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Research Article

Effect of Platelet Concentrates (PCs) Leucodepletion on the Activation and Efficacy of Platelet Transfusion

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ABSTRACT

Platelet concentrates (PCs) are blood components transfused in thrombocytopenic patients, including patients with blood malignancies. PCs contain leukocytes, which can pose potential side effects and activate platelets, reducing the efficacy of platelet transfusion. The leucodepletion process can be carried out by filtration to reduce the leukocyte count. This study aims to determine the difference between the CD62P expression of nonleucodepleted and leucodepleted PCs and to determine the difference between the Corrected Count Increment (CCI) of patients transfused with nonleucodepleted and leucodepleted PCs. This analytic observational study with a cross-sectional design was carried out on PCs obtained from platelet-rich plasma (PRP-PC). PCs were transfused into 48 blood malignancy patients, Yogyakarta, consisting of two groups i.e the group transfused with nonleucodepleted PCs (24 patients) and the group transfused with leucodepleted PCs (24 patients). CD62P expression in PCs was measured by flow cytometry method, and the CCI of the patients was calculated based on the CCI formula. The difference between the median CD62P expression and CCI of the two groups was analyzed using the Mann-Whitney Test with a significance of $p<0.05$. The median CD62P expression of the nonleucodepleted and leucodepleted groups were 34.4% (16.8–94.4%) and 21.7% (6.2–34.0%), respectively. There was a statistically significant difference between the CD62P expression of the two groups ($p = 0.00$). The group transfused with nonleucodepleted, and leucodepleted PCs showed respective median CCI of 18.8 x 10^9/L (2.4–94.8 x 10^9/L) and 14.7 x 10^9/L (2.4–124.0 x 10^9/L). There was no statistically significant difference between the CCI of the two groups ($p = 0.42$). It can be concluded that the CD62P expression in the PCs of the leucodepleted group was significantly lower than those of the nonleucodepleted group and that there was no significant difference between the CCI of both groups.

Key words: *platelet concentrates, CD62P, corrected count increment*

INTRODUCTION

Platelet concentrates (PCs) are one of the blood components in transfusion therapy utilized in a medical procedure to maintain primary hemostatic function. The need for platelet concentrates is increasing for therapeutic transfusions in diseases that cause thrombocytopenia, such as patients with blood malignancies and those who experience thrombocytopenia after chemotherapy or major invasive procedures such as heart surgery.[1-3]

Although platelet transfusions are recognized to provide benefits in various cases of thrombocytopenia, they still carry risks. One of the efforts to reduce platelet transfusion reactions is leucodepletion on platelet concentrates. Leucodepletion is aimed to remove leukocytes from blood components using a special filter.[4] Leukocytes, especially mononuclear cells in allogeneic blood products, both packed red cells and platelet concentrates, can cause various side effects, including immunomodulation (Transfusion-Related Immunomodulation), acute lung damage, Graft versus Host Disease, and virus transmission.[5] Transfusion-related immunomodulatory mediated by allogeneic mononuclear cells can be prevented by reducing leukocytes both before and after storage and autologous transfusion. The allogeneic transfusion effects caused by soluble HLA peptides can be overcome by autologous transfusion, while the allogeneic transfusion effects caused by soluble mediators produced by leucocytes can be prevented by reducing them before storage.[6,7]

Leucocytes can influence platelet function by releasing various substances such as cathepsin G, reactive oxygen species, nitric oxide,[8,9] and myeloperoxidase (MPO).[10] MPO is an enzyme derived from PMN cell granules that reflects PMN degranulation and is also produced by PMN cells that experience necrosis during storage. These enzymes are excreted along with several other biochemical substances, such as proteases and peroxidative enzymes, into the extracellular environment.[11] Platelet activation by MPO is characterized by increased expression of P-selectin (CD62P) and PECAM-1.[12-14] If platelet activation occurs, there will be a release of platelet granules, and the process is called degranulation. One of the degranulation markers due to platelet activation is the expression of P-selectin (CD62P) on the platelet surface, which can be assessed using flow cytometry.[1,15,16] High CD62P expression is the cause of faster platelet destruction in the reticuloendothelial system.[5]

Therefore, an assessment of the efficacy of platelet transfusions can be measured by calculating the CCI and platelet recovery. In addition, the in vivo hemostatic efficacy after PC transfusion can be measured by the level of CD62P expression and the formation of leukocyte-platelet aggregates. This study aims to determine the difference between CD62P expression in nonleucodepleted and

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leucodepleted platelet concentrates and to determine the difference between the CCI of patients transfused with nonleucodepleted and leucodepleted platelet concentrates.

