Volume 6, No. 1 January, 2025
p ISSN 2723-6927-e ISSN 2723-4339

The Effect of Platelet Aditive Solution In Maintaining the Quality and Function of Concentrated Platelets In The Pooling Method of Buffy Coat Leukofiltered

Idah Wido Sari Nawangsih[1*], Sri Widia A. Jusman[2], Saptuti Chunaeni[3]
Universitas Indonesia, Indonesia
Email: iid.nawangsih@gmail.com

Abstract
The availability of platelets in the Indonesian Red Cross (PMI) Blood Transfusion Unit (UDD) has been significantly high, with 767,680 bags collected in 2019, increasing to 957,397 bags in 2021. The dengue fever outbreak alongside the COVID-19 pandemic led to a nearly twofold increase in the demand for concentrated platelets (TP) in hospitals from 2021. However, platelet additive solution (PAS), which can extend platelet shelf life, is not yet utilized in Indonesia. This study aims to investigate whether TP obtained using the buffy coat leukofiltered pooling method and stored with PAS can improve platelet quality during storage. A series of laboratory tests were conducted to evaluate platelet count, pH levels, and aggregation function using ADP inspection, monitored over a storage period from day zero to day seven. The results indicate that the addition of PAS effectively maintains platelet quality, pH stability, and platelet function throughout the storage duration. This is attributed to the nutrient-rich composition of PAS, which contains glucose, sodium acetate, sodium chloride, phosphate, magnesium, and potassium, ensuring the preservation of platelet integrity. The findings highlight the potential benefits of PAS implementation in Indonesia to enhance platelet storage efficiency and availability, particularly during periods of high demand. Future research should explore the optimal PAS formulation for different storage conditions, cost-effectiveness, and clinical efficacy in transfusion settings to support broader adoption of this technology in blood banking.
Keywords: platelet additive solution, quality, concentrated platelets, buffy coat leukofiltered

Introduction
Blood transfusion is the process of transferring complete blood or blood components from a healthy person (donor) to a patient (resipien). As a country with a population of around 275 million people (BPS, 2022), Indonesia needs a large blood availability for blood transfusions. Based on World Health Organization (WHO) standards, the minimum amount of blood needed is 2% of the total population of Indonesia or around 5.4 million bags of blood in a year. To meet this need, the Blood Donor Unit (UDD) of the Indonesian Red Cross (PMI) plays a role in preparing the national availability of blood, including platelets. The availability of platelets in the PMI UDD every year is quite large, as seen from the national data in 2019 there were 767,680 bags collected, and in 2021 it increased to 957,397 bags. However, with the outbreak of dengue fever as the COVID-19 pandemic progresses, the need for concentrated platelets (TP) in hospitals has almost doubled from 2021.

The TP needs in the last three years are 9998 bags but 4073 bags expire first before use. (National Data of UDD PMI Center). Therefore, it is necessary to add a solution that can increase the shelf life of platelets. The addition of platelet additive solution (PAS), to extend the shelf life of platelets, is currently not used in Indonesia.

In some countries such as Malaysia and Taiwan, PAS has been used for thrombophoresis. Research in the country proves that the shelf life can last for seven days or increase by two days from the original shelf life. PAS is a crystalline nutrient medium used as a substitute for plasma of 60% - 70% in platelet components for the in vitro shelf life process. PAS contains sodium

chloride, sodium acetate, sodium citrate, dibasic sodium phosphate, monobasic sodium phosphate and water. Phosphate is useful for maintaining ATP levels so that the storage time of TP in vitro increases to seven days. In addition, TP plus PAS was shown to reduce the risk of allergic transfusion reactions, hemolysis and transfusion related acute lung injury (TRALI) reactions.[5] In addition, the cost of processing also becomes cheaper if using the pooling method of buffy coat leukofiltered, so that quality and safe TP is obtained, and can be used in areas with limited equipment.

