Comparing P-Selectin (CD62P) expression in patients receiving non-leukodepleted versus leukodepleted thrombocyte concentrates

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ABSTRACT

Thrombocyte concentrate (TC) transfusion is an important supportive therapy in patients with thrombocytopenia. The risks in platelet transfusions may be related to the content of TC including the contaminant leukocytes. The aim of this study was to assess the risk of increased level of P-Selectin (CD62P) expression of non-leukodepleted TC transfusions. This was a quasi-experimental study. Subjects were children patients aged 1-18 years who received a non-leukodepleted or a leukodepleted TC transfusions. Comparison of the proportion of increased expression of CD62P in both groups expressed as relative risk. The subjects consisted of 51 patients who received non-leukodepleted and 52 patients who received leukodepleted TC transfusions. The risk of increased expression of CD62P in patients receiving non-leukodepleted TC transfusions were 2.38 (95%CI:1.60-3.53) times higher than those who received leukodepleted TC. Non-leukodepleted have significant higher risks of increased CD62P expression than leukodepleted TC transfusions.

Keywords: thrombocyte concentrate – transfusion – CD62P expression - thrombocytopenia – adverse effect

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INTRODUCTION

Globally, total blood donations are approximately 107 million units annually, which is almost fifty percents collected in high-income countries. In Indonesia, 4,644,863 units of blood products were processed in 2014, which were consisted of 16.18% whole blood and 83.82% components. From those components, 58% were packed red cells (PRC), 22% were fresh plasma, 13% were thrombocyte concentrate (TC), 6% were fresh frozen plasma (FFP), 0.18% were washed erythrocyte, 0.65% were cryoprecipitate, and 0.17% were apheresis platelet. At Dr. Sardjito General Hospital, Yogyakarta, approximately 36,000 units of blood components transfusion are registered annually. The increase of TC use has been noted from 29.52% in 2011 to 37.2% in 2015. Pediatrics ward was the largest TC user, and there has been a steady increase of TC use from 51.20% in 2011 to 67.01% in 2015.

Platelet transfusion is associated with post-transfusion reaction including allergic reaction and febrile non-hemolytic reaction. To reduce the risk of leukocyte-associated transfusion adverse event, blood component should be leukoreduced before usage. So far, there is little concern about leukocyte contaminants within blood component especially in developing countries. One of many methods described to eliminate thrombocyte transfusion reaction is by doing TC leukodepletion. Leukodepletion is a procedure to remove leukocyte contaminants present within blood component, using a specific filter.

Nielsen et al. reported accumulation of chemical mediators as a result of leukocyte intracellular granule release during blood component storage (PRC or TC) such as histamine, eosinophil cationic protein, eosinophil protein X, myeloperoxidase, and plasminogen activator inhibitor-1. Leukocyte could affect the function of platelets by releasing any chemical substances for example cathepsin G, reactive oxygen species, nitric oxide, and myeloperoxidase (MPO).

Myeloperoxidase is originally an enzyme stored inside the granule of polymorphonuclear cell (PMN), its detectable level in circulation reflects the degranulation of PMN and PMN cells that undergo necrosis during blood component storage also produce it. This enzyme is secreted along with other biochemical substances like protease and peroxydase to surrounding extracellular environment. Myeloperoxidase is secreted along with other biochemical substances like protease and peroxydase to surrounding extracellular environment. This study was aimed to compare the CD62P expression in patients receiving non-leukodepleted versus leukodepleted thrombocyte concentrates.