METHODS

This study was an analytic observational study with a cross-sectional design, conducted in Dr. Sardjito Hospital, Yogyakarta, Indonesia from January–September 2014. The target population included all pediatric patients aged 1-18 years who received platelet concentrate transfusions. The inclusion criteria were pediatric patients aged 1-18 years diagnosed as having blood malignancy with an indication of platelet transfusion. In comparison, the exclusion criteria were patients with bleeding manifestations, fever, DIC, hypersplenism, undergoing antithrombotic therapy, and transfusion of other components.

Patients who qualified for the inclusion criteria were examined for their platelet count before transfusion. The subjects were divided into two transfusion groups, i.e the nonleucodepleted and leucodepleted PC transfusion groups. The platelet yield and CD62P expression of the PCs to be transfused was initially examined. One hour after the transfusion, the platelet count was rechecked for the CCI calculation.

Platelet Concentrate Production

The platelet concentrates were obtained from whole blood through a centrifugation process using a refrigerated centrifuge. The platelet concentrates were then stored in a horizontal agitator at a temperature of 22°C, to make the platelet concentrate homogeneous and prevent platelet aggregation. The agitator was placed in an air-conditioned room with temperature set at 22±2°C.

Examination of CD62P expression using the flow cytometry method

The nonleucodepleted and leucodepleted platelet concentrates were processed in two separate tubes. Flow cytometry analysis was conducted using anti-CD41 as a marker of platelets and anti-CD62P as a marker of platelet activation. For analysis, at least 5,000 platelets were analyzed using Celquest software, gates were made on predetermined platelet areas with the addition of anti-CD41. Data are presented in the percentage of positive CD41 and CD62P.

Statistical Analysis

The percentage (%) of CD62P-expressing platelets are shown in the median (95% CI). The Mann-Whitney test was used to determine the difference between the mean CD62P expression in the nonleucodepleted, and leucodepleted platelet concentrates, as well as to determine the difference between the mean CCI of the nonleucodepleted and leucodepleted groups. The basic characteristics of the subjects are displayed descriptively. Statistical calculations were performed using a significance limit of $p < 0.05$ with a 95% confidence interval.

Ethical considerations

The subjects were asked for consent to participate in this study by signing an informed consent form. This study was approved by the Biomedical Research Ethics Commission of the Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta.

RESULTS

The characteristics of the platelet concentrates (PCs) in this study are presented in Table 1 as follows.

Nonleucodepleted and leucodepleted PCs were transfused in 24 blood malignancy patients with characteristics as shown in table 2 below.

Table 1: Characteristics of platelet concentrates in the nonleucodepleted and leucodepleted groups.

Platelet concentrates (unit)	Nonleucodepleted group n = 24	Leucodepleted group n = 24
Number of transfused PC, units	130	142
Mean platelet yield/unit, x 10^{10}	5.8	4.4
Median platelet storage, days(min-max)	1 (1-4)	2 (0-5)

Table 2: Characteristics of subjects.

Characteristics	nonleucodepleted group n = 24	Leucodepleted group n = 24
Average Age (years)	8.1	10.3
Sex n (%)		
Male	11 (45.8%)	12 (50%)
Female	13 (54.2%)	12 (50%)
Mean Body Weight (kg)	24.1	26.9
Mean Height (cm)	115.2	129.8
Mean Body Surface Area	0.857	0.989
Diagnosis		
ALL	16 (66.7%)	15 (62.5%)
AML	8 (33.3%)	9 (37.5%)
Transfusion indications n (%)		
Therapy	17 (70.8%)	16 (66.7%)
Prophylaxis	7 (29.2%)	8 (33.3%)
Blood group n (%)		
A	4 (16.6%)	3 (12.5%)
B	10 (41.7%)	12 (50.0%)
O	10 (41.7%)	9 (37.5%)
Median platelet count (min-max), /µL		
Before transfusion	12,500 (2,000-40,000)	9,500 (3,000-140,000)
After transfusion	51,000 (10,000-136,000)	32,500 (10,000-104,000)

Table 3: Comparison of platelet CD62P expression in the nonleucodepleted and leucodepleted groups.