Platelets are blood clotting plates that are smaller in size than other blood cells and amount to about 150,000−400,000/μL in adults. Platelets play an important role in the blood clotting mechanism by releasing A2 thromboxine at the site of injury or wound and together with other blood clotting factors will form a strong protein webbing (fibrin). The coagulation process will end with the formation of platelet plugs that can stop further bleeding. Platelets have a shorter lifespan than red blood cells and only survive between 8−10 days in vivo. The life span in the in vitro environment is even shorter, namely three days without agitation (shaking) and five days with agitation using platelet agitators. Agitation is needed to allow oxygen exchange through diffusion, thus preventing hypoxia, and metabolism turning into anaerobic glycolysis accompanied by increased lactic acid production. During storage, TP can undergo various changes, so storage during in vitro should be observed and efforts should be made to reduce possible changes. This is due to environmental differences between in vitro and in vivo conditions. The principle of in vitro storage is that TP must remain alive and functioning until the time it is transfused into the patient's body.

PAS has three generations, namely PAS I, first developed in the 1980s, then PAS II is the simplest additive, containing only sodium chloride, sodium citrate and sodium acetate. Then in PAS III there is an addition of phosphate, potassium and magnesium which are useful for increasing glucose metabolism. PAS III containing phosphate inhibits glycolysis rate and better pH retention. PAS contains ingredients to support platelet storage at room temperature; and acetate is one of the substances present in PAS solution that is negatively charged so that it is oxidized to neutral. Acetate obtains H+ ions from its environment, so it can increase pH levels when oxidized. In addition, platelet metabolism can alter the oxidation of glucose as a substrate (which produces lactic acid), and cause a decrease in pH during shelf life.

To find out whether the TP from the pooling method of buffy coat leukofiltered (four) stored with the addition of generation III PAS is still of good quality until the desired shelf life, in this study several tests were carried out on the platelets, starting from the quality test by looking at the amount of platelet yield, aggregation test and pH of TP products; shelf life of the first day to the shelf life of the seventh day.

Research Methods

This study uses an experimental analytical design. The research was carried out at the PMI Central Blood Transfusion Unit, the PMI Tangerang City Blood Transfusion Unit and the Pramita Laboratory. The research was conducted in April – May 2024. This research has received approval from the Ethics Commission of the Faculty of Medicine, University of Indonesia No. KET-215/UN2. F1/ETIK/PPM.00.02/2024. Platelet donors were given an explanation of the purpose and purpose of the research as well as the expected benefits of this research. After understanding clearly, the donor is asked to sign a letter of approval. If there is no objection, then the donor will be included as a research subject. The research sample was platelets from an

additional pouch (sample pouch) of platelets from conventional blood collection by pooling and then filtered leukocytes using triple top and bottom buffycoat leukofiltered bags from donors at the PMI Central Blood Transfusion Unit and the PMI Tangerang City Blood Transfusion Unit. The sample was divided into two groups, namely the group with PAS (group 1) and the group without PAS (group 2).

The data obtained was calculated using Microsoft Excel, then a statistical analysis test was carried out with Graphpad prism ver 10. Statistical analysis begins by conducting normality and homogeneity tests to find out whether the data meets the requirements of the parametric test. If the distribution of the data obtained is normal and homogeneous, a one-way ANOVA test is carried out to determine the average difference between the groups, as well as a post-hoc Least Significance Differences (LSD) test to determine the significance of the average difference between the groups. Correlation analysis on parametrically qualified data will be performed using the Pearson test. Meanwhile, if the distribution of data is abnormal and homogeneous so that it does not meet the requirements of the parametric test, statistical analysis will be carried out using non-parametric tests, namely Kruskal Wallis and Mann-Whitney as post-hoc tests, and Spearman tests for correlation analysis.

Results and Discussion

Sample Characteristics

In this study, 32 conventional platelet samples were obtained with 16 samples inserted into the TC Pool Buffycoat Leukofilter group without PAS with the same 4 blood types and 16 samples were put into the TC Pool Buffycoat Leukofilter group with PAS with the same 4 blood types. The samples came from platelet donors taken conventionally at the PMI Central Blood Transfusion Unit and at the PMI Tangerang City Blood Transfusion Unit.

Table 1. Characteristics of concentrated platelet groups Without PAS and with PAS

Parameters	TP without PAS	TP with PAS	P value
Number of platelets (cells/uL)	826750	664500	0,548
Ph	7,4	7,2	0,116
% Aggregation	7,9	19,5	0,226

In Table 4.1, it appears that after the processing of concentrated platelet products by the buffy coat pool leukofiltered method, it appears that the platelet count, pH, ADP level and lekocyte count at the addition of PAS and without the addition of PAS, do not differ significantly.

