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Effects of Storage Temperature and Duration on the Hematological Characteristics of Allogeneic Leukocyte-Poor Platelet-Rich Plasma

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Abstract

Allogeneic PRP (Al-PRP) was platelet concentrate from voluntary donors as therapy. The composition within a PRP preparation other than platelets (PLT) may also include white blood cell (WBC) and red blood cells. The biochemical parameters as inflammatory markers such as platetet to white blood cell ratio (PWR) indices in a variety of medical conditions. Aims to investigate the effects of different storage temperatures and storage durations on the hematological characteristics of Al-LP-PRP. Methods: This pilot study used 3 different donors as source of allogenic PRP (a, b and c) with informed consent. The samples were three aliquots and stored at 22°C (room temperature), 4°C (refrigerator), and -20°C (deep freezer). The samples were observation by time analysis (1 day, 3 days, and 7 days). After storage, the parameters were assessed using Hematology Analyzer XP-100. PWR was defined as PLT divided by WBC. Result: The age of male donors were 34.67 ± 2.1 (mean ± SD). Platelet and WBC level were significant difference at storage conditions, whereas red blood cell levels did not differ significantly. WBC level had negative correlation to platelet to white blood cell ratio. Conclusion: Room-temperature storage was the most effective condition for preserving the hematological characteristics of allogeneic leukocyte-poor platelet-rich plasma (Al-LP-PRP). The findings of this pilot study provide preliminary evidence supporting the development of standardized storage protocols for future regenerative medicine applications, however, further validation in studies involving a larger sample size is needed.

Keywords: *Allogeneic, Biochemistry, Donors, PRP*

1. INTRODUCTION

Platelet-rich plasma (PRP) is an autologous or allogeneic blood-derived biological preparation that is characterized by a platelet concentration higher than that found in whole blood. Platelets contained within PRP possess several types of intracellular granules, including alpha, dense, and lysosomal granules, each of which stores distinct bioactive molecules involved in various physiological processes (Lana et al., 2019). Among these granule types, alpha granules are considered the most biologically significant because they contain a wide range of growth factors, cytokines, adhesive proteins, and other regulatory molecules that are released upon platelet activation.

The principal bioactive components stored in alpha granules include platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), and vascular endothelial growth factor (VEGF), all of which play fundamental roles in tissue repair and regenerative processes. PDGF is involved in stimulating cell migration, proliferation, and extracellular matrix production, whereas TGF-β regulates cellular differentiation and matrix remodeling. VEGF primarily promotes angiogenesis by stimulating the formation of new blood vessels, thereby improving oxygen and nutrient delivery to damaged tissues. Through the coordinated actions of these growth factors and other bioactive proteins, PRP facilitates cell proliferation, enhances extracellular matrix synthesis, supports neovascularization, and accelerates the overall process of tissue regeneration and wound healing (S. Gupta et al., 2021; Mifune et al., 2013; Patel et al., 2023).

Allogeneic platelet-rich plasma (Al-PRP), which is prepared from platelet concentrates obtained from healthy voluntary blood donors, has emerged as a promising biological therapeutic option for regenerative medicine. Compared with autologous PRP, Al-PRP offers several practical advantages, particularly for elderly individuals and patients with age-related disorders who may have impaired platelet function, reduced platelet counts, or underlying comorbidities that limit the quality of autologous

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PRP. The use of donor-derived platelet concentrates also enables the production of standardized PRP preparations with more consistent biological characteristics and immediate availability for clinical application.

The safety of Al-PRP is an important consideration for its clinical use. Blood donors are rigorously screened according to established blood donation regulations to minimize the risk of transfusion-transmitted infections. Consequently, Al-PRP products are prepared only from donations that have tested negative for mandatory serological and infectious disease screening markers, including Human Immunodeficiency Virus (HIV), hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV), and syphilis, thereby ensuring a favorable safety profile for therapeutic applications (Akbarzadeh et al., 2021).