Groups	N	CD62P Expression Median (Min-Max)	p
Nonleucodepleted	24	34.4 (16.8-94.4)	0.00
Leucodepleted	24	21.7 (6.2-34.0)	

Table 4: Comparison between the Corrected Count Increment (CCI) of the group transfused with nonleucodepleted platelet concentrates and those transfused with leucodepleted platelet concentrates.

Groups	N	CCI (x 10^9/L) Median (Min-Max)	p
Nonleucodepleted	24	18.8 (2.4-94.8)	0.42
Leucodepleted	24	14.7 (2.4-124.0)	

The platelet activation was assessed from CD62P expression by platelets in PCs. CD62P expression was measured using monoclonal antibodies against CD41 and CD62P using flow cytometry method, as plotted and shown in Figure 1 below.

The effect of leucodepletion on platelet activation was assessed by comparing CD62P expression in the non-filtered PC group (nonleucodepleted group) and the filtered PC group (leucodepleted group) using the Mann-Whitney test as shown in Table 3.

The above table presented that the median CD62P expression in the leucodepleted group was significantly lower than those in the nonleucodepleted group ($p<0.05$).

The efficacy of platelet transfusion was assessed by the Corrected Count Increment (CCI) formula, which was calculated one hour after

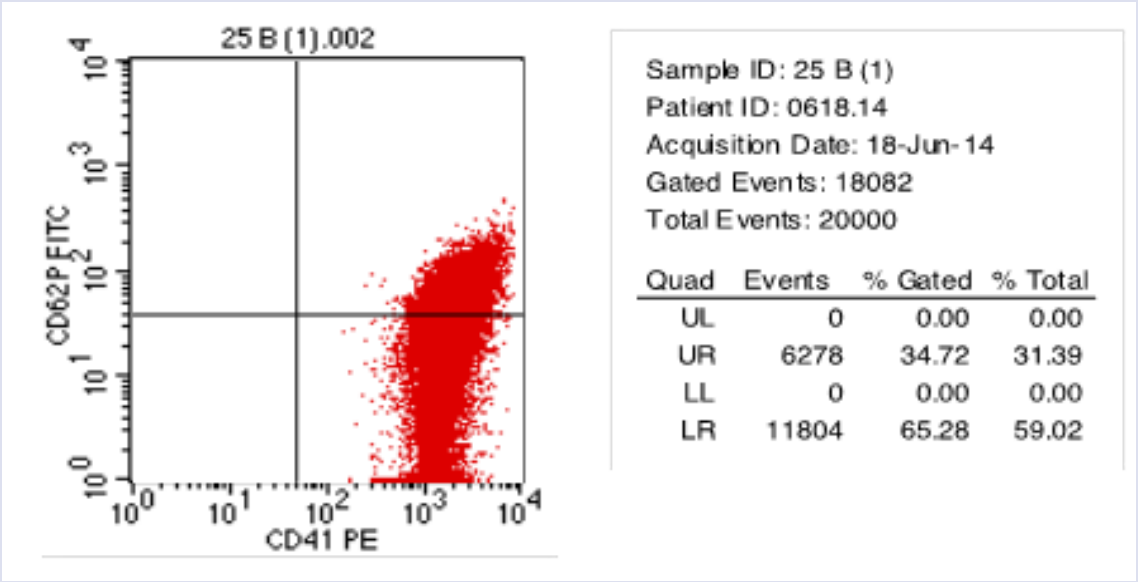


Figure 1: CD62P expression plot using flow cytometry method.

the platelet concentrate product was transfused to the patient. The Mann-Whitney Test statistic was performed to compare the CCI values between two groups as shown in Table 4.

Table 4 above depicts that, the CCI of the two groups indicated good platelet transfusion efficacy i.e more than 7.5 x 10^9/L, however, there was no significant statistical difference ($p > 0.05$).

DISCUSSION

All PCs in this study were obtained using the platelet-rich plasma (PRP-PC) method. The mean platelet yield in the nonleucodepleted group was 5.8 x 10^{10} with a median storage time of one day, and the mean platelet yield in the leucodepleted group was 4.4 x 10^{10} with a median storage time of two days. The platelet yield obtained in this study was similar with various references which was approximately 5.5 x 10^{10}.

Leucodepletion that can be performed by filtration on PCs aims to remove or reduce the contaminant leucocyte. It is acknowledged that leucocytes in blood components can cause various harmful side effects to recipients.[17] In addition, leukocytes can also cause activation of platelet contained in PCs to reduce the efficacy of platelet transfusions.

