

## Pre-Storage Leukoreduced Filtration (PLF) Decrease Soluble Human Leukocyte Antigen-I (sHLA-I) Level in Thrombocyte Concentrate Stored for Five Days

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**Background:** Thrombocyte concentrate is one of the important blood component to improve patient's clinical condition. In order to provide thrombocyte, concentrate with good therapeutic effect, the preparation process and storage condition should be maintained properly. One attempt to maintain good quality of thrombocyte concentrates is by doing Pre-storage Leukoreduced Filtration (PLF) method during preparation of thrombocyte concentrates. The purpose of this study was to determine the effect of PLF on sHLA-I level in thrombocyte concentrates stored for 1, 3 and 5 days. **Methods:** This is an experimental study with posttest only Control Group design, enrolling 34 thrombocyte concentrates and randomly assigned into PLF Group and Control Group. **Results:** It was obtained that PLF Group has lower mean sHLA-I level than Control Group stored for 1, 3 and 5 days and statistically significant. Mean sHLA-I level in PLF Group at day 1 is  $2,75 \pm 0,82$   $\mu\text{g/ml}$  and Control Group is  $3,66 \pm 0,87$   $\mu\text{g/ml}$ , which is statistically significant ( $p < 0,05$ ). Mean sHLA-I level in PLF Group at day 3 is  $3,07 \pm 0,75$   $\mu\text{g/ml}$  and Control Group is  $4,05 \pm 1,29$   $\mu\text{g/ml}$ , which is statistically significant ( $p < 0,05$ ). Mean sHLA-I level in PLF Group at day 5 is  $3,81 \pm 0,97$   $\mu\text{g/ml}$  and Control Group is  $5,61 \pm 3,26$   $\mu\text{g/ml}$ , which is statistically significant ( $p < 0,05$ ). **Conclusions:** It can be concluded that PLF decrease sHLA-I level in thrombocyte concentrates stored for five days compared with Control Groups.

**Keywords:** PLF, sHLA-I, thrombocyte concentrate

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### INTRODUCTION

The use of blood components has been increased in the last decade because of the progress in the field of bone marrow transplantation and the increased use of chemotherapy in hematologic malignancy. Thrombocyte concentrate is one of the important blood component to improve patient's clinical condition. Thrombocyte concentrate can be obtained from the pooled random donor platelet concentrate and from single donor platelet apheresis. Thrombocyte concentrate from pooled random donor platelet concentrate can be prepared by Platelet Rich Plasma (PRP) method which is well known in United States and Indonesia and Buffy Coat (BC) method which is mostly used in Europe<sup>1,2,3</sup>.

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In order to provide thrombocyte, concentrate with good therapeutic effect, the preparation process and storage condition should be maintained properly for good viability of thrombocyte concentrates. Viability of thrombocyte concentrate is the ability of thrombocyte concentrate which is transfused to the recipients to circulate without experiencing early destruction in the recipient's body<sup>2,4</sup>.

Thrombocytes contained in thrombocyte concentrates undergo various changes during the collection, processing and storage which cause changes in the structure and function of thrombocyte and adversely may decrease thrombocyte transfusion effectiveness. These changes are known as platelet storage lesion. Platelet storage lesion causing loss of integrity of platelet function, changes in the process of aggregation and granule release of platelets, changes in the cytoskeleton of platelet, phosphatidylserine exposure on the outer surface membrane of platelets and microvesiculation<sup>5,6</sup>.

Many research related to platelet storage lesion have been carried out, aiming to maintain the viability of platelets in vitro and in vivo. One attempt to maintain thrombocyte concentrates quality is by doing preparation of thrombocyte concentrates with pre-storage Leukoreduced Filtration (PLF). PLF aims to reduce residual leukocytes contained in thrombocyte concentrates. Several studies have shown that residual leukocytes, especially monocytes are a major source of pro-inflammatory cytokines such as interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) that play a role in transfusion reactions<sup>4,5,7,8,9</sup>. Residual leukocytes in thrombocyte concentrates associated with Human Leukocyte Antigen (HLA). HLA is a heterodimer and highly polymorphic glycoprotein. HLA class I antigens expressed on all nucleated cells, while expression of HLA class II antigen is limited to Antigen Presenting Cells (APC) and activated T lymphocytes. The presence of HLA-I antigen in thrombocyte concentrates is considered as a marker of immunological reactivity and cellular fragmentation therefore it can be used as an indicator of the performance of PLF in thrombocyte concentrates. The purpose of this study was to determine the effect of PLF on sHLA-I level in thrombocyte concentrates stored for 1, 3 and 5 days<sup>5,10</sup>.

## MATERIAL AND METHODS

### Data and specimen collection

This study was an experimental study with posttest only Control Group design that was conducted during the years 2013 – 2014. A total of 34 thrombocyte concentrates were used for this study. They were randomly assigned into PLF Group and Control Group. PLF Group was thrombocyte concentrate with PLF treatment and Control Group without PLF treatment.

