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The Effect of Storage Time on the Whole Blood (WB) Quality at the Blood Bank of Dr. Soetomo General Hospital

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Abstract---Several changes occur during whole blood (WB) storage in the blood banks. The structure of erythrocytes and thrombocytes changes, and their viability decreases. Leukocyte degradation causes the release of cytokines and enzymes that can trigger a transfusion reaction. In addition, a decrease in pH will damage the WB components. The coagulation factors will be degraded during the storage process. All of these changes will impact the WB quality and the recipient. Therefore, this study aims to analyze the effect of storage time on WB quality. This research employed an analytical study with a time series design conducted at the Clinical Pathology Installation and Blood Bank at Dr. Soetomo General Hospital, Surabaya, on February-June 2020. The researchers utilized a sample of sixteen WB bags. The sample will be tested with several examinations, including a complete blood count examination (to determine the number of erythrocytes, leukocytes, thrombocytes, and hemoglobin), the coagulation examination (to determine PPT and APTT), and a BGA examination (to determine pH, pO₂, and pCO₂). All parameters were checked on day 0, day 5, day 10, day 20, and day 30. The statistical analysis was carried out with the same-subject analysis of variance and Friedman's test. The different test results using the same subject analysis of variance on the number of erythrocytes resulted in a p-value of 0.07, meaning that there was no significant difference in the number of erythrocytes. In addition, Friedman's test on the number of leukocytes, thrombocytes, hemoglobin, PPT, APTT,

pH, and pO₂ indicated a p-value of less than 0.001, implying there was a significant difference. Meanwhile, the analysis results of the same variance of pCO₂ subjects demonstrated a p-value of less than 0.001, indicating that there were significant differences in WB storage time on day 0, day 5, day 10, day 20, and day 30. There is no difference in the number of erythrocytes on the storage time of whole blood. On the contrary, differences appeared in the number of leukocytes, thrombocytes, hemoglobin, PPT, APTT, pH, pO₂, and pCO₂ on the storage time.

Keywords---BGA, coagulation, complete blood, storage lesion, whole blood.

Introduction

Blood is a bodily fluid carrying nutrients and oxygen into the cells while also removing unnecessary materials from the cells. Blood supplies oxygen to the tissues with hemoglobin. In addition, the blood also supplies nutrients, such as glucose and amino acids, and removes carbon dioxide, urea, and lactic acid. This assists in the regulation of pH and body temperature. Blood consists of several components, including erythrocytes, leukocytes, and thrombocytes suspended in plasma (Vani et al., 2015).

The utilization of whole blood (WB) to treat and prevent hemorrhagic shock began during World War I. The use of WB with citrate indicated effective results in rescuing wounded soldiers during the war. As a result of the excessive use of WB during the war, the "walking blood bank" concept emerged in the early 2000s. In addition, the numerous issues that developed during the usage of WB in World War I have shifted the use of WB to the use of blood components after the Vietnam War (Meledeo et al., 2019).

In 2018, the demand for whole blood at Dr. Soetomo General Hospital was 21,768 bags, with just 39% (8,325) of the 21,371 bags that were crossmatched being taken and donated to patients. The incidence of transfusion reactions on WB administration was 18.7% of all transfusion reactions. The number of unutilized WB is quite large, so it is necessary to investigate its feasibility (Instalasi Transfusi Darah RSUD Dr. Soetomo, 2018).

Several studies have revealed an increased risk of complications after transfusion with long storage of whole blood. It has been discovered that blood cells undergo structural and functional changes during storage, reducing these cells' function and life span following transfusion (Adias, Moore-igwe, and Jeremiah, 2012). On top of that, erythrocytes undergo changes in structure and function and decrease in viability. Likewise, the oxygen affinity of erythrocytes also decreases during the storage process. Thrombocytes also undergo a change in shape and a decrease in the aggregation response during the storage process (Vani et al., 2015).

