Risk of Increased IL-6 Level in Adult Malignancy Patients Receiving Nonleucodepleted Packed Red Cell Transfusion

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Abstract

Blood transfusion in patients with malignancy can trigger transfusion reactions, including immunomodulation in the patients, which is known as Transfusion–Related Immunomodulation (TRIM). The clinical effects of TRIM include recurrence of malignancy. The mechanism of TRIM has not been comprehensively comprehended, but it is considered related to leukocytes as the main producers of cytokines, including IL-6, in transfused blood units. This study aims to determine the risk of increased IL-6 levels in adult malignancy patients who receive nonleucodepleted Packed Red Cells (PRC) transfusions compared to those receiving leucodepleted PRC. A quasi-experimental design was applied in this study. The 64 subjects included were adult malignancy patients who required PRC transfusions and visited the outpatient care at the "Tulip" Integrated Cancer Department of Dr. Sardjito Central Public Hospital (RSUP Dr. Sardjito). The patients were divided into nonleucodepleted (NLD) and leucodepleted (LD) groups, and the before and after transfusion IL-6 levels were tested. There was no significant difference between median IL-6 levels in the NLD and LD groups before transfusions (p = 0.25). The median after transfusion IL-6 levels in the NLD and LD groups were 2.45 (0.60 - 7.80) pg/mL and 1.90 (0.90 - 5.00) pg/mL, respectively. There was no significant difference of IL-6 levels in two groups (p = 0.18). In the NLD group, a slight increase of after transfusion IL-6 level was identified, but a decrease of after transfusion IL-6 level was showed in the LD group. This study showed that PRC transfusions have no significant effect on IL-6 levels measured 1 hour after transfusion. Nonleucodepleted PRC transfusions resulted in a non-statistically significant increase in IL-6 levels compared to leucodepleted PRC transfusions.

Keywords: *PRC transfusion; nonleucodepleted;IL-6 level; malignancy recurrence*

1. Introduction

Hematological malignancies accounted for 6.5% of all cancer incidence and 2.4% of deaths worldwide in 2012 [1]. Hematological malignancies can be found in various places, with different geographical conditions, and include various ethnicities with varying ages of patients