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Platelet Morphological Assessment Based on Shelf-Life of Concentrated Platelet Components

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ABSTRACT

Concentrated platelet components are the components or platelets that are processed through centrifugation from whole blood or a single donor. The components of the concentrate platelets are stored in an agitator at a temperature of 20-24°C with a maximum shelf-life of five days. During the storage, a change in component metabolism occurs, which is characterized by alteration in platelet morphology as an indicator of decreasing quality of platelet components. The purpose of this study was to determine the quality of the components of platelets based on the shelf-life by measuring the platelet morphological values with the Kunicki method. This study applied an experimental method by conducting inspections of the regional blood transfusion unit (UTDD) of the Indonesian Red Cross of Jakarta Capital Special Region (PMI DKI Jakarta). Platelet morphological values of six components of platelets were examined by the Kunicki method on day 0 to day 5 of shelf-life. Quality assessment was carried out based on a significant difference-dependent t-test and comparison with normal value. The results of the study by performing statistical analysis of the significant difference-dependent t-test showed a significant difference of values between day 0 and day 1 to day 5 of shelf-life. Comparison with normal values depicted that all samples stored on day 4 had a morphological value of more than 200. On day 5 of shelf-life, the platelet morphology values of five samples were below 200. The quality of the concentrated platelet components is said "good" if it is above 200. This study concluded that there was a significant difference in the platelet morphology values between the components of concentrated platelets on day 0 and day 1 to day 5 of shelf-life. All samples were of good quality until day 4 of storage. However, on day 5 of storage, only one sample was good in quality.

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INTRODUCTION

Thrombocyte concentrate (TC) transfusion or platelet component transfusion is one of the therapies for thrombocytopenia patients. This component is stored at a temperature of 20-24°C with a shelf-life of 5 days in the agitator (Ravindra, 2009). Data from the Ministry of Health and Information Center recorded that Indonesia experienced an increase in the production of concentrated platelet components for transfusion each year. The national production of concentrated platelet components in 2014 was 13% of the 4,644,863 bags and it increased to 20.40% in 2016, from 4,201,578 bags of blood produced by 281 blood transfusion units throughout Indonesia (INFODATIN, 2014, 2018).

The manufacturing and storing of concentrated platelet components need to pay attention to standards and quality control because, during the storage period, changes in the structure and function of platelets can occur, which may result in damage to the components of the concentrated platelets ((Jain A, Neelam Marwaha, Ratti Ram Sharma, Jyotdeep Kaur, 2015; Diyanti, Luh Putu Sukma, 2017). In vivo storage of platelets can stimulate changes in biochemical and platelet function called a platelet storage lesion (Six, Compernolle and Feys, 2020).

The parameters used to determine the quality of platelets in the components of the concentrated platelets are platelet count, pH, volume, and residual leukocytes. Moreover, in assessing platelet function and the effect of platelet storage, another parameter, the calculation of the platelet morphological value, can also be utilized (Levin,E, Craig Jenkins, Brankica Culibrk, Maria IC, Gyongyossy-Issa, Katherine Serrano, no date). The calculation is based on the changes in the morphology of the platelets, from discoid or disc (normal) into round or dendritic. This change in shape can occur due to an activation process in storing the concentrated platelet components, particularly if the storage does not meet the standards or if the storage is done for a long period (Kunicki, TJ, M. Tucelli, G.A Becker, 1975; Dewland, 2017). Therefore, in quality control, it is necessary to assess the morphology of platelets to determine the suitability of process and concentrated platelet component storage (Snowball, 2016). The quality of platelet morphology can be measured through platelet morphological evaluation with the Kunicki method, by assessing the structural integrity of the platelets. Morphological determination is based on four categories of shape, including discoid or disc-like, sphere or round, dendritic, and balloon-like (Kunicki, TJ, M. Tucelli, G.A Becker, 1975). This assessment can be carried out using an improved Neubauer counting chamber with 1% ammonium oxalate reagent or preparation with May Grunwald Giemsa staining (Jain A, Neelam Marwaha, Ratti Ram Sharma, Jyotdeep Kaur, 2015; Snowball, 2016). Of the two techniques, the more common and easier technique for assessing platelet morphology is using preparations stained with May Grunwald Giemsa (Snowball, 2016).

Platelet morphological assessment using the Kunicki technique has not yet become a routine examination of platelet component quality in the regional blood transfusion unit (UTDD) of the Indonesian Red Cross of Jakarta Capital Special Region (PMI DKI Jakarta). Moreover, platelet quality has never been assessed based on morphology during the shelf-life of concentrated platelet components. Therefore, the study on the morphological assessment of platelets for controlling the quality of concentrated platelet components on day 0 to day 5 of storage was conducted. This study aimed to investigate whether or not a significant difference in the platelet morphology value between the components of platelet concentrate on day 0 and day 1 to day 5 of sheld-life happened.