MATERIALS AND METHODS

Subjects

Study subjects of this research were pediatric patients receiving transfusion of either non-leukodepleted or leukodepleted TC during their care in Estella ward Dr. Sardjito General Hospital, Yogyakarta. The subjects were selected in consecutive sampling basis according to inclusion and exclusion criteria. Randomization could not be performed properly because of a limitation in preparing leukodepleted TCs. Inclusion criteria of this study were all pediatric patient age 1-18 years old diagnosed with malignancy and indicated to receive platelet transfusion. The study subjects would be excluded from research if he had bleeding manifestation, DIC, hypersplenisms, fever, receiving antipiretic or antithrombotic therapy or other blood component transfusions. Study subjects were categorized into two groups, first group received non-leukodepleted TC and another...
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... grp received leukodepleted TC transfusion. Protocol of this study has been approved by the Medical and Health Research Ethics Committee of Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta. Leukodepleted thrombocyte concentrates were platelet-pooled (2-4 units) derived from whole blood with CPDA1 processed using PRP method and filtered to remove leukocyte contaminants before stored in agitator at 20 – 24 °C for maximum 5 days. Non-leukodepleted thrombocyte concentrates were produced using the same process without undergone leukocyte filtration.

**Measurement of CD62P expression using flowcytometry method**

The method applied in this research refered to previous method developed by Kim and Lim,\cite{13} which minimalize platelet activation artificially, eliminating the need of centrifugation for washing and fixation before labelling and simultaneously label monoclonal antibody to the patient platelets. CD62P expression was measured by flowcytometry method using monoclonal antibody anti-CD62P-FITC against whole blood sample without addition of agonist (ADP). The measurement was conducted on blood sample before and 1 hour after TC transfusion (pre vs. post-transfusion). For analytical purpose, gates were made in predetermined platelet area with addition of anti-CD41-PE (FIGURE 1). Data were presented as platelet percentage that positively expressed CD62P.

![FIGURE 1. Dot plot and statistic quadrans of CD62P measurement](image-url)
Data analysis

Subject characteristics was presented descriptively. The percentage of platelet (%) that expressed CD62P was presented as mean value. The comparison of increased CD62P expression in both groups was presented as relative risk. Statistical analysis was performed at significance value p < 0.05 with confidence interval of 95%.

RESULTS

Total subjects enrolled in this study were 103 patients, divided into 2 groups; first group consist of 51 patients receiving non-leukodepleted TC and another group of 52 patients receiving leukodepleted TC transfusion. Subject characteristics were summarized in TABLE 1. Overall, there were no statistically significant difference (p >0.05) between two groups in term of gender, age, body weight, height, body surface area (BSA) and body mass index (BMI).

| Characteristic       | Non-leukodepleted (n=51) | Leukodepleted (n=52) | p  |
|----------------------|--------------------------|----------------------|----|
| Gender               |                          |                      |    |
| Male                 | 32 (62.7%)               | 30 (57.7%)           | 0.600 |
| Female               | 19 (37.3%)               | 22 (42.3%)           |    |
| Age (mean ± SD year) | 8.69 ± 4.83              | 9.93 ± 5.10          | 0.209 |
| 1-5                  | 16                       | 12                   |    |
| 5-10                 | 17                       | 17                   |    |
| 10-14                | 7                        | 6                    |    |
| 14-18                | 11                       | 17                   |    |
| Weight (mean ± SD kg)| 26.12 ± 15.84            | 26.67 ± 12.84        | 0.847 |
| Height (mean ± SD m) | 1.22 ± 0.28              | 1.28 ± 0.29          | 0.279 |
| BSA (mean ± SD m²)   | 0.925 ± 0.379            | 0.975 ± 0.354        | 0.491 |
| BMI (mean ± SD kg/m²)| 16.048 ± 4.10            | 15.279 ± 2.64        | 0.259 |