Although the clinical application of biological therapies, particularly platelet-rich plasma, has increased substantially over the past decade, evidence regarding the use of Al-PRP for age-related diseases remains relatively limited. Most published studies have focused on autologous PRP, whereas investigations evaluating the biological properties, therapeutic efficacy, and clinical outcomes of Al-PRP are still scarce. This knowledge gap highlights the need for further research to establish standardized protocols and determine the optimal clinical indications for donor-derived PRP. Another important challenge in PRP therapy is the considerable variability in preparation techniques, which can result in substantial differences in platelet concentration, leukocyte content, growth factor release, and overall biological activity. Such heterogeneity has complicated the interpretation and comparison of clinical outcomes across studies. Furthermore, there is currently no consensus regarding the most appropriate PRP formulation for different clinical conditions. Increasing evidence suggests that leukocyte-rich PRP may induce excessive inflammatory responses through the release of pro-inflammatory cytokines and proteolytic enzymes. Therefore, several studies have recommended the use of leukocyte-poor PRP (LP-PRP), particularly for age-related musculoskeletal disorders such as knee osteoarthritis (OA), where minimizing inflammation while preserving the regenerative potential of platelet-derived growth factors may improve therapeutic outcomes (A. Gupta et al., 2023; Mifune et al., 2013)

In the present study, we conducted a series of experiments using allogeneic leukocyte-poor platelet-rich plasma (Al-LP-PRP) prepared from platelet concentrates obtained from healthy voluntary blood donors. This pilot study aimed to investigate the effects of different storage temperatures and storage durations on the hematological characteristics of Al-LP-PRP. By evaluating changes in platelet, leukocyte, and erythrocyte profiles during storage, we sought to identify the storage condition that best preserves the hematological characteristics associated with the biological quality of Al-LP-PRP. The findings of this study are expected to provide baseline evidence for the standardization of Al-LP-PRP preparation and storage protocols and to support future investigations on its application in regenerative medicine, particularly for age-related disorders.

2. METHOD

Subject

Three units of whole blood collected from healthy voluntary donors were used as the source of allogeneic platelet-rich plasma (Al-PRP) in this study. The donor blood units were designated as samples A, B, and C. Written informed consent was obtained from all donors by the Indonesian Red Cross Semarang before blood collection. Eligible donors were healthy individuals between 17 and 40 years of age who fulfilled the standard blood donation criteria. Blood donation and sample collection were performed on 28 June 2024.

Preparation of Allogeneic

A total of 350 mL of peripheral venous whole blood was collected from each donor into sterile JMS blood bags containing Citrate Phosphate Dextrose Adenine (CPDA-1) as the anticoagulant. The collected whole blood was subsequently centrifuged at 2,000 × g for 4 min at 22 °C to obtain platelet-rich plasma (PRP). Prior to inclusion in the study, all donated blood units underwent routine serological screening in accordance with standard blood donation regulations. Only blood samples that tested negative for transfusion-transmissible infectious markers, including Human Immunodeficiency Virus (HIV),

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hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV), and syphilis, were included for Al-PRP preparation (He et al., 2020; Kim et al., 2020).

Platelet and Hematological Parameters Count Assay

The storage protocol was adapted from the method described by (Kim et al., 2020) with appropriate modifications according to the experimental setting and facilities available in our laboratory. Previous studies have demonstrated that PRP can be stored under a range of temperature conditions, including room temperature, refrigeration at 4°C, and sub-zero temperatures, depending on the intended duration of storage and subsequent application. Based on these previous findings, three storage conditions were selected in the present study: 22°C (room temperature), 4°C (refrigerated condition), and −20°C (deep-freezer condition), representing commonly applicable storage environments with different temperature ranges. The room temperature condition was set at 22°C to represent the ambient temperature routinely maintained in the laboratory, thereby reflecting a practically relevant condition for PRP handling and short-term storage without active temperature control. The 4°C condition was selected to represent refrigerated storage, which is commonly used for the short-term preservation of biological samples and has been investigated in previous studies of PRP storage. Meanwhile, −20°C was selected as the sub-zero storage condition based on the temperature capacity of the deep freezer routinely available in our laboratory. This condition was intended to represent a practical frozen-storage condition that could be implemented using the existing laboratory infrastructure. Each Al-PRP sample was divided into three aliquots for storage, and evaluations were performed after 1, 3, and 7 days of storage.

