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The differences in the levels of interleukin 1 beta (IL-1 β) and tumor necrosis factor alpha (TNF-A) based on the storage of thrombocyte concentrates

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Abstract---Thrombocyte concentrate (TC) transfusion plays an important role in preventing bleeding in patients with severe thrombocytopenia. Febrile non hemolytic transfusion reaction (FNHTR) may occur after TC administration. IL-1 β and TNF- α cytokines released by thrombocytes and leucocytes during TC storage play important roles in the occurrence of FNHTR after TC transfusion. The purpose of this study was to analyze changes in levels of IL-1 β and TNF- α on the duration of TC storage. This was an observational analytical research with time series design carried out at the Clinical Pathology Laboratory and Blood Bank of the Dr. Soetomo Hospital Surabaya in September - October 2019. IL-1 β and TNF- α levels in 20 bags of Thrombocyte Concentrate blood components derived from Platelet Rich Plasma during storage for day 1, day 3 and day 5 were measured using ELISA Sandwich method. The statistical analysis was performed using the Subject Same Variant Test or Friedman Test. The results showed no significant differences in the levels of IL-1 β and TNF- α based on the storage duration of TCs on day 1, 3 and 5, $p = 0.262$ and $p = 0.534$ respectively. There was a significant difference of IL-1 β levels between day 1 and day 3 ($p=0.032$).

Keywords---IL-1 β , TNF - α , thrombocyte, concentrate.

Introduction

Thrombocyte transfusions in the United States of America have increased with the decrease of red blood cells and plasma transfusion. 2,2 million thrombocytes or also known as platelets, are transfused each year in the USA (Whitaker, 2013). There are two types of Thrombocyte Concentrate (TC) components, TC derived from Whole Blood (WB) and TC derived from apheresis. TC that is made from WB consists of pooled thrombocyte made from WB, and single or pooled thrombocytes made from Leukodepleted Whole Blood. Apheresis TC consists of thrombocytes from apheresis and thrombocytes from leukodepleted apheresis (Yuan & Goldfinger, 2018). The use of thrombocytes from apheresis or leukoreduced blood is done to increase the safety of thrombocyte transfusion, because the fragmented and activated leukocytes in TC will produce cytokines (TNF- α , IL-1 β , IL-6 dan IL-8) that can cause several transfusion reactions such as febrile non hemolytic transfusion reaction (FNHTR) dan transfusion related acute lung injury (TRALI) (Mittal & Kaur, 2015). Thrombocyte Concentrate blood components used in Dr. Soetomo Hospital Surabaya are TC's derived from WB that are stored up to 5 days that could undergo platelet storage lesions (PSL).

Platelet storage lesions (PSL) are all changes that can destruct the function or structure of thrombocytes that appear since the blood is taken from the donor until the thrombocytes are transfused into the recipient. The mechanisms that cause PSL are multifactorial and aren't completely understood. These lesions are related with the decrease of thrombocyte number, durability and in vivo hemostatic activity, after transfusion (Mittal & Kaur, 2015). The incidence of thrombocyte transfusion reactions relate to the length of thrombocyte storage. The Blood Bank of Dr. Soetomo Hospital Surabaya produces 29,679 bags of TCs throughout January to December 2018 and there were 73 cases of fever due to TC transfusion (BDRS Surabaya, 2018). Transfusion reactions multiplied two fold in recipients of 3 – 5 days old TC compared to 1 – 2 days old TC (BDRS Surabaya, 2018). This shows that there is an active blood component contained in the plasma of stored TC. The concentration of IL-1 β and TNF- α is highly correlated with the frequency of febrile reaction. Mullye et al, found that IL-1 β and TNF- α increased in the plasma of TC due to active synthesis and/or the release of both cytokines. IL-1 β and TNF- α are a strong mediator in the acute phase response, and has the highest correlation in the increase of these cytokine concentrations due to PS L (Muylle et al., 1992). This experiment was done on TC blood components derived from WB or plasma rich platelet (PRP) and measurement of IL-1 β and TNF- α concentration were done on the 1st, 3rd and 5th day of storage. IL-1 β and TNF- α were chosen because these cytokines had an immediate effect of inducing fever through the synthesis of prostaglandin E₂ in the hypothalamus (Elabscience (a), 2017). Choosing day 1, 3 and 5 for the measurement was based on sources/literature stating that TC can be stored up to day 5 or 7 to avoid contamination of bacteria.