MATERIALS AND METHODS

This research was an experimental study by examining the concentrated platelet components on days 1, 2, 3, 4, and 5, with day 0 as the control. The population of this study included the concentrated platelet components produced by the centrifugation process at the regional blood transfusion unit (UTDD) of the Indonesian Red Cross of Jakarta Capital Special Region (PMI DKI Jakarta). Samples taken from this study were the components of concentrated platelets in six bags of blood produced by the centrifugation process. The samples were taken by purposive sampling method, considering the inclusion criteria: (1) the concentrated platelet components were derived from whole blood through centrifugation, (2) the concentrated platelet components were stored in an agitator at a temperature of 20˚C - 24˚C, (3) the concentrated platelet components had a platelet count > 60 x 109, following the Regulation of Minister of Health Number 91 Year 2015, and (4) the samples were not components of concentrated platelets obtained by the apheresis technique and were not lysed or lipemic.

A light microscope, agitator, sealer machine, slide glass, 20 µL drop stirrer, 3 mL syringe, alcohol swab, dropper pipette, staining rack, hand sealer, and Eppendorf cup were used to perform this research. The materials used were May-Grunwald Giemsa stain, Wright stain, buffer (pH 6.5), absolute methanol, and immersion oil.

The research was completed through several stages of procedures. Samples were prepared from the components of concentrated platelets stored in an agitator at a temperature of 20˚C - 24˚C. Based on standard operating procedures applied at the regional blood transfusion unit (UTDD) of the Indonesian Red Cross of Jakarta Capital Special Region (PMI DKI Jakarta), sampling was done by squeezing the bag hose using a hand sealer and then homogenizing the concentrated platelet components for ten times and repeating the same procedure for three times, to ensure that the platelet concentrate components in the hose could accurately represent the entire bags. ± 1 mL samples were taken aseptically through the hose of the bag of concentrated platelet components using a syringe. After that, the end of the bag hose was closed again using a sealer machine. The samples were transferred to the Eppendorf cups for preparation and staining (Levin,E, Craig Jenkins, Brankica Culibrk, Maria IC, Gyongyossy-Issa, Katherine Serrano, no date; Bethesda, 2005).

The samples were then prepared with homogenization and then taken with a pipette, approximately 20 µL, and placed on a slide about one cm from one side of the slide. The sliding glass was placed in front of the drop at an angle of 30˚ - 45˚. The sliding glass touched the sample and allowed the droplets to spread on the slide. The slide was then slowly but steadily pushed forward. The smear was allowed to dry, then fixed using absolute methanol for five minutes or longer. After fixation, the preparation was rinsed with running water, and then Wright staining was performed for two minutes and the preparation was rinsed again with running water. The preparation was inundated with May-Grunwald Giemsa staining, with a concentration of 20% for 20 minutes, and rinsed with running water. The preparation was read on a microscope with 1000 times magnification by categorizing four platelet shapes: oval, round, dendritic, and bizarre (AS Adewoyin, 2014; Rukman, 2014; Snowball, 2016).

The smear preparation was observed and calculated using a light microscope with a magnification of 1000 times. The calculation was performed on 200 platelets based on oval, round, dendritic, and bizarre categories. The percentage of each platelet was multiplied by shape: oval (x4), round (x2), dendritic (x1), and

bizarre (x0). The maximum value is obtained if 100 percent of the platelets are oval with a total value of 400 (Snowball, 2016).

Statistical data analysis was carried out in two stages, covering normality test and significant different statistical test with a significance level of $p<0.05$ and a 95% confidence level. A normality test was performed to determine whether or not the data were normally distributed. If the data are normally distributed, a difference dependent t-test is carried out to determine whether there is a significant difference in the results of differences in morphological values based on the storage time of the component of the platelet concentrate, whereas if the data are not normally distributed, the Wilcoxon test is carried out.

RESULTS AND DISCUSSION

The microscopic platelet morphological assessment based on the shelf-life of the platelet components (day 0 to day 5) produced the following results (Figure 1). The platelet morphological value is good or the quality is acceptable if it is higher than 200. Table 1 demonstrates the results of platelet morphological assessment based on the shelf-life of platelet components from day 0 to day 5. A significant difference test was carried out to compare the platelet components on day 0 and day 1 to day 5. The normality (Shapiro-Wilk) test was previously performed to determine whether or not the obtained data were normally distributed. Table 2 presents that the overall sig value was more than 0.05, meaning that all data were normally distributed; and therefore, the difference-dependent t-test was carried out.