Following each storage period, hematological parameters were determined to evaluate the effects of storage temperature on Al-PRP quality. Platelet count, white blood cell (WBC) count, and red blood cell (RBC) count were measured using a Sysmex XP-100 Automated Hematology Analyzer (Sysmex, Indonesia).

Statistical Analysis

Data are presented as mean ± SD (standar deviation) for the 3 groups temperature were collected. The normality test was used to determine parametric or nonparametric ($p > 0.05$). The Statistical significance was indicated at $P < 0.05$. Kruskal-Wallis test was used for platelet and RBC count with Mean Rank shows the average rank of each treatment. Two Way Anova test was used for WBC count with post-hoc Bonferroni. The correlation of WBC with PWR in PRP was analyzed using Pearson correlation. All statistical analyses were performed using SPSS 21.0 for Windows (IBM Corp) and Graph pad Prism Software (version 8).

3. RESULT AND DISCUSSION

The characteristics of the healthy voluntary blood donors included in this study are summarized in Table 1. A total of three male donors participated in the study, with a mean age of 34.67 years (range: 30–40 years), a mean body weight of 69.67 kg, and a mean hemoglobin concentration of 14.53 g/dL. Following blood processing, plasma units obtained from each donor were used for the preparation of allogeneic platelet-rich plasma (Al-PRP), as illustrated in Figure 1. The donor plasma units were identified as samples A, B, and C throughout the study.

Table 1. Donors Characteristics

	Value
Sex (male)	3
Age, mean (range)	34,67 (30-40)
weight (mean)	69,67 kg
Hb (mean)	14.53

Hb, hemoglobin (g/dL);

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(a) (b) (c)

Figure 1. Plasma units from three healthy voluntary blood donors used for allogeneic platelet-rich plasma (Al-PRP) preparation.

The baseline hematological characteristics of freshly prepared Al-PRP (Day 0) are presented in Table 2. Platelet counts ranged from 266.67 × 10^3/µL to 317 × 10^3/µL among the three donor samples, while white blood cell (WBC) and red blood cell (RBC) counts remained low. Hemoglobin and hematocrit values were undetectable in all Al-PRP samples, whereas the platelet-to-white blood cell ratio (PWR) varied among donors.

Table 2. Baseline Hematological Profile

	Day 0		
	A	b	C
PLT	306±0.81	317±0.81	266,67±1.24
WBC	0.3±0.08	0.3±0.08	0.13±0.04
RBC	0.01±0.004	0.01±0.004	0.02±0.008
HGB	0.0	0.0	0.0
HCT	0.0	0.0	0.0
PWR	1104.167±314	1145.56±332	2225±636

PLT, Platelet (10^3/µL); WBC, white blood cell (10^3/µL); RBC, red blood cell (10^6/µL); HGB, hemoglobin (g/dL); HCT, hematocrit (%); PWR, platetet to white blood cell ratio; a: donor 1; b: donor 2; c: donor 3.Day 0 mean immediately measurement after production.The data shown as mean±SD.

Compared with the baseline hematological profile obtained immediately after Al-PRP preparation, platelet counts were generally maintained during the 7-day storage period, although their stability varied according to storage temperature. Room temperature storage preserved platelet levels closest to baseline, whereas refrigerated storage resulted in the greatest reduction in platelet counts over time. Deep-freezing better maintained platelet levels than refrigeration, despite minor fluctuations during storage. Overall, these findings indicate that storage temperature significantly influences the preservation of platelet stability during 7 days of storage ($p < 0.05$).

The effects of storage conditions on WBC counts are shown in Figure 2. Significant differences were observed according to both storage duration and storage temperature ($p < 0.05$). Changes in RBC counts during storage are presented in Figure 3. No statistically significant differences in RBC counts were detected among the different storage temperatures or storage durations ($p > 0.05$).