Methods

This experiment was a time series analytic observational study conducted in the Blood Bank of Clinical Pathology Installation of dr Soetomo Hospital Surabaya. The samples were Thrombocyte Concentrate (TC) blood components that were

produced and stored at room temperature (20 – 24°C) with agitation and fulfilled the inclusion and exclusion criteria. Samples were taken with the consecutive sampling technique from April – October 2019. This experiment was conducted on 20 thrombocyte concentrate blood components that were produced during the experimental period, at the Blood Bank of dr Soetomo Hospital Surabaya, that has passed quality control in concordance with Permenkes no 91 year 2015 on the first, third and fifth day of storage. TC's that had a change of color and didn't have swirling were excluded from this experiment. This experiment was stated ethical with the no: 1426/KEPK/VII/2019 from the Ethical Committee of Health Research of RSUD Dr Soetomo Hospital Surabaya.

Three ml of first day TC components stored at 20 – 24°C with agitation, were stored in plain tubes. Samples were obtained by using the tube of the TC blood bag in a BSC to maintain sterility. The blood bag was homogenized carefully, the tube clamped, and the tip of the tube was cut so the blood could be stored in plain tubes. The tube was sealed using a heat sealer. The plain tube consisting of 3 ml of TC were centrifuged at 4000 g for 15 minutes at 20 – 24°C. The supernatant was transferred to aliquots with the volume of 1 ml each. The transfer of TC to aliquots were all done in a biosafety cabinet to prevent bacterial contamination. TC bags that were already sampled, were stored at 20 – 24°C with agitation and underwent the same process for samples extracted on the 3rd and 5th day of storage. Aliquots were stored in a deep freezer at – 80°C to maintain the stability of IL-1 β and TNF- α for 3 months. The measurement of IL-1 β and TNF- α were done using the ELISA sandwich method according to the insert kit by *Elabscience*® (Elabscience (a), 2017; Elabscience (b), 2017). IL-1 β and TNF- α data would be analyzed by Same Subject Varian Analysis if they were normally distributed and Friedman analysis if they were not normally distributed, with a p value < 0.05 deemed as statistically significant.

Results and Discussion

Quality assurance of the examinations in this experiment was conducted before measuring the samples. Quality assurance was done by checking the expiration date, the lot number of each kit and if the storage of the reagents fulfilled the kit instructions. Quality control was also done by checking if the control levels for both high and low examination were within limit. The number of samples used in this experiment were 20 samples. This number fulfills the minimum limit of samples for this type of experiment. Sampling was done during April – October 2019 in The Blood Bank of Dr. Soetomo General Hospital Surabaya. The sample characteristics are the blood types of the donors as seen in table 1.

Table 1
Experiment Sample Characteristics'

Blood type	Thrombocyte Concentrate (Bag)	Amount (%)
A+	2	10
B+	10	50
AB+	2	10
O+	6	30
Total	20	100

The sample size of this study used Thrombocyte Concentrate (TC) blood component as many as 20 bags and the most obtained TC bags were blood type B+ as many as 11 bags (40.7%) and the least with AB blood type and A+ blood type as many as 2 bags each (10%) such as seen in table 1. This was due to the consecutive sampling technique adjusted to the availability of TC reserved in the Blood Bank of the Dr. Soetomo Hospital Surabaya.

