical Pathology and Medical Laboratory*, 20(3).
- Slichter J, Corson J, Jones KM, Christoffel T, Pellham E, Bolgiano D. 2012. Platelet concentrates prepared after a 20- to 24-hour hold of the whole blood at 22°C, *Transfusion* ,52(9):2043-8
- Sherwood L, 2016, Darah, In: Sherwood L, Fisiologi Manusia dari Sel ke Sistem, 8 ed, Jakarta: EGC pp. 415-436.
- Victor W. Rodwell, David Bender, Kathleen M. Botham (2018), Harper illustrated Biochemistri, 30 td edition, Mc-Graw Hill.
- Zhang Y, Hai Jia, Yuhang Jin, Ning Liu. 2020. Glycine Attenuates LPS-Induced Apoptosis and Inflammatory Cell Infiltration in Mouse Liver. *J Nutr*;150(5):1116-1125