In this study, CD62P expression on platelet was measured from patient venous blood sample before and 1 hour after TC transfusions. Mean expression of CD62P before transfusion was not statistically significant difference between two groups i.e. 25.42 ± 19.52% in non-leukodepleted group and 27.16 ± 10.31% in leukodepleted group respectively (p > 0.05). On the contrary, there was statistically significant difference in mean expression of post-transfusion CD62P (p < 0.05), with the group receiving non-leukodepleted TC expressed higher level (35.85 ± 20.31%) than the group receiving leukodepleted TC transfusion (24.12 ± 7.16%). Mean expression of post-transfusion CD62P in group receiving non-leukodepleted TC post-transfusion was increased than pre-transfusion, meanwhile in leukodepleted group, it was slightly decreased as seen in TABLE 2.
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TABLE 2. Mean expression of pre and post-transfusion CD62P

| Group          | Non-leukodepleted (n = 51) | Leukodepleted (n = 52) | p    |
|---------------|---------------------------|------------------------|------|
| Pre-transfusion | 25.42±19.52               | 27.16±10.31            | 0.574|
| Post-transfusion | 35.85±20.31              | 24.12±7.16             | 0.000|

Relative risk of increased CD62P expression in patients receiving non-leukodepleted TC compared to leukodepleted group was presented in the TALE 3. It was seen that the increased CD62P expression was 2.38 times higher in the group receiving non-leukodepleted TC than leukodepleted TC transfusions.

TABLE 3. Relative risk of increased CD62P expression in non-leukodepleted group

| Group          | Increment (+) | Increment (-) | Total | p    |
|---------------|---------------|---------------|-------|------|
| Non-leukodepleted | 42            | 9             | 51    |      |
| Leukodepleted  | 18            | 34            | 52    | 0.000|
| Total         | 60            | 43            | 103   |      |

Relative risk = 2.38; (95%CI=1.60-3.53)

DISCUSSION

The increased expression of post-transfusion CD62P was greater in the group receiving non-leukodepleted TC than leukodepleted TC probably caused by addition effects of pretransfusion activated donor platelets or the effect of increased level of MPO in transfused TCs. Nomura et al. have conducted a research with transfusion of 100 TCs involved 28 hematologic and non-hematologic malignancy patients, it was concluded that platelet CD62P expression was diminished significantly after leukocyte filtration process or leukodepletion.

There were some markers to evaluate platelet activation such as β-Thyroglobulin (β-TG), Glycoprotein-1β (GPIB/CD42), platelet aggregation test or GPIIb/IIIa (CD41), soluble P-selectin, surface P-selectin (CD62P), and among them CD62P is the best marker. CD62P also known as α-granule platelet membran protein-140 (GMP-140), expressed on the surface membrane during platelet activation, but remain undetectable in resting platelets. CD62P reported to be expressed rapidly on the surface membrane when platelet is activated. CD62P is the most sensitive and specific marker which reflects activation of platelets. Among other activated thrombocyte surface marker, CD62P expression shows the most significant difference between activated and resting platelet state.

There was a correlation between P-selectin expression and platelet survival after transfusion, stronger expression of P-Selectin on the platelet surface, associated with easier destruction of platelet after being transfused. After activation, platelets will release proinflammatory chemokine, cytokine and adhesion molecule appear onto its surface, including CD62P and CD40 ligand (CD40L). Interaction between platelet and neutrophyl triggers recruitment of neutrophyles to the inflammed tissue, activates the neutrophyles.
and it contribute toward host immunity and thrombosis process. Human neutrophil α-defensins (HNPs) consist of 50% protein from azurophilic granules. It can be released from azurophilic granule into extracellular environment after PMN cell activation and then undergo degranulation, leakage, cell death and lysis during inflammation.\(^{20}\)

Clinical relevance of CD62P expression level to predict in vivo platelet behaviour after transfusion is still debatable and not fully understood. It is partly based on fact that dynamicity of platelet and loss of CD62P surface protein.\(^{21}\) There was evidence that P-selectin level increased during storage, whereas its activatability undergo reduction.\(^{22}\) Increased level of CD62P significantly unrelated to body mass index (BSA), carotid intima media thickness and inflammatory mediator (CRP).\(^{17}\)

**CONCLUSION**

In conclusion, non-leukodepleted have significant 2.38 times higher risk of increased post-transfusion CD62P expression than leukodepleted TC transfusions.

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