Leukocytes have a shorter lifespan than RBCs. Neutrophils have a life span of about 10 hours, whereas, when activated due to the presence of bacteria, their life

span is only 30 minutes. Damaged leukocytes will release enzymes that will attack the erythrocyte membrane. One of the key outcomes of releasing enzymes by leukocytes is the formation of platelet-activating factor (PAF). The formation of PAF is thought to be responsible for the incidence of transfusion-related acute lung injury (TRALI). This damage caused by leukocytes can be reduced by isolating leukocytes using filtration (Sparrow et al., 2006; Ashton, 2013). The viability of thrombocytes stored at cold temperatures is considered to last up to day 14 of storage. Whole blood with leukoreduced can result in longer platelet viability (Kristoffersen and Apelseth, 2019).

The decrease in coagulation factors during the storage process has long been known. Factors V and VIII are the most rapidly degraded coagulation factors, and as a result, they are classified as labile factors. The coagulation process requires all the factors to function properly. Plasma prothrombin time (PPT) and activated partial thromboplastin time (APTT) are laboratory tests used to investigate the coagulation function of both the intrinsic and extrinsic pathways. The loss of one or more coagulation factors will affect the overall coagulation process, thus affecting the prolongation of PPT and APTT examinations (Pidcoke et al., 2013; Palta, Saroa and Palta, 2014).

Research Method

This research employed an analytical study with an observational time-series design. The sample was collected using a consecutive sampling technique from February to June 2020. The inclusion criteria were WB components (stored on day 0, day 5, day 10, day 20, and day 30) obtained during the study at the Blood Bank of Dr. Soetomo General Hospital, which is in accordance with the Regulation of the Minister of Health (Permenkes) number 91 of 2015. Meanwhile, the exclusion criteria for this study were damaged WB components (with signs of hemolysis and lipemia). WB stored at day 0, day 5, day 10, day 20, and day 30 at a temperature of 2-6°C were taken according to the storage time for measurements of Hb, RBC, WBC, PLT, pH, pCO₂, pO₂, PPT, and APTT.

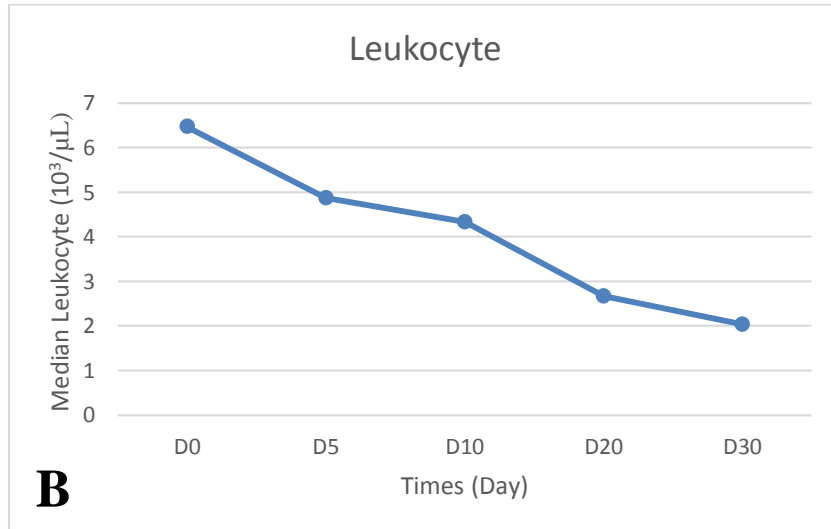
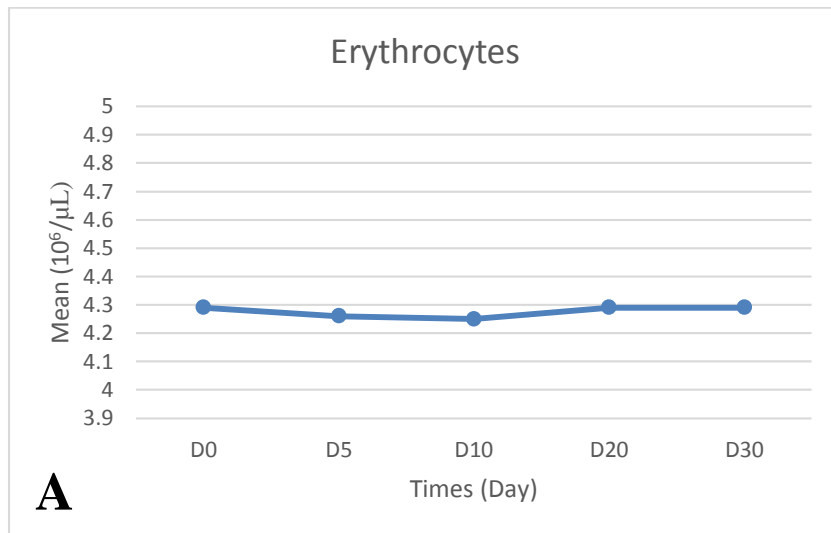
Results and Discussion