The composition and biological quality of platelet-rich plasma are influenced by several donor-related factors, including age and sex. Previous studies have demonstrated that these biological characteristics affect platelet physiology, growth factor content, and inflammatory mediator profiles, thereby contributing to variability in PRP composition (Taniguchi et al., 2019). To minimize donor-related variability, only healthy male voluntary blood donors who fulfilled the blood donation criteria were included in the present study. Characterization of freshly prepared Al-PRP is an essential step to ensure product quality prior to storage and potential therapeutic application. The hematological profile obtained in this study demonstrated an adequate platelet concentration accompanied by minimal contamination with leukocytes and erythrocytes, indicating that the prepared product could be classified as leukocyte-poor PRP (LP-PRP) (Buford & Sherman, 2024).

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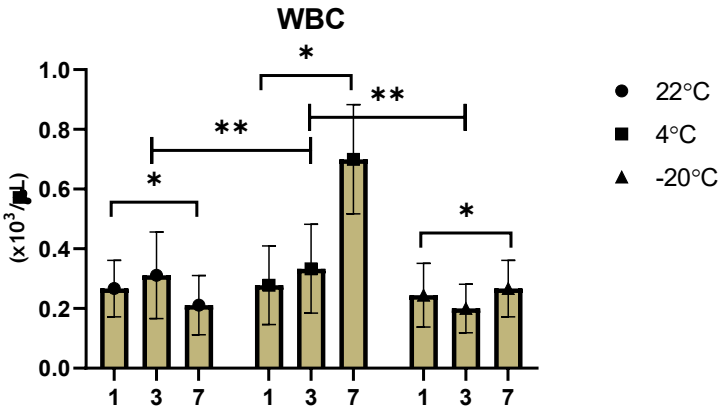

Figure 2. WBC Count on Al-PRP by Storage Conditions
*Significantly difference by day of storage;**Significantly difference by temperature of storage;
Statistical significance level of p<0.05 (p=0.00)

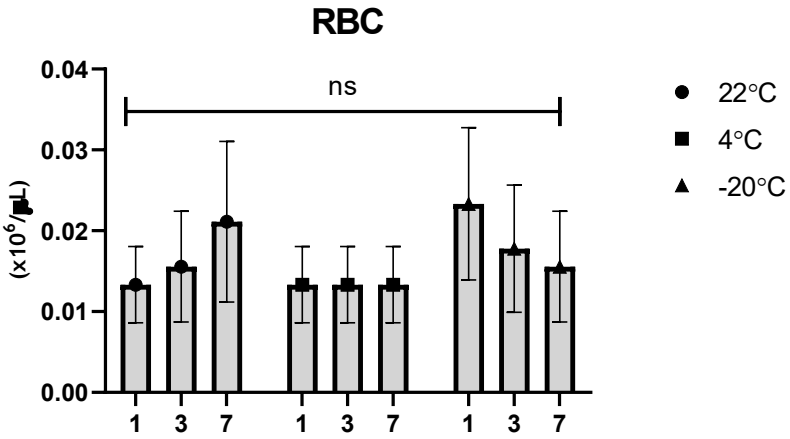

Figure 3. RBC count on Al-PRP by Storage Conditions;
ns: no significance (p>0.05)

This type of PRP has been widely recommended for regenerative therapies, particularly for age-related musculoskeletal disorders such as osteoarthritis, because reduced leukocyte content may decrease the release of pro-inflammatory cytokines and proteolytic enzymes while preserving the regenerative activity of platelet-derived growth factors (Lin et al., 2022).

Although the present study evaluated only hematological parameters, these measurements are closely associated with the biological quality of platelet-rich plasma. Platelets represent the primary reservoir of growth factors and bioactive proteins, including platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), and vascular endothelial growth factor (VEGF), which are released upon platelet activation and play essential roles in tissue regeneration (Hariprasad et al., 2021). In addition, residual leukocyte and erythrocyte contents may influence the inflammatory microenvironment and consequently affect the biological properties of PRP. Therefore, maintaining an appropriate cellular composition during storage is considered an important prerequisite for preserving the regenerative potential of allogeneic leukocyte-poor platelet-rich plasma (Al-LP-PRP). However, because growth factor concentrations and platelet activation status were not directly evaluated in the present study, the biological quality of Al-PRP should be interpreted based on its hematological characteristics rather than direct biochemical measurements.

