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**ORIGINAL PAPERS** 



# Effect of Storage Period on Platelet Count and Erythrocyte Morphology in Whole Blood Bags

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## Abstract

Platelets and erythrocytes undergo transformation during storage, which may cause them to become inactive or non-functional. In transfusion medicine, the storage period of platelets and erythrocytes is very important because they change shape during storage, such as during echinocytosis, spherocytosis, and crenation. This study aims to determine the effect of storage period on platelet count and erythrocyte morphology in whole blood samples. Methods: This study used simple random sampling method to determine the effect of time on the occurrence of a disease. The study was conducted from January to April 2022 at the Semarang Regency Blood Donor Unit. This study focuses on the effect of time on the occurrence of a disease in whole blood samples. This study used automated sampling with hematology analyzer to analyze the data. Data collection was done with informed consent from donors and laboratory data from Blood Donor Unit Semarang Regency. The results showed a significant difference in changes in platelet count and erythrocyte morphology between day 0 and day 30 storage periods, with a p value of 0.014. It is recommended that in the process of storing blood bags in the Blood Donor Unit, excessive shelf life is avoided to maintain blood quality.

Keywords: Cerythrocyte, platelet, storage period, whole blood.

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#### INTRODUCTION

Whole blood is a blood product consisting of red blood cells, white blood cells, and platelets stored in a plastic bag with a preservative solution.<sup>1,2</sup> Storage of blood in plastic bags with preservative solution aims to maintain blood quality so that it remains usable and safe for transfusion. Storage of blood in plastic bags with preservative solution can affect blood quality. Some factors that affect blood quality during storage include storage temperature, type of preservative solution, and length of storage. One of the parameters used to evaluate blood quality is platelet count and erythrocyte morphology. Platelets are blood cells that function in the blood clotting process. A sufficient platelet count is important in blood transfusion as a lack of platelets can cause bleeding. In addition, erythrocyte morphology is also important as it can provide information about the patient's condition such as anemia<sup>3-5</sup>.

WB with CPDA-1 (Citrate Phosphate Dextrose Adenine-1) anti-coagulant is stored at 2-6 °C with a storage period of up to 35 days. The storage period of WB will cause effects in the form of changes in erythrocyte membrane integrity. Blood during the storage period will experience changes in blood components. One of the blood components that play an important role in hemostatis is platelets or blood clots. Platelets play an important role in repairing the chain of blood vessel damage reactions and initiating the cessation of bleeding resulting in blood clots (thrombus). Decreased platelet levels are found in some cases such as Von Willebrnad's disease, vitamin K deficiency and others.<sup>6</sup>

The storage period of platelets and erythrocytes is a crucial factor in transfusion medicine. Platelets are usually stored for up to 5 days prior to transfusion, although in some blood services the storage period is extended to 7 days<sup>7</sup>. Platelets and the medium in which they are stored undergo transformations while they are being stored, and these transformations can cause platelets to become activated or dysfunctional. The implications of these alterations for clinical practice are not yet clear.<sup>7</sup>

Erythrocytes can be preserved in a solution containing a preservative and kept at 4 degrees Celsius for up to 42 days.<sup>8</sup> Erythrocytes go through a variety of morphological changes while they are being stored, including echinocytosis, spherocytosis, and crenation<sup>3</sup>. These alterations can have a negative impact on the quality of the erythrocytes that are transfused and may cause adverse reactions in the recipient<sup>9</sup>. A systematic review of 18 studies analyzed the relationship between the length of time platelets were stored and clinical outcomes in allogeneic platelet transfusions. Five of the 18 studies included 4719 critically ill patients and 8569 patients with hematology conditions. None found any association between the length of time platelets were stored and the risk of death, even when platelets were kept for up to five days. Seven out of thirteen studies on hematology patients found that fresh platelets (less than two or three days old) were associated with a significant increase in corrected count increment (CCI) compared to older platelets<sup>7</sup>.