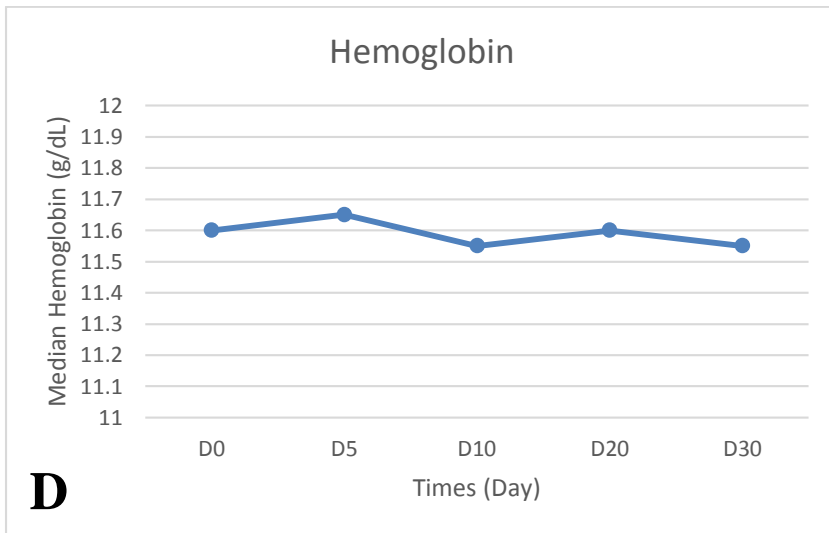
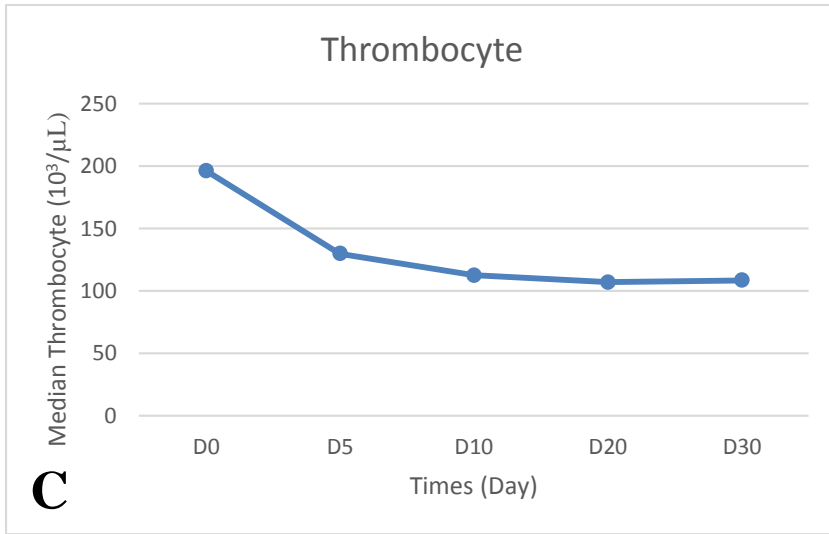
The samples used in this study were 16 bags of WB. This amount has met the minimum sample size set in this study. The sampling was carried out from February to June 2020 at the Blood Bank of Dr. Soetomo General Hospital, Surabaya. The characteristics of the sample in the form of donor blood type are presented in Table 1.