The platelet-to-white blood cell ratio (PWR) has recently been proposed as a novel hematological indicator associated with inflammatory status in several pathological conditions, including hepatitis B

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virus infection (Zhang et al., 2020). Because WBC count directly influences the PWR value, the low leukocyte content observed in the present study contributed to relatively high PWR values, further supporting the successful preparation of a leukocyte-poor platelet concentrate. Although PWR was not evaluated as a clinical biomarker in this study, it may serve as an additional parameter for assessing the hematological quality of Al-PRP preparations.

Storage conditions are recognized as important factors influencing platelet preservation and biological activity. In the present study, platelet counts were significantly affected by storage temperature, with room-temperature storage maintaining higher platelet counts than refrigerated and frozen storage during the observation period. This finding may be related to the physiological stability of platelets under ambient conditions. Platelets are metabolically active cells that remain sensitive to changes in their storage environment, and exposure to low temperatures may induce platelet activation, cytoskeletal rearrangement, membrane alterations, aggregation, and other storage-related lesions. These changes may compromise platelet integrity and recovery and consequently reduce the number of freely measurable platelets. Previous studies have similarly demonstrated that storage temperature can influence platelet morphology and platelet preservation. Kim et al (2020) reported that platelet counts remained relatively stable in PRP stored at room temperature and under refrigerated conditions, whereas a significant reduction was observed in the deep-freezer group on the first day, which was suggested to be associated with cell lysis during thawing. In addition, Jennes et al (2023) reported that PRP stored at 4°C exhibited platelet clumping, morphological alterations, and an apparent reduction in platelet numbers, whereas these changes were less pronounced in samples stored at −20°C. Low-temperature exposure has also been associated with increased platelet aggregation, which may contribute to alterations in platelet morphology and recovery.

These previous findings provide a biological basis for the relatively better preservation of platelet counts observed at room temperature in the present study. Maintaining PRP at 22°C may minimize cold-induced platelet activation and aggregation during the storage period, thereby allowing a greater proportion of platelets to remain morphologically intact and detectable during hematological analysis. Moreover, previous studies have shown that storage temperature and duration can influence the release and preservation of growth factors in PRP, suggesting that temperature-dependent changes may extend beyond platelet count and potentially affect the biological characteristics of the preparation. However, the effects of storage temperature are not necessarily uniform across all PRP preparations and may depend on the storage duration, preparation protocol, platelet concentration, activation status, and specific biological parameters evaluated. Therefore, the finding that room temperature provided the most favorable preservation of platelet counts in the present study should be interpreted within the experimental conditions evaluated and should not be considered evidence that room-temperature storage is universally optimal for all PRP applications.

White blood cell counts were also significantly affected by storage temperature and duration, indicating that residual leukocytes in Al-LP-PRP are likewise susceptible to storage-related changes. In contrast, RBC counts remained relatively unchanged throughout the storage period. Given that erythrocyte contamination in the prepared Al-LP-PRP was already minimal, the storage conditions may have had a limited measurable effect on RBC levels. Taken together, these findings suggest that storage temperature and duration primarily influenced platelet preservation and leukocyte characteristics, whereas erythrocyte contamination remained relatively stable under the conditions evaluated. This observation further supports the importance of considering multiple hematological parameters when assessing the quality and stability of Al-LP-PRP during storage.

Although PRP is generally expected to contain platelet concentrations approximately three to five-fold higher than those of peripheral whole blood while minimizing leukocyte and erythrocyte contamination (Cole et al., 2010; KARADAĞ SARI, 2022). The platelet concentrations obtained in the present study remained within or close to the physiological range of peripheral blood. This finding may have implications for the biological potency of the resulting Al-LP-PRP, as platelet enrichment is an important determinant of the amount of platelet-derived mediators and growth factors available for subsequent biological activity. Nevertheless, platelet concentration alone should not be considered the

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sole determinant of PRP quality, particularly for leukocyte-poor preparations, in which the cellular composition and preservation of platelet functionality are also important considerations.