The results of difference dependent t-test presented in Table 3 show that the significance value of concentrate platelet components in day 0 and day 1 to day 5 was lower than the alpha value (0.05); and therefore, Ho was rejected. This exemplifies the significant difference of platelet morphological values of concentrate platelet components between day 0 and day 1 to day 5 of shelf-life. Graph showing the decrease pattern in platelet morphological values are detailed in Figure 2. Figure 2 demonstrates a decrease in the platelet morphological values in the six components of the concentrated platelets up to day 5 of shelf-life. Based on the normal platelet morphological values, only one sample of concentrated platelet components (sample 1 with a value of 201) fell into a good category until day 5 of shelf-life. Moreover, five samples (sample 2, 177; sample 3, 200; sample 4, 190; sample 5, 200; and sample 6, 149) of concentrated platelet components were considered bad.

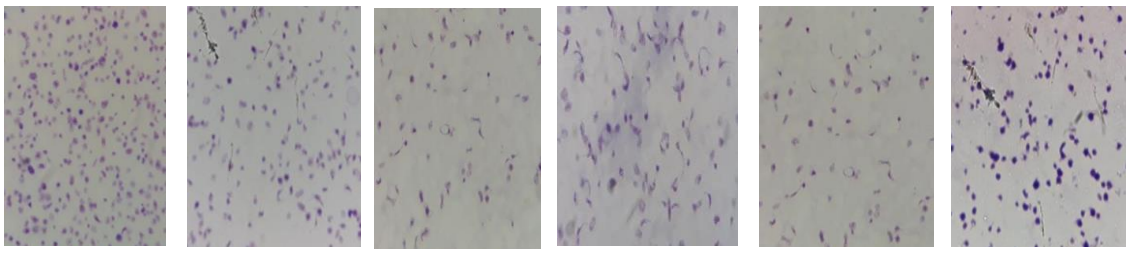

Figure 1. The platelet morphological assessment on the shelf-life of day 0 to day 5 (1000x Microscope Magnification)

Table 1. Minimum and maximum number, and the average morphological value of platelets based on shelf-life

Platelet component shelf-life of day -	Minimum number	Maximum number	Average value
0	360	391	376
1	313	357	335
2	289	321	306
3	279	299	289
4	243	280	264
5	149	201	186

Table 2. Normality Test on Data Distribution of Platelet Morphological Values of Concentrated Platelet Components

Platelet component shelf-life of day -	Shapiro-Wilk Sig.	Data distributionz
0	.823	Normal
1	.881	Normal
2	.708	Normal
3	.354	Normal
4	.759	Normal
5	.057	Normal

Table 3. The Results Difference Dependent T-Test on Samples Concentrated Platelet Components

Statistical Test (Day 0 to Day 5)	Sig.	Notes
Day 0 vs Day 1	.007	Significant difference
Day 0 vs Day 2	.000	Significant difference
Day 0 vs Day 3	.000	Significant difference
Day 0 vs Day 4	.000	Significant difference
Day 0 vs Day 5	.000	Significant difference

Technically, platelet morphological assessment is carried out to evaluate the structural changes in platelets during storage and their function (Kunicki, TJ, M. Tucelli, G.A Becker, 1975). In the Kunicki method, platelet morphology is said to have acceptable quality if the Kunicki score is more than 200 (Dewland, 2017). In this study, the morphological value of platelets varied in each component until day 5 of shelf-life, with a maximum value of 391 on day 0 and a minimum value of 149 on day 5 (Table 1). Examination on day 0 was carried out a few hours after the collection of donor blood and the preparation of concentrated platelet components. Along with the length of the shelf-life, a decrease in the morphological value of platelets occurred. This was evidenced by the minimum platelet morphological value of 149 obtained from the concentrated platelet component on day 5 of shelf-life (Table 1). Likewise, the assessment of concentrated platelet components on day 5 of shelf-life produced the minimum average value of 186, while the evaluation on day 0 of shelf-life resulted in the maximum average value of 376. The study by Jain et al (2015) recorded a decrease in the platelet morphological value on day 1 to day 3 of shelf-life of concentrated platelet components (Jain A, Neelam Marwaha, Ratti Ram Sharma, Jyotdeep Kaur, 2015)