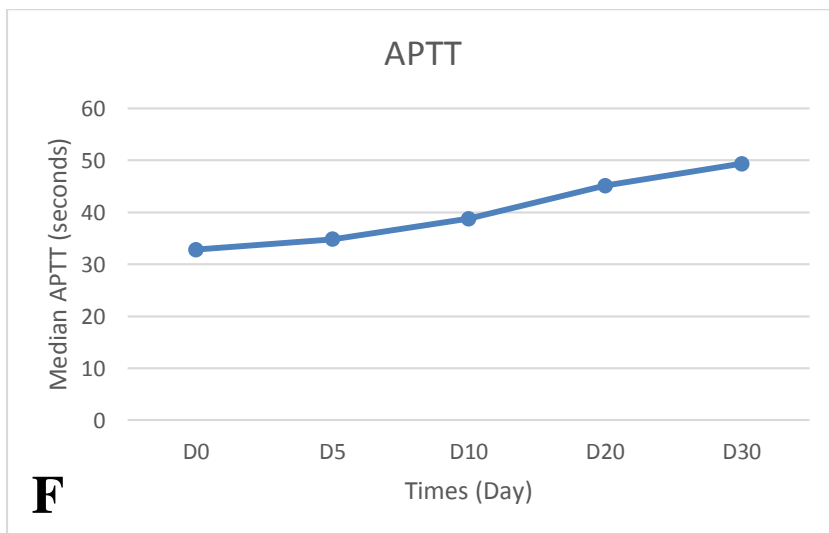
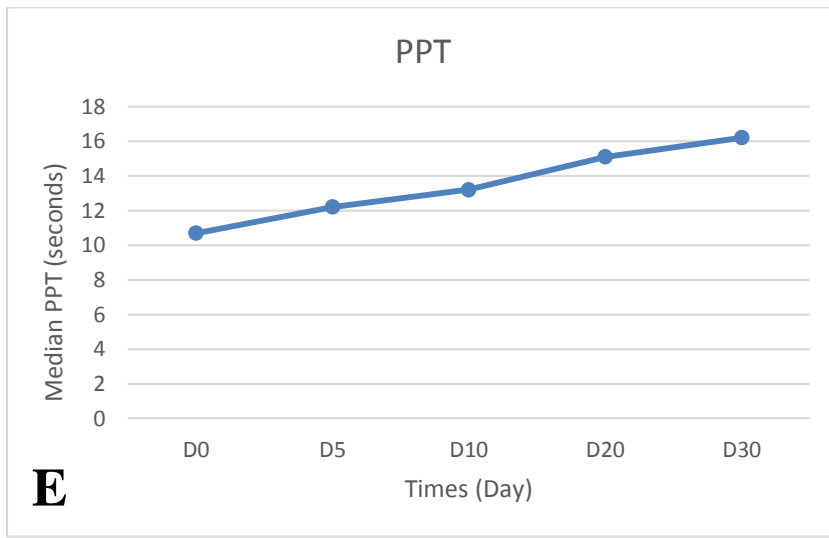
Table 1
Characteristics of the research sample