The relatively low platelet enrichment observed in the present study may be related to the centrifugation protocol used for Al-LP-PRP preparation. Platelet recovery and enrichment are influenced by several centrifugation-related parameters, including relative centrifugal force, centrifugation duration, number of centrifugation steps, acceleration and braking conditions, as well as the volume and fraction of plasma collected after centrifugation. Previous studies have demonstrated that double-centrifugation protocols can effectively increase platelet concentration; however, excessive centrifugal force may adversely affect platelet morphology and induce platelet activation or spontaneous aggregation. Therefore, optimization of the centrifugation protocol, rather than simply increasing the centrifugal force, may provide an opportunity to improve platelet recovery while maintaining platelet integrity and minimizing leukocyte and erythrocyte contamination. Future studies should systematically evaluate different combinations of centrifugation force and duration to determine an optimal protocol for producing Al-LP-PRP with greater platelet enrichment and consistent cellular characteristics.

Standardization of Al-PRP remains challenging because both donor characteristics and processing methods contribute to considerable variability in product composition. Rossi et al. reported that donor age significantly influences platelet yield in PRP, whereas Xiong et al. demonstrated that sex affects the concentrations of inflammatory cytokines and growth factors, with male donors exhibiting higher levels than female donors (Bausset et al., 2012; Düregger et al., 2016a, 2016b; Jennes et al., 2023; Kim et al., 2020; Raveendran et al., 2019). In addition to donor-related variability, storage conditions should also be carefully optimized to preserve platelet viability and maintain the biological properties of Al-PRP before clinical application.

Safety is another critical consideration for the clinical use of donor-derived PRP. Since Al-PRP originates from donated blood, rigorous screening for transfusion-transmissible infections is mandatory. In the present study, all donor blood units tested negative for Human Immunodeficiency Virus (HIV), hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV), and syphilis in accordance with blood donation regulations. Together with the low leukocyte and erythrocyte content of the final preparation, these findings indicate that the produced Al-PRP met essential safety and quality requirements for potential regenerative applications (Lin et al., 2022; Sharma & Marwaha, 2010).

Study Limitations

Despite these promising findings, this study should be interpreted with caution because of the limited number of donor samples included. The relatively small sample size may not fully represent the biological variability among blood donors and therefore limits the generalizability of the findings. In addition, this study primarily evaluated hematological parameters and did not assess platelet activation status, growth factor release, or functional biological activity following storage, which are important determinants of the therapeutic efficacy of allogeneic platelet-rich plasma (Al-PRP). Nevertheless, the present findings provide initial evidence regarding the influence of different storage conditions on the quality of Al-PRP and may serve as a basis for the development of standardized preparation and storage protocols. Future studies involving larger donor populations, comprehensive biochemical characterization, and functional analyses are warranted to validate these findings and further optimize the clinical application of Al-PRP.

4. CONCLUSION

This pilot study demonstrated that the prepared allogeneic platelet-rich plasma (Al-PRP) exhibited a leukocyte-poor profile with low white blood cell contamination. Among the evaluated storage conditions, room-temperature storage was the most effective condition for preserving platelet counts, whereas storage temperature significantly affected both platelet and white blood cell levels. In contrast, red blood cell counts remained relatively stable across the different storage conditions. In addition, white blood cell counts showed a negative correlation with the platelet-to-white blood cell ratio (PWR), indicating that changes in leukocyte levels were associated with variations in the PWR during storage. Overall, these findings provide preliminary evidence supporting room-temperature storage as a favorable

condition for maintaining the hematological characteristics of Al-PRP and may contribute to the development of standardized storage protocols. However, the findings should be interpreted as preliminary due to the limited number of donors included in this pilot study. Further studies involving a larger number of donors and evaluating additional biological parameters, including growth factor concentrations and platelet activation, are needed to provide a more comprehensive assessment of Al-PRP quality and storage stability.

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