Differential Concentrations of IL-1 β

The results of the Friedman test showed that there was no significant difference between the concentrations of IL-1 β day 1, day 3 and day 5 ($p > 0.05$). Degradation and activation of thrombocytes is multifactorial, that can occur due to mechanical exposure during the production process, PSL can happen due to bacterial contamination that grows well on TC blood component due to storage in room temperature (Devine & Serrano, 2010). The production process of TC blood components which sterilization is guarded, lessens the degradation of thrombocyte and the release of IL-1 β .

The day-per-day analysis showed a significant difference between IL-1 β concentrations on day 1 and day 3, but no significant differences between day 3 and 5 or day 1 and 5. This shows that during TC storage on day 1 and day 3 there is an increase in activation and destruction of thrombocytes and/or leucocytes. This can be caused by production of the TC component that can activate cells causing a higher release of chemokines on day 3. Devine et al, stated that the production of TC caused thrombocytes to have many stressors and stimulation. Mechanical force towards thrombocytes produced by Wb or PRC can cause more thrombocytes to activate compared to TC's derived from apheresis or using buffy coat method. This is shown by temporary aggregation of the thrombocytes, so TCs must be diamkan after production, so the aggregation will decrease. This mechanical force is stated as the first activation of thrombocytes causing PSL (Devine & Serrano, 2010). This also indirectly shows that activation of thrombocytes due to mechanical stimulation decreases after day 3.

Tunjung Putri, et al shows that the number of thrombocytes effects the concentration of IL-1 β , and thrombocyte degranulation is what increases the production capacity of IL-1 β by leucocytes. Thrombocytes can also synthesize IL-1 β and inflammasome through *splicing intra-platelet IL-1 β pre-messenger RNA* (mRNA) (Tunjungputri et al., 2018). This is what underlies the significant increase of IL-1 β , because it is produced by degradation of thrombocytes and leucocytes. Thrombocyte and leucocyte count were not performed in this experiment. PSL is a multifactorial process that is influenced by activation and degradation of leucocytes and thrombocytes, this can happen due to different conditions that cause thrombocytes and leucocytes to be metabolically active due to room temperature storage and stressor in the production of TC. A sterile production process of TC components and good storage procedure exclude these factors in influencing IL-1 β . Friedman test shows that there is no significant difference between IL-1 β according to time of observation ($p > 0.05$). Data is shown using median because it is abnormally distributed.

Table 2
Results of Differential Test of IL-1 β between Times of Observation

IL1- β	n	Mean $\pm$ SD Median (min – max)	Mean $\pm$ SD Median (min – max) Differentiation	P value
Day-1	20	1.76 $\pm$ 1.027	0.83 $\pm$ 1.593	0.032
Day -3	20	2.58 $\pm$ 1.807		
Day -1	20	1.78 (0,25 – 3.62)	-0.025 (-1.59 – 4.70)	0.765
Day -5	20	1.73 (0,25 – 8.32)		
Day-3	20	2.58 $\pm$ 1.807	-0.47 $\pm$ 2.051	0.320
Day -5	20	2.12 $\pm$ 2.092		

Results for paired t test shows a significant difference for IL-1 β concentrations on day 1 compared to day 3 ($p < 0.05$), but there were no significant differences of IL-1 β between day 3 and 5 ($p > 0.05$). Wilcoxon tests shows no significant differences of IL-1 β concentrations of day 1 and 5 ($p > 0.05$).