The clinical implications of transfusing long-stored blood are that patients may experience adverse side effects due to the metabolic and biochemical changes that occur in red blood cells during storage. One of these adverse effects is related to red blood cells losing their deformability during storage. In vivo, red blood cells need to adjust their shape, especially when passing through capillaries and the spleen, to maintain their viability. During storage, morphological changes, biochemical changes, and oxidative stress can damage the red blood cell membrane, leading to impaired deformability. Additionally, damage to the protein cytoskeleton can reduce membrane elasticity, increase the fragility of red blood cells, and reduce their viability.<sup>10</sup>

#### METODE

The type of research method used is an experiment that aims to determine the gelaja or influence that arises as a result of treatment. The research sampling technique with simple random sampling. Voluntary donor blood samples that were successfully tapped and passed the IMLTD filter test examination at the Semarang Regency Indonesian Red Cross Blood Donor Unit. This study was conducted in January-April 2022 at the Semarang Regency Blood Donor Unit. Storage periods tested were 0, 10, 20 and 30 days in the blood bank refrigerator. Tests were conducted on platelet levels and erythrocyte morphology in whole blood. Measurement of platelet cell levels using automatic method with Hematology Analyzer. Secondary data in the form of supporting data derived from archives of donor informed consent data and archives of laboratory examination data at the Semarang Regency Blood Donor Unit.

## DATA ANALYSIS

Data analysis was carried out descriptively, to obtain an overview of the data or variable scores measured. Data were analyzed analytically to determine the effect of storage time on the characteristics of whole blood platelet levels at blood bank refrigerator temperature. To find differences in storage time on the characteristics of platelet levels in the blood bank refrigerator using Saphiro wilk two independent samples, then comparative hypothesis testing of paired samples for interval and ratio data types using One-Way Anova statistical techniques. The data obtained were normally distributed with a value > 0.05.

## RESULTS

Based on the results of the examination conducted at the Ungaran Regional Health Laboratory on the effect of storage period on platelet levels in whole blood.

Tabel 1. Population Characteristics

Study Population Characteristics	All n (%) or mean (Min± Max)			
Observations (n)	30			
Gender				
Male	17 (56.7%)			
Female	13 (43.3%)			
Age (Years)	46 (32±55)			
Hemoglobin (g/dL)	15.2 (14.2±15.8)			

Table 1 shows that the average age of respondents is 46 years, minimum 32 years and maximum 55 years. The average hemoglobin of the respondents was 15.2 g/dL, minimum 14.2 and maximum 15.8 g/dL. Most of the respondents were Muslim (86.6%) while the gender of the respondents were 17 (56.7%) males and 13 (43.3%) females respectively. Platelet values based on storage duration of 0 days to 30 had a mean on day 30 of 170.8 with a decrease value of 85.5 (56.5%).

 Table 2. Results of Comparative Analysis of Platelet Levels.

Thrombosis	n	Mean ±SD	P value	
0 (Day)	30	272.9±68.6		
10 (Day)	30	239.8±66.3	.0.001	
20 (Day)	30	192.2±48.1	<0.001	
30 (Day)	30	170.8±45.6		

According to Table 2, the mean value of platelet levels with 0 days storage is 272.9 with a standard deviation of 68.6 and a p-value of less than 0.001. The mean value of platelet levels with 10 days storage is 239.8 with a standard deviation of 66.3 and a p-value of less than 0.001. The mean value of platelet levels with 20 days storage is 192.2 with a standard deviation of 48.1 and a p-value of less than 0.001. Finally, the mean value of platelet levels with 30 days storage is 170 with a standard deviation of 45.6 and a p-value of less than 0.001.

**Table 3.** Differences in erythrocyte morphology changes in each group.

parameter	ties	Postiif rank	Negative rank	P value
Changes in erythrocyte morphology of the 10th day shelf-life group	9	1	0	0.32
Changes in erythrocyte morphology of the 20th day storage period group	6	4	0	0.07
Changes in erythrocyte morphology of the 30th day shelf life group	4	6	0	0.01

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In the table 3, it appears that in the 30th day shelflife group, the Wilcoxon test results obtained a p value of 0.01, so it can be concluded that there are significant changes in erythrocyte morphology between the 0th day shelf life and the 30th day shelf life. The results of the p value in the 10th day storage period group and the 20th day storage period group obtained results  $\geq$  0.05, so it can be concluded that there are no significant changes in erythrocyte morphology. The morphological shape of erythrocytes from storage period 0 to 30 days can be seen in Figure 1.



Figure 1. Edge Blood Smear Preparation (Erythrocyte Morphology)

### DISCUSSION

Whole blood transfusion is one of the life-saving measures through the process of transferring healthy donor blood which is generally given to recipients with massive bleeding. Prevention of transfusion reactions is by maintaining the quality and quality of blood products. Inappropriate blood storage process will change the blood components. One of the components of whole blood is platelets which play a role in repairing the blood vessel damage reaction chain and initiating the cessation of bleeding which results in the formation of blood clots (thrombus)<sup>11-13</sup>.

Drug-induced thrombocytopenia is diagnosed by noting the time relationship between drug administration and the onset of thrombocytopenia, through immune mechanisms, GPIIb/IIIa inhibition-induced thrombocytopenia within 24 hours of exposure. Increased platelet destruction is associated with the use of thiazide diuretics, ethanol, esterogen, trimethropime-sulfamethoxazole, and chemotherapeutic agents. Increased platelet destruction is suspected in patients given quinine, quinidine, heparin, gold salts, rifampin and sulfonamides. Platelet abnormalities can be the cause of decreased platelet levels, namely changes in cell structure. a decrease in pH (<6.8) causes platelet cell morphology to change shape irreversibly and can reduce platelet viability<sup>14-16</sup>.

The blood quality of both erythrocytes and platelets in blood bags is based on several examination parameters, namely the number and morphology of platelets and erythrocytes. Based on the results, only the 30th blood storage period group experienced significant changes in erythrocyte morphology. The existence of blood cell damage can be seen with indicators of changes in blood cell morphology<sup>17,18</sup>.

Platelets are typically kept in storage for a period of up to 5 days before being used in a transfusion; however, the storage period may be extended to 7 days in some blood services. Platelets and the medium in which they are stored undergo transformations while they are being stored, and these transformations can cause platelets to become activated or dysfunctional. Uncertainty persists regarding these changes' implications for clinical practice. In patients who were receiving allogeneic platelet transfusions, a systematic review was carried out to investigate the association between the length of time that platelets were stored and the clinical or transfusion outcomes. The findings of the review indicate that the amount of time that platelets are kept in storage do not appear to be associated with any clinical outcomes in critically ill patients or hematology patients. These outcomes include bleeding, sepsis, and even death in some cases<sup>7</sup>.

The morphology of erythrocytes is a crucial factor that determines their function. Changes in erythrocyte morphology have been associated with the development of various diseases, including sickle cell anemia, thalassemia, and hereditary spherocytosis<sup>19</sup>. A study was conducted to compare the erythrocyte morphology in blood smears stained with K3EDTA and Na2EDTA, with a focus on the effects of varying storage times20. According to the research findings, there were no discernible differences in the shape of erythrocytes between K3EDTA and Na2EDTA, regardless of the length of storage time.

### CONCLUSION

There is a significant difference in changes in platelet levels and changes in erythrocyte morphology between the storage period of day 0 and day 30 with a p value of 0.014. It is recommended that in the storage process of blood bags in the Blood Donor Unit, excessive shelf life be avoided to maintain blood quality.

**Ethical Approval**. The research protocol was approved by the Health Ethics Commission of the Poltekkes Kemenkes Semarang with registration No. AHU/ KEPK-SMRG/0365/2023

**Informed Consent**. For this type of study, informed consent is not required.

**Consent for publication.** For this type of study, consent for publication is not required.

**Competing interests.** The author(s) declare no competing interests

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