Among the various techniques to remove or reduce leukocyte count in PCs, filtration is quite efficient, cost-effective, and easy to apply clinically. The working principle of the leukocyte filter is based on differences in deformability and adhesive properties of the cells. In general, this filter can remove more than 95% of the leukocytes in one unit of blood product.[18]

The effect of leucodepleted platelet concentrates on platelet activation

Platelet activation in this study was assessed from CD62P expressed by platelets in PCs using flow cytometry method. Platelets can be activated during the preparation and storage of PCs, and this activation can be assessed by the expression of P-selectin (CD62P) by platelets.[19] P-selectin (CD62P) is a protein in the alpha granule of platelets, which will be expressed on the cell surface a few seconds after platelet activation. Flow cytometry analysis of CD62P expression alone or in combination with other markers has been utilized as the gold standard for assessing platelet activation.[20,21]

There are not many studies regarding the effect of leucodepletion on platelet activation as assessed through CD62P expression in PCs. In this study, CD62P expression in the nonleucodepleted group was found to be higher than those in the leucodepleted group. It showed that reducing the leukocyte yield or known as leucodepletion, can reduce platelet activation. Nomura et al. (2000), who conducted a study of 100 PC units transfused in 28 patients with hematological and nonhematological malignancies, found that platelets expressing CD62P (P-selectin) were significantly reduced after leukocyte filtration.[22] Average CD62P expression in the leucodepleted group in this study (20.8%), was not much different from that obtained in a study by Dijkstra-Tiekstra et al. (19.2%).[23]

Most of the studies regarding platelet activation in PCs relate the activation with storage time. Dijkstra-Tiekstra et al. (2004) found that there was an increase in platelet activation if stored for more than eight days (CD62P 41.6 ± 30.7%) compared to those stored for 2-7 days (CD62P 12.3 ± 4.8%).[23] A study by Ali (2012), which compared platelet activation during storage based on two PC preparation methods, found that with storage for more than five days, PRP-PCs showed a significant increase in CD62P expression compared to buffy coat-derived PCs.[24] The shelf life of PCs in this study was no more than five days, so the effect of storage on platelet activation in this study could be ruled out.

The PCs in this study were obtained from platelet-rich plasma (PRP-PC) method. The mean platelet yield in the leucodepleted group was 4.4 x 10^{10}, lower than that in the nonleucodepleted group, which was 5.8 x 10^{10}. The filtration process could reduce platelet count in PCs due to the platelet-leukocytes aggregation on the filter.

The effect of leucodepleted platelet concentrates on transfusion efficacy

The efficacy of platelet transfusion in this study was assessed by calculating the Corrected Count Increment (CCI) one hour after the PCs were transfused to the patients. Patients with blood malignancies have prolonged thrombocytopenia due to bone marrow involvement or medication. Platelet transfusions, mostly from random donors, are given to patients with platelet counts < 10,000/μL to reduce the risk of bleeding.[25,26] In this study, the median platelet count before transfusion was 12,500/μL in the nonleucodepleted group and 9,500/μL in the leucodepleted group.

The mean CCI of patients transfused with nonleucodepleted platelet concentrates was 23.6 x 10^9/L, while those transfused with leucodepleted platelet concentrates was 22.1 x 10^9/L. Both CCI results showed good efficacy because they were more than 7.5 x 10^9/L, but according to the statistical test, there was no significant difference between the CCI of the two groups. The influencing immunological factors include anti-HLA antibodies, anti-HPA antibodies, ABO mismatch, and immune complexes in the circulation, while the influencing non-immunological factors include splenomegaly, drugs, bleeding, disseminated intravascular coagulation, fever, sepsis, etc.[27,28]

Besides using CCI, the efficacy of platelet transfusion can also be assessed by calculating platelet recovery, but this parameter was not measured in this study. Many platelet transfusions do not produce the expected platelet recovery due to the presence of immunological and non-immunological factors. In a study of 97 patients with hematological malignancies who received leucodepleted platelet transfusions from random donors, it was found that the low platelet recovery was caused by immune factors, in this case, anti-HLA antibodies and anti-HPA antibodies, as well as non-immune factors, in this case, splenomegaly and PC storage time of longer than three days.[27]

The limitation of this study was that authors unable to rule out clinical splenomegaly and bleeding, which may affect the efficacy of platelet transfusions because these two symptoms are commonly found in patients with blood malignancies, who were the subjects of this study. The potential significant difference between the CCI of the group transfused with nonleucodepleted PCs and those transfused with leucodepleted PC in this study due to this effect cannot be ruled out.