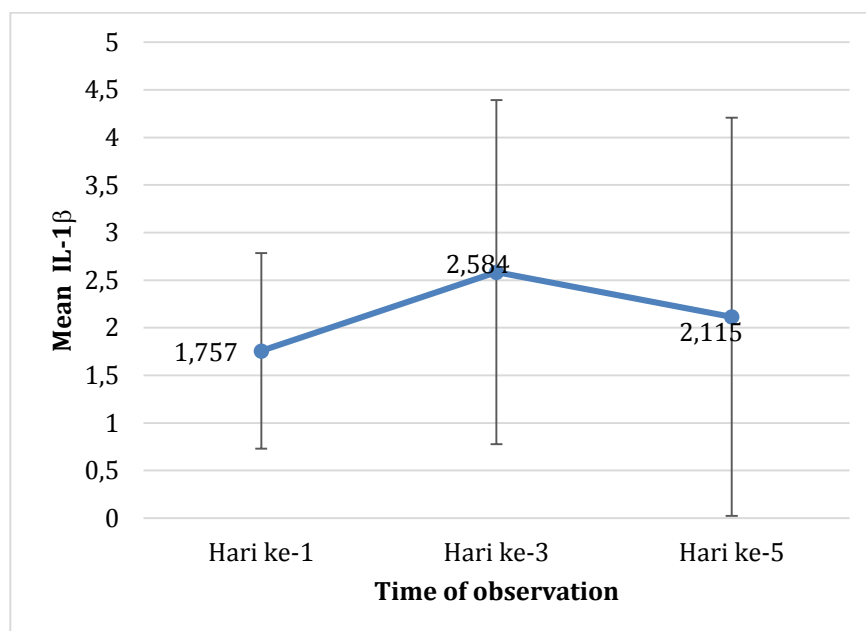


Figure 1. Line diagram of Mean Concentration of IL-1 β towards time of observation

Picture shows that the variation of IL-1 β concentrations on day 1 has a narrow range with a median of 1.757 pg/ml; and the concentrations on both day 3 and 5 are wide with a median of 2.584 pg/ml and 2.115 pg/ml respectively.

Results of TNF- α Concentration Differential Test

The results of Friedman show that there are no significant differences between TNF- α concentrations on day 1, 3 or 5.

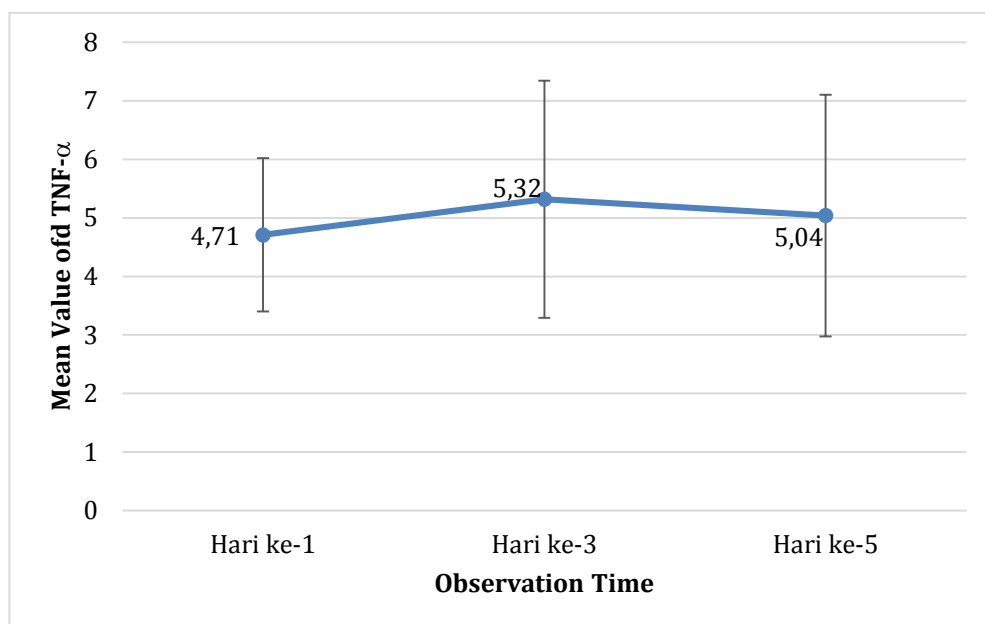


Figure 2. Line diagram of mean concentration of TNF- α and observation time

The mean concentration of TNF- α on day 1, 3 and 5 do not show a significant difference, with a median of 4.71 pg/mL; 5.32 pg/mL and 5.04 pg/mL respectively. These results are different from Shaegian et al (2002), that showed a significant increase of TNF- α in TC made by Random Donor Concentrate on day 0 to day 3. Shaegian also found that TNF- α reached its peak on day 3 and decreased on the days after (Devine & Serrano, 2010). Data normality test using Shapiro-Wilk on table 13 shows that the difference between TNF- α was normally distributed ($p > 0.05$), hence TNF- α was analyzed using a paired t test.