### Preparation of thrombocyte concentrates

Whole blood (350 ml) was collected into triple blood bags containing CPDA-1 anticoagulant. Criteria for inclusion include preparation of thrombocyte concentrate by PRP method with screening test result of Hepatitis B, Hepatitis C, HIV and VDRL were negative. Criteria for exclusion include hemolysis in thrombocyte concentrate, low platelet count (< 150 x 10<sup>3</sup>/ $\mu$ l) and use of any drug known to affect platelet function in the 72 hours prior to blood donation. After collection of whole blood, within 4 hours was done centrifugation 375 x g for 15 minutes at 22°C to form platelet rich plasma (PRP). The PRP was expressed into second satellite bag, then centrifuged again at 1.500 x g for 15 minutes at 22°C to obtain platelet poor plasma (PPP). Plasma was expressed into third satellite bag and

leaving 40 – 60 ml in the prepared thrombocyte concentrates unit which was left undisturbed for 1 – 2 hours<sup>2,3</sup>. PLF Group were 17 units of thrombocyte concentrates undergo PLF with Acrodose<sup>TM</sup> Plus System (Haemonetics), and Control Group were 17 units of thrombocyte concentrates that did not undergo PLF. All the thrombocyte concentrates were then placed on a horizontal shaker at 22°C for 5 days.

Samples from all thrombocyte concentrates were taken on day 1, day 3 and day 5 to determine the concentration of sHLA-I using an Enzyme Linked Immunosorbent Assay (ELISA) (Bioassay Technology Laboratory).

### Statistical analysis

Data was expressed as mean  $\pm$  SD. Independent Samples T-test procedure was used to compare means of PLF Group and Control Group. Repeated measure one way anova was used to compare the difference of sHLA-I level in serial measure in both Groups. Statistical significance for these tests using a value of 95% (CI = 95 %) with a p-value less than or equal to 0.05 as the limit of significance.

### Ethical approval

The study protocol was approved by Institutional Ethics Committee of Sanglah Hospital / Faculty of Medicine Udayana University.

## RESULTS

Laboratory and basic characteristic of thrombocyte concentrate were listed in table 1.

Table 1  
Laboratory and basic characteristic of thrombocyte concentrate

Parameter	PLF Group	Control Group
Mean thrombocyte count/unit	2,58 x 10 <sup>10</sup>	2,47 x 10 <sup>10</sup>
Mean leucocyte count/unit	0,66 x 10 <sup>6</sup>	1,99 x 10 <sup>6</sup>
Mean volume/unit	44,06 ml	42,29 ml
Swirling phenomena	+2	+2
Mean pH	7,38	7,39

Data of mean sHLA-I level in Control Group and PLF Group were listed in table 2.

Table 2  
The difference of mean sHLA-I level in platelet concentrate stored on Day 1, 3, 5 for Control Group (without PLF) and treatment Group (with PLF)

Storage	sHLA-I level ( $\mu$ g/ml)		Mean difference of sHLA-I	p value
	Control (without PLF)	Treatment (with PLF)		
Day 1	3,66 $\pm$ 0,87	2,75 $\pm$ 0,82	0,91	0,004*
Day 3	4,05 $\pm$ 1,29	3,07 $\pm$ 0,75	0,98	0,011*
Day 5	5,61 $\pm$ 3,26	3,81 $\pm$ 0,97	1,80	0,036*

\* significant p<0,05

Mean sHLA-I level in PLF Group at day 1 is  $2,75 \pm 0,82$   $\mu\text{g/ml}$  and Control Group is  $3,66 \pm 0,87$   $\mu\text{g/ml}$ , which is statistically significant ( $p < 0,05$ ).

Mean sHLA-I level in PLF Group at day 3 is  $3,07 \pm 0,75$   $\mu\text{g/ml}$  and Control Group is  $4,05 \pm 1,29$   $\mu\text{g/ml}$ , which is statistically significant ( $p < 0,05$ ). Mean sHLA-I level in PLF Group at day 5 is  $3,81 \pm 0,97$   $\mu\text{g/ml}$  and Control Group is  $5,61 \pm 3,26$   $\mu\text{g/ml}$ , which is statistically significant ( $p < 0,05$ ). It indicates that PLF Group has lower mean sHLA-I level than Control Group and statistically significant.

To determine differences of sHLA-I level due to the length of storage time, then performed repeated measure one way anova, listed in table 3.

Table 3  
The difference of mean sHLA-I level between storage time in Control Group (without PLF) and treatment Group (with PLF)

Group	Mean difference of sHLA-I level	p value
<b>Treatment (with PLF)</b>		
Storage at first day vs third day	0,32	0,028*
Storage at third day vs fifth day	0,74	0,001*
Storage at first day vs fifth day	1,06	0,000*
<b>Control (without PLF)</b>		
Storage at first day vs third day	0,39	0,046*
Storage at third day vs fifth day	1,56	0,038*
Storage at first day vs fifth day	1,95	0,011*

\* significant  $p < 0,05$

During storage, both treatment Group (with PLF) and the Control Group (without PLF) seemed there were increment of sHLA-I level, but increment of sHLA-I in treatment Group (with PLF) is lower than the Control Group (without PLF).