Concentrated platelet quality of the pool buffy coat leukofiltered method at storage

Platelet Count during Storage

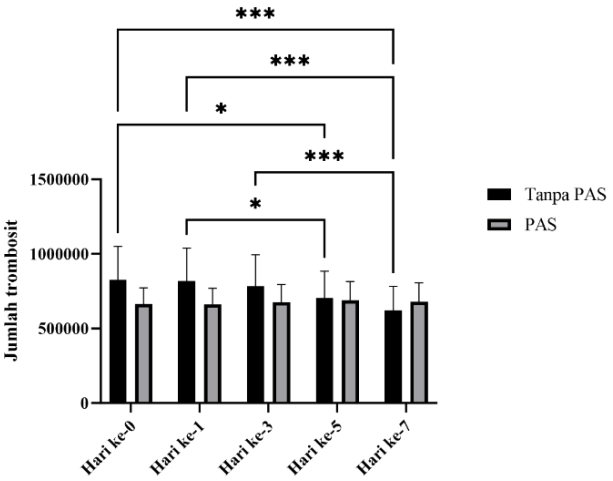

Figure 1. Changes in concentrated platelet count at 0, 1, 3, 5, and 7 day shelf life

In Figure 4.1, it appears that the platelet count in TP products with PAS is relatively constant and does not differ significantly during the shelf life of 0, 1, 3, 5 and 7 days. The number of platelets in TP products without PAS decreased significantly ($p<0.005$) between H1 and H7 and H3 with H7. In the group without PAS, there was a significant difference between H0 and H5 and H7. Also between H1 and H5 and H7, as well as between H3 and H7.

pH during Storage

In Figure 4.2, the pH of TP with PAS is relatively stable between 7.2 (H0); 7.25 (H1); 7,175 (H3); 7,175 (H5); and 7.1 (H7). Meanwhile, the pH of TP without PAS shows a pH higher than 7.4 (H0); 7.5 (H1); 7,675 (H3); 7,625 (H5) and remained at 7,625 (H7).

There was no significant difference in pH ranging from H0 to H7, both in the PAS and non-PAS groups.

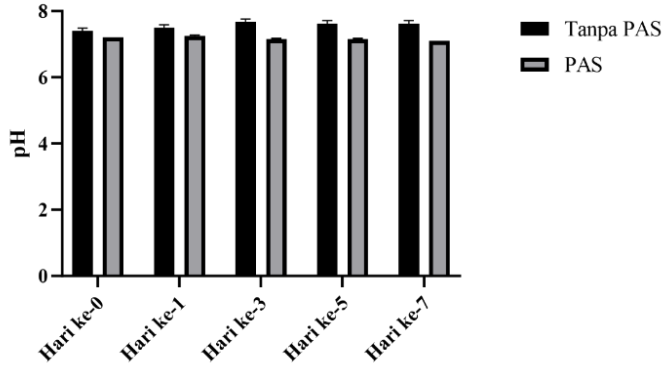

Figure 2. Changes in Ph of concentrated platelets at 0,1,3,5 and 7 days shelf life

Aggregation Percentage during Storage

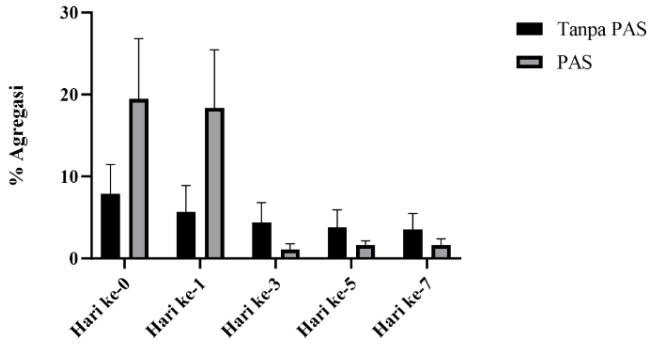

Figure 3. Percentage of Concentrated Platelet Aggregation at 0,1,3,5 and 7 Day Storage Period

Figure 4.3 shows that the % of aggregation on TP with PAS at H0 is 19.5 %; H1 was 18%, which then decreased to 0.7% in H3. Then it increased to 0.9% on H5, and increased to 1.1% on H7.