CONCLUSIONS

This study indicated that there was a significant difference between CD62P expression in the nonleucodepleted group and that in the leucodepleted group. CD62P expression in the leucodepleted group is significantly lower than that in the nonleucodepleted group. In addition, the study also showed that there was no significant difference between the Corrected Count Increment (CCI) of both groups.

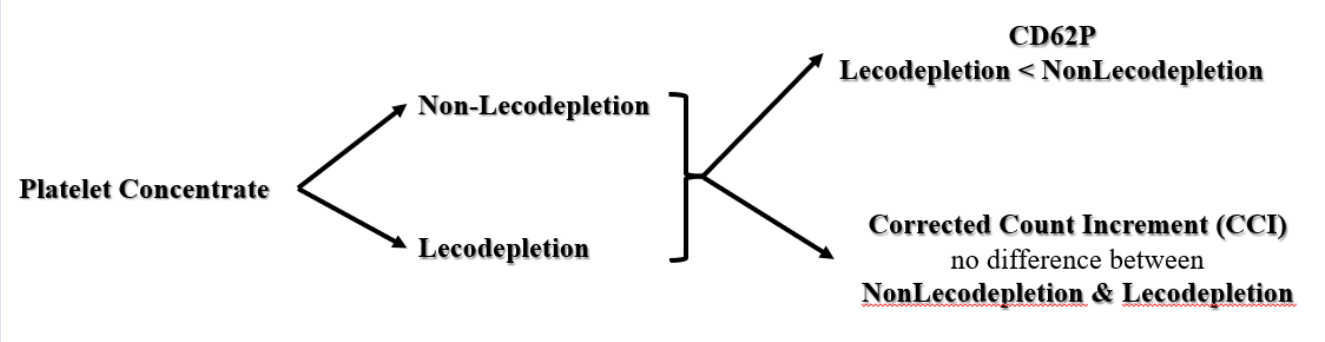
REFERENCES

1. Schubert P, Devine DV. Towards targeting platelet storage lesion-related signaling pathways. Blood Transfus. 2010;8 Suppl 3(Suppl 3):s69-s72. doi:10.2450/2010.011S
2. Chandra T, Gupta A, Kumar A, Afreen S. Morphological and functional changes in random donor platelets stored for seven days in platelet additive solution. IJBTI. 2011.1:20-25
3. Schubert P, Culibrk B, Karwal S, Slichter SJ, Devine DV. Optimization of platelet concentrate quality: application of proteomic technologies to donor management. J Proteomics. 2012;76 Spec No.:329-336. doi:10.1016/j.jprot.2012.06.023
4. Sharma RR, Marwaha N. Leukoreduced blood components: Advantages and strategies for its implementation in developing countries. Asian J Transfus Sci. 2010;4(1):3-8. doi:10.4103/0973-6247.59384
5. Vucic M, Tijanic I, Govedanevic L, Pavlovic Z. The Effect of A Leukodepletion model on The activation Stage of Platelets. Centr. Eur. J. Med. 2011;6(2):181-84
6. Vamvakas EC, Blajchman MA. Transfusion-related immunomodulation (TRIM): an update. Blood Rev. 2007;21(6):327-348. doi:10.1016/j.blre.2007.07.003.
7. Apriastini NKT, Ariawati K. Risk factors of acute blood transfusion reactions in pediatric patients in Sanglah General Hospital, Bali-Indonesia. Bali Med J. 2017;6(3):534-8.
8. Faraday N, Scharpf RB, Dodd-o JM, Martinez EA, Rosenfeld BA, Dorman T. Leukocytes can enhance platelet-mediated aggregation and thromboxane release via interaction of P-selectin glycoprotein ligand 1 with P-selectin. Anesthesiology. 2001;94(1):145-151. doi:10.1097/00000542-200101000-00025.
9. Faint RW. Platelet-neutrophil interactions: their significance. Blood Rev. 1992;6(2):83-91. doi:10.1016/0268-960x(92)90010-n
10. Nielsen HJ, Reimert CM, Pedersen AN, Brunner N, Edvardsen L, Dybkler L. Time-dependent, Spontaneous Release of White Cell and Platelet-Derived Bioactive Substances from Stored Human Blood. Transfusion. 1996;36:960-965
11. Mcfaul SJ, Corley JB, Mester CW, Nath J. Packed Blood Cells stored in AS-5 Become Proinflammatory during Storage. Transfusion. 2009;49:1451-1460
12. Kolarova H, Klinke A, Kremserova S, et al. Myeloperoxidase induces the priming of platelets. Free Radic Biol Med. 2013;61:357-369. doi:10.1016/j.freeradbiomed.2013.04.014.
13. Eiserich JP, Baldus S, Brennan ML, et al. Myeloperoxidase, a leukocyte-derived vascular NO oxidase. Science. 2002;296(5577):2391-2394. doi:10.1126/science.1106830.
14. Baldus S, Heitzer T, Eiserich JP, et al. Myeloperoxidase enhances nitric oxide catabolism during myocardial ischemia and reperfusion. Free Radic Biol Med. 2004;37(6):902-911. doi:10.1016/j.freeradbiomed.2004.06.003.