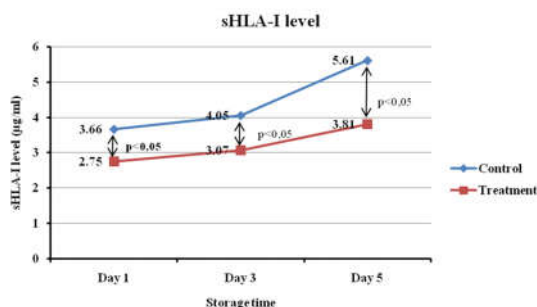


Figure 1

The difference of mean sHLA-I level in treatment Group (with PLF) and control (without PLF) in platelet concentrate stored on day 1, 3 and 5

## DISCUSSION

The mean ( $\pm$  SD) sHLA-I level in PLF Group were statistically lower than the Control Group both on the first day of storage ( $2,75 \pm 0,82$

$\mu\text{g/ml}$  vs  $3,66 \pm 0,87$   $\mu\text{g/ml}$ ,  $p = 0,004$ ), storage on the third day ( $3,07 \pm 0,75$   $\mu\text{g/ml}$  vs  $4,05 \pm 1,29$   $\mu\text{g/ml}$ ,  $p = 0,011$ ), and the fifth day of storage ( $3,81 \pm 0,97$   $\mu\text{g/ml}$  vs  $5,61 \pm 3,26$   $\mu\text{g/ml}$ ,  $p = 0,036$ ). sHLA-I level in PLF Group was statistically lower than the Control Group indicating the role of PLF in immunological reactivity. PLF is a procedure to reduce the number of residual leukocytes contained in thrombocyte concentrates prior to storage using a filtration method. PLF eliminate residual leukocytes before undergoing apoptosis and necrosis and before release cytokines<sup>6,7,11,12</sup>.

Residual leukocytes in thrombocyte concentrates express HLA antigens. APC will present donor HLA antigens to recipient T lymphocytes. Interaction of HLA antigens and recipient T lymphocytes cause the first signal that increases the expression of IL-2 receptor. The second signal is required to induce a variety of cytokines which then resulted in the proliferation and differentiation of specific T- lymphocytes. Immunogenicity of HLA antigens on blood components depends on the ability of APC to stimulate recipient T cell by sending 2 different signals<sup>6,10</sup>.

In vitro studies revealed that the HLA Antigen-I plays a role in modulating the function of immunocompetent cells, so the presence of sHLA-I in thrombocyte concentrates is a marker of immunological reactivity. HLA-I antigen inhibit lymphocyte responses and cytotoxic T cell activity. HLA-I antigen modulate the function of immunocompetent cells through two ways: (1) HLA-I molecule binds to its physiological ligand and inhibits the T cell function through blockade of receptors and/or induce apoptosis, and (2) through indirect presentation in which HLA-I phagocytosis by APC, and then degraded into peptides and presented to CD4 T-cells that will cause immune tolerance or immune activation which depends on the activation capacity or tolerance capacity of HLA-I peptide<sup>13,14</sup>.

Our findings are in agreement with Ahmed et al (2010) which reported a significant difference between sHLA-I level in thrombocyte concentrate with leukoreduction and thrombocyte concentrates without leukoreduction, where the sHLA-I level was lower in leukoreduction platelet concentrate ( $p < 0,05$ ). Also reported the accumulation of pro-inflammatory cytokines (IL-1, TNF- $\alpha$ , IL-6 and IL-8) and chemokines in platelet concentrates without leukoreduction<sup>5,15</sup>.

In this study also done further analysis of sHLA-I levels during storage, both in the treatment Group (with PLF) and the Control Group (without PLF). There were found elevated sHLA-I level during storage either in the treatment Group and the Control Group which is statistically significant, but relatively higher in the Control Group (without PLF) than the treatment Group (with PLF). In this

case the PLF is able to suppress elevated of sHLA-I level during storage of platelet concentrates, which can suppress platelet destruction and immunological reactivity in platelets. In platelet concentrates with PLF can still measure sHLA-I level, because associated with residual leukocyte which is found in platelet concentrates ( $< 1 \times 10^6$ ), but much lower than the amount of residual leukocytes contained in platelet concentrates without PLF. During storage, the release of antigen HLA-I from residual leukocytes due to membrane damage or cell death. In addition, increment of sHLA-I level during storage can be caused by in vitro aging which platelet loss its biological reactivity and viability, associated with platelet apoptosis. Platelet apoptosis related because platelets contain enough mitochondria and give an overview of apoptosis-like morphologic changes. So, the increment sHLA-I level during storage associated with residual leukocyte apoptosis and platelet apoptosis<sup>6,11,12,16-17</sup>.

#### CONCLUSION

The present study concludes that PLF decrease sHLA-I level of thrombocyte concentrates stored for five days compared with Control Groups.

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