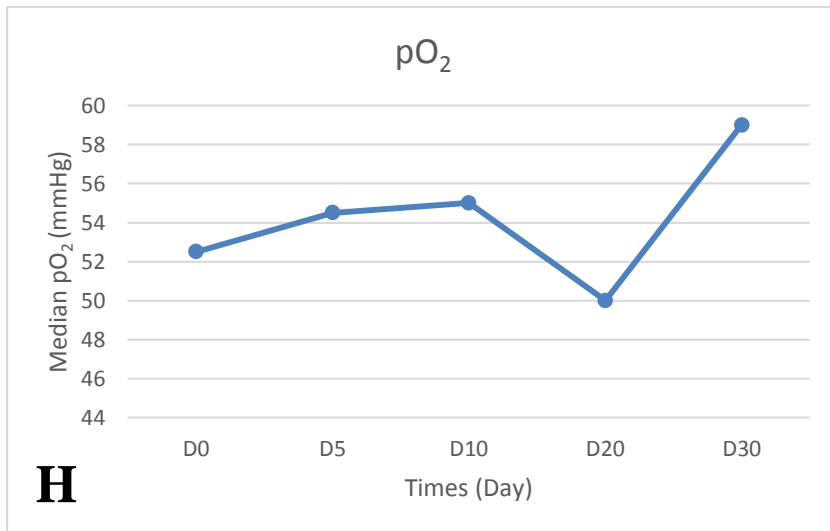
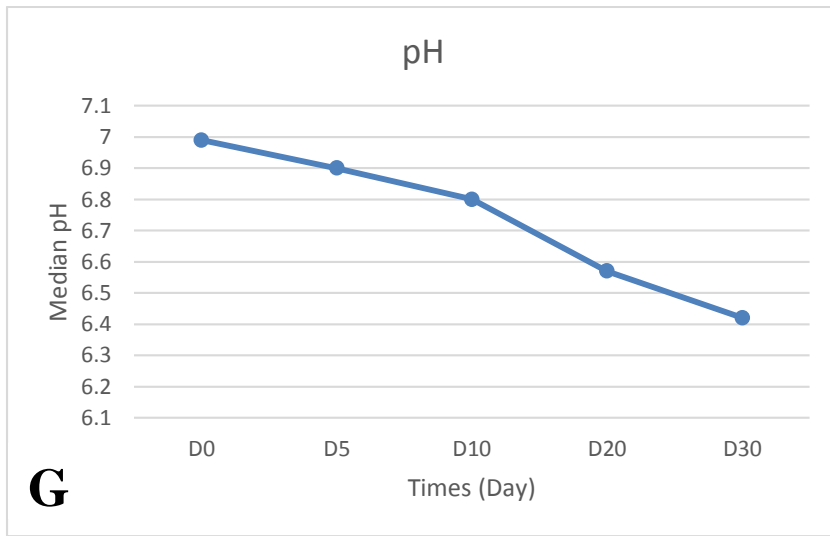
Blood type	Whole Blood (bag)	Total (%)
A+	6	37.5
B+	5	31.25
O+	5	31.25
AB+	0	0
Total	16	100

There was no significant difference in the number of erythrocytes during WB storage with a p-value of 0.07. Otherwise, significant differences were found in other parameters, such as Hb, leukocytes, thrombocytes, PPT, APTT, pH, pO₂, and pCO₂. The graph of changes in each parameter can be seen in Figure 1. Graph of changes for each parameter is shown in Figure 1.









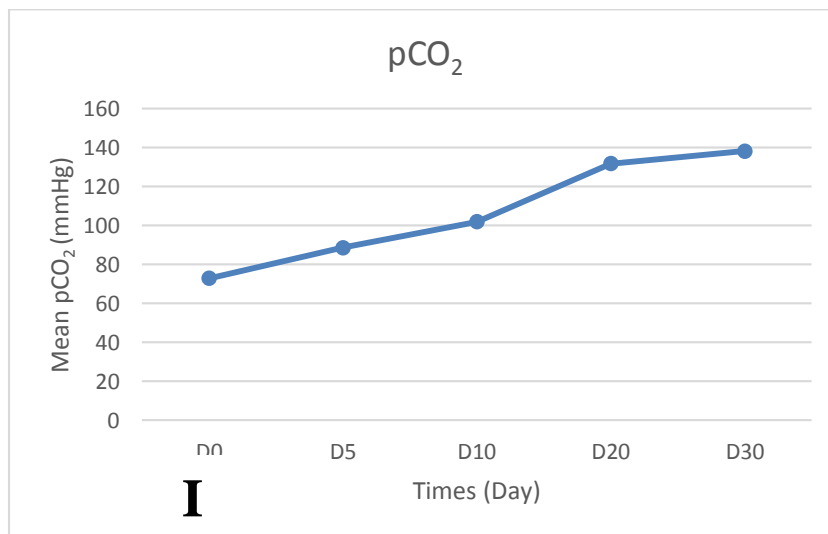


Figure 1. Graph of changes in each parameter during storage time: A Erythrocytes; B Leukocytes; C Thrombocytes; D Hemoglobin; E PPT; F APTT; G pH; H pO₂; I pCO₂

The difference in the number of erythrocytes is still included in the coefficient of variation (CV) range, meaning that there is no significant change in the number of erythrocytes during the storage time (Sysmex Corporation, 2012). The storage time of erythrocytes varied from 0 – 120 days. Erythrocytes with a shorter storage time can last longer, while erythrocytes with longer storage time (closer to 120 days) will be immediately degraded (Tuo et al., 2014). The results of this study are similar to those of previous studies conducted by Adias et al., indicating no significant difference in the mean of erythrocytes number during WB storage (Adias, Moore-igwe, and Jeremiah, 2012).