15. Kaufman RM. Platelets: testing, dosing and the storage lesion--recent advances. Hematology Am Soc Hematol Educ Program. 2006;492-496. doi:10.1182/asheducation-2006.1.492.
16. Thon JN, Schubert P, Devine DV. Platelet storage lesion: a new understanding from a proteomic perspective. Transfus Med Rev. 2008;22(4):268-279. doi:10.1016/j.tmrv.2008.05.004.
17. Chu RW. Leukocytes in blood transfusion: adverse effects and their prevention. Hong Kong Med J. 1999;5(3):280-284.
18. Bruil A, Beugeling T, Feijen J, van Aken WG. The mechanisms of leukocyte removal by filtration. Transfus Med Rev. 1995;9(2):145-166. doi:10.1016/s0887-7963(05)80053-7.
19. Lindahl TL, Ramstrom S. Methods for evaluation of platelet function. Transfusion and Apheresis Science. 2009;41(2):121-125.
20. Javela K. Laboratory analysis for evaluation of platelet disorders and platelet concentrates. Academic Dissertations from the Finnish Red Cross Blood Service, number 50. 2006.
21. Nagy B Jr, Debreceni IB, Kappelmayer J. Flow Cytometric Investigation of Classical and Alternative Platelet Activation Markers. EJIFCC. 2013;23(4):124-134. Published 2013 Jan 16.
22. Nomura S, Okamae F, Abe M, et al. Platelets expressing P-selectin and platelet-derived microparticles in stored platelet concentrates bind to PSGL-1 on filtrated leukocytes. Clin Appl Thromb Hemost. 2000;6(4):213-221. doi:10.1177/107602960000600406.
23. Dijkstra-Tiekstra MJ, Pietersz RN, Huijgens PC. Correlation between the extent of platelet activation in platelet concentrates and in vitro and in vivo parameters. Vox Sang. 2004;87(4):257-263. doi:10.1111/j.1423-0410.2004.00573.x.
24. Ali SF. Platelet activation in stored platelet concentrates: comparison of two methods preparation. J Hematol. 2012;1(1):15-19.
25. Wandt H, Frank M, Ehninger G, et al. Safety and cost effectiveness of a 10 x 10(9)/L trigger for prophylactic platelet transfusions compared with the traditional 20 x 10(9)/L trigger: a prospective comparative trial in 105 patients with acute myeloid leukemia. Blood. 1998;91(10):3601-3606.
26. Liumbruno G, Bennardello F, Lattanzio A, Piccoli P, Rossetti G; Italian Society of Transfusion Medicine and Immunohaematology (SIMTI) Work Group. Recommendations for the transfusion of plasma and platelets. Blood Transfus. 2009;7(2):132-150. doi:10.2450/2009.0005-09.

27. Levin MD, de Veld JC, van der Holt B, van 't Veer MB. Immune and nonimmune causes of low recovery from leukodepleted platelet transfusions: a prospective study. Ann Hematol. 2003;82(6):357-362. doi:10.1007/s00277-003-0648-7.

28. Slichter SJ, Davis K, Enright H, et al. Factors affecting posttransfusion platelet increments, platelet refractoriness, and platelet transfusion intervals in thrombocytopenic patients. Blood. 2005;105(10):4106-4114. doi:10.1182/blood-2003-08-2724.

GRAPHICAL ABSTRACT



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