In TP without PAS in H0 it was 7.9 %; H1 was 5.3%, then decreased in H3 at 4.4%, then decreased to 3.6% on H5, and decreased again to 3.1% on H7.

Discussion

Sample Characteristics

In this study, 32 conventional platelet samples were obtained with 16 samples inserted into the TC Pool Buffycoat group without PAS with the same 4 blood types and 16 samples were put into the TC Pool Buffycoat group with PAS with the same 4 blood types. The samples came from platelet donors taken conventionally at the PMI Central Blood Transfusion Unit and at the PMI Tangerang City Blood Transfusion Unit. The characteristics of the sample of the No PAS and PAS groups are seen in Table 4.1. There was no significant difference in platelet count and pH, % Aggregation between the two groups of TP. The results are in accordance with the purpose of the study, namely there is no significant difference at the beginning of the observation.

TP Quality in Storage

Platelet count at 0, 1, 3, 5, and 7 days of storage

During storage starting from H1 to H7, the number of platelets remained unchanged, meaning the number of platelets in the TP group with PAS. This shows that there is no platelet fragmentation which causes the number of platelets to increase during the storage period. This means that the addition of PAS to TP can maintain the quality of platelets. This is possible because PAS contains sodium acetate, sodium chloride, phosphate, magnesium and potassium, among others, so that platelets remain alive during storage. In TP without PAS, it was seen that the number of platelets increased at the end of the shelf life observation. This increase in platelet count may be due to platelets enlarged (swollen) and further fragmentation forming platelet derived procoagulant microparticles (PDMPs) in the process of apoptosis but Hematology Analyzer Still count them as platelets so that the number of platelets increases. According to the literature, during the storage period, platelets can undergo membrane fragmentation, cytoskeleton damage, micropartkel/microvesicle formation, and exposure of PS phospholipids in the membrane.

pH Changes during Storage

The pH of TP in PAS addition was relatively stable during storage and was within the physiological pH range in cells (7.1-7.2). This is possible due to the phosphate buffer that can

resist pH changes during storage. Meanwhile, TP without PAS is higher from H1 to H7.

PAS administration affects pH stability at the time of TP storage. The presence of acetate can provide an energy source for oxidative phosphorylation in platelets and prevent anaerobic glycolysis which can lead to a decrease in pH.

Platelet Function During Storage

Aggregation Percentage during Storage

In TP with the addition of PAS there was no significant difference between Ho and H1, then there was a decrease during the storage period from H3 to H7. This means that PAS administration does not improve the function of platelet aggregation after H1. This can be due to hypoaggregation even though the examination uses ADP concentration of 10 μM. Bock also found that there was a decrease in platelet aggregation during the storage period. Sinzinger also obtains a decreasing platelet aggregation function either by using ADP agonists. This decreased aggregation function may be due to the pH in the platelet preparation <7.7.In TP without PAS, there was a decrease in aggregation percentage. This can be caused by platelet function decreasing during shelf life.

Conclusion

The administration of Platelet Additive Solution (PAS) has been shown to effectively maintain platelet count and pH stability during storage when using the leukofiltered buffy coat pooling method. This preservation of platelet integrity is crucial for ensuring transfusion efficacy, particularly in high-demand scenarios such as dengue fever outbreaks and the COVID-19 pandemic. However, while PAS helps sustain platelet quality, platelet function, as measured by aggregation percentage tests, showed a decline between day-3 and day-7 of storage. This suggests that while PAS extends shelf life, it may not fully prevent functional degradation over time.

Future research should explore strategies to optimize platelet function preservation, including adjustments to PAS composition, alternative storage temperatures, or supplementary preservation agents. Additionally, comparative studies assessing PAS efficacy across different platelet processing methods and clinical transfusion outcomes would provide valuable insights. Investigating cost-effectiveness and scalability of PAS implementation in Indonesia's blood banking system would also support its broader adoption, ultimately improving platelet availability and transfusion success rates nationwide.

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