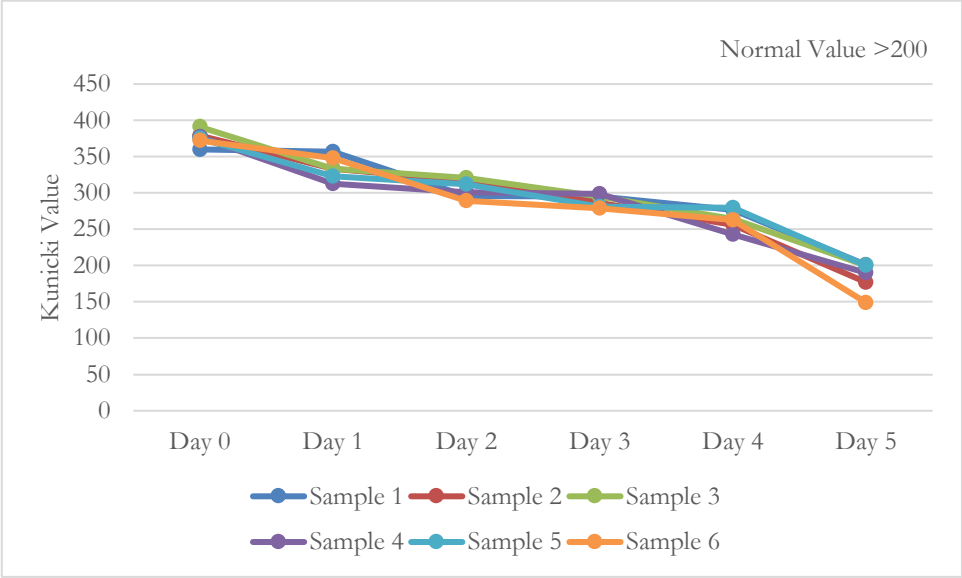

Figure 2. Graph of Platelet Morphological Assessment

.The statistical analysis of the significant difference test showed a significant difference in platelet morphological values on day 0 and day 1 until day 5 of shelf-life (Table 3). This difference occurred due to different average platelet morphological values resulted each day of shelf-life. The difference also depicted the decreased pattern in platelet morphological values (Figure 1).

Based on the normal platelet morphological value measured with the Kunicki method, only one sample of the concentrated platelet components in this study was categorized as "good" until day 5 of shelf-life. Five components of concentrated platelets had platelet morphological values of less than normal value. A decrease in the platelet morphology could occur during the process of collecting, processing, and storing platelet components.

Changes in platelets can happen during shelf-life due to several factors, such as the collection, preparation, and storage of the components (Raveendran *et al.*, 2019). During the storage process of concentrated platelet components, several changes in platelet metabolism that affect platelet morphology occur (Tynngård,N., Wallatesdt,M., Södergren,L.A., Faxälv,L., and Ramström, 2014). Platelet morphological changes occur when the platelets are activated and the platelet granules release their contents. This activation can be caused by several aspects, such as exposure to foreign surfaces, trauma, low pH, agonists, Gp Ib-IX-V, and Gp VI (Mittal and Kaur, 2015; Seong-Hoon Yun, Eun-Hye Sim, Ri-Young Goh, Joo-In Park, 2016). This reaction causes a change in the structural integrity of platelets which then causes the morphology of the platelets to change from oval to round, and then dendritic and balloon, where the platelets have swollen after losing their osmotic resistance capacity (Kunicki, TJ, M. Tucelli, G.A Becker, 1975; Mittal and Kaur, 2015).

With the long shelf-life of the concentrated platelet components, many platelets turned round and dendritic, thus affecting the platelet morphological value. In this study, on day 0 and day 1 of shelf-life, platelets were generally oval or disc-shaped, while on days 2, 3, and 4, they started to look round and dendritic, and on day 5, they were dominated by a round shape and almost no oval

or disc shape was found.

Platelet morphological changes occur due to the alteration in the platelet metabolism in the blood bags. The reduced oxygen pressure (pO_2) in the pocket of the concentrated platelet component can cause an increase in glycolysis rate during the shelf-life of the concentrated platelet component. This also triggers an increase in glucose consumption and lactic acid production, as well as a decrease in the pH of the concentrated platelet component during storage. A decrease in pH is associated with a loss of platelet viability. When the pH drops below 6.2, the platelets will swell and transform from a disc shape to a spherical shape (Harmening, 2012). The morphology of platelets that become round and then dendritic generally shows an increase in activation as the shelf-life of the platelet concentrated component increases (Dewland, 2017).

CONCLUSION

This study concluded a significant difference in the platelet morphological values between the concentrated platelet components on day 0 and other days (day 1 to day 5) of shelf-life. All components of the concentrated platelets were good in quality until day 4 shelf-life, but only one sample was in good quality on day 5 of shelf-life.

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CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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