Table 3
Differential t-Paired Test of TNF- α according to time of Observation

TNF- α	n	Mean $\pm$ SD	Mean $\pm$ Difference in SD	P value
Day 1	20	4.71 $\pm$ 1.308	0.61 $\pm$ 1.832	0.152
Day 3	20	5.32 $\pm$ 2.025		
Day 1	20	4.71 $\pm$ 1.308	0.34 $\pm$ 1.827	0.419
Day 5	20	5.04 $\pm$ 2.064		
Day 3	20	5.32 $\pm$ 2.025	-0.27 $\pm$ 1.500	0.425
Day 5	20	5.04 $\pm$ 2.064		

Table 3, shows that there was no significant difference of TNF- α on day 1 compared to day 3; day 3 compared with day 5 or day 1 compared with day 5 ($p > 0.05$). Table 3, shows that there was no significant difference of TNF- α on day 1 compared to day 3; day 3 compared with day 5 or day 1 compared with day 5 ($p > 0.05$).

Cytokine formation happens due to new synthesis and the release of cytokines from white blood cells that are left from the production of TC components. This is in line with the peak of TNF- α being on the third day, but the increase in this research is insignificant and has a wide variation. The decrease of TNF- α levels after day 3 shows that activation and degradation of leucocytes after day 3 also decreases (Shaiegan, 2006). Mulleye, et al found an increase of cytokines such as IL-1 β and TNF- α during TC storage. The comparison of IL-1 β and TNF- α levels from day 0 to day 7 indicates that the increase of cytokines are a result of active synthesis or cytokine release. This effect is related to a minimum leucocyte concentration of 3×10^9 /L (Elabsience (a), 2017). Active cytokine synthesis is not found if the leucocytes are lower than that number. Shukla et al, found a relationship between the number of leucocytes and the TNF- α concentration (Shaiegan, 2006). The variation of TNF- α production in this study is due to the different number of leucocytes left in the TC component, but in this study, the number of leucocytes per bag was not measured (Shukla et al., 2014).

A previous study by Mulleye, et al (1992) showed an accumulation of cytokines in the plasma of TC blood components such as TNF- α were lower in apheresis products compares to products derived by WB, but Bayraktarogglu et al (2007) found an increase of TNF- α in apheresis products that was predicted to happen due to the pheresis machine that activated more leucocytes than the other studies. This shows that not only the number of leucocytes but also the mechanical stress from production can influence TNF- α concentration (Bayraktarogglu et al., 2007). The day by day analysis showed that there were no significant differences between TNF- α based on the days of storage. This is shown from the differential test of TNF- α stated in Table 13. It is different from IL-1 β where a significant difference from day 1 to day 3 can be found, this is due to IL-1 β being produced not only by leucocytes but also by thrombocytes, when TNF- α is produced by leucocytes only. Leucocytes in the plasma of TC components that are activated and degraded can release TNF- α . A sterile production process and proper storage will not affect the concentration of TNF- α .

Conclusion and Advice

Concentrations of IL-1 β and TNF- α chemokine that are contained in Thrombocyte Concentrate blood component on day 1, day 3 and day 5 do not differ during storage. The number of leukocytes and thrombocytes in each blood bag should be examined to see how big a role they play in the increase of IL-1 β and TNF- α , further studies should also be done to compare the levels of IL-1 β and TNF- α in TC blood components made with different methods also calculating the number of thrombocytes and leucocytes per bag, to get a more detailed result.

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