The results of the leukocyte test revealed a substantial difference from day 5 of the examination. This indicated that leukocytes had degraded significantly within five days of storage. The leukocytes degradation in the blood bag caused the release of enzymes, such as proteases, lipases, and glycosidases. These enzymes could attack the erythrocyte membrane and cause erythrocyte damage. Lipase can dealkylate *trialkyl-glycerols* to form *lysophospholipids*. This process triggered the formation of platelet-activating factor (PAF), which was responsible for several cases of transfusion-related acute lung injury (TRALI) (Bux and Sachs, 2007). This study showed a significant difference starting on day 5 of observation, indicating that thrombocytes began to damage on that day. The stored thrombocytes would experience changes in shape and a decrease in function. This phenomenon is known as platelet storage lesion (PSL). The mechanism responsible for PSL is still not fully understood, but it is associated with decreased safety and hemostatic activity of thrombocytes after transfusion (Mittal and Kaur, 2015).

The measured hemoglobin was the hemoglobin in the erythrocytes and plasma. Therefore, there was no major change in the hemoglobin level. Since changes occurring during storage were also still included in the CV of the equipment used,

there were no clinically substantial differences even if statistically significant differences were found (Sysmex Corporation, 2012). The different PPT tests demonstrated a significant difference on day 5 of the examination. The most influential coagulation factor on PPT examination was factor VII. Factor VII had the shortest half-life than other factors (3-6 hours). The loss of factor VII will affect the PPT examination (Bolliger, Görlinger, and Tanaka, 2010). The results of the different APTT tests indicated an increase during storage time. This was also found in the study of Pidcoke et al., where from the 8 WB stored at 4°C, all of them demonstrated APTT prolongation. The APTT prolongation occurring due to the degradation of coagulation factors during storage time had long been known. Each coagulation factor has various levels of degradation, but factors V and VIII are the most rapidly degraded factors (Pidcoke et al., 2013).

This study revealed a progressive decrease in pH during storage time. The mean decrease in pH was also discovered in the study carried out by Nogueira et al., who used PRC. Nogueira et al. conducted a study on PRC stored at a temperature of 2°C – 6°C and observed on day 0, day 7, day 14, day 21, day 28, and day 35. On day 0, the pH was 7.33. By the end of the examination (day 35), the pH had decreased to 6.528. The primary cause of this progressive decrease in pH was lactate accumulation from anaerobic glycolysis during the storage process (Nogueira et al., 2015). A decrease in pH also would affect the function of WB for tissue oxygenation. An environment with a pH of less than 7.2 will trigger the breakdown of 2,3-diphosphoglycerate (DPG), playing an essential role in the affinity of erythrocytes for oxygen (Hess, 2010). The results of the examination revealed a decrease and increase in pO₂ levels. This was similar to the research of Oh et al., concluding that contamination occurred while sampling for examination (Oh et al., 2020). In addition, pCO₂ levels in this study also increased during the storage process. The increase in pCO₂ can be produced by HCO₃⁻, converting H⁺ to compensate for the excess lactic acid (Verma and Dahiya, 2015).

This study observed changes in several parameters in the WB bag during the storage process. Several parameters, such as erythrocytes and thrombocytes, were only examined for changes in their number rather than their functional capabilities. The researchers examined ten parameters of the WB components stored at 4°C for 30 days. The examination result showed a decrease in erythrocytes, leukocytes, thrombocytes, hemoglobin, and pH during the WB storage time. Conversely, other parameters, such as PPT, APTT, and pCO₂, increased during storage time. All changes to the components in the WB bag will certainly affect the donor-recipient. Based on the parameters of hemoglobin and erythrocytes, WB stored for 30 days is still feasible. On the other hand, other parameters warn that changes after day 5 should be monitored. This evidences that WB should be distributed before day 5. However, it is hard to realize since the ideal WB supply does not always available. This can be overcome by using WB with the shortest storage days or under 20 days of storage. This is due to several parameters that have changed significantly since the beginning of the sampling. Consequently, Leukocytes and thrombocytes decreased by almost 50% from the initial sampling. Compared to the initial examination, PPT and APTT parameters increased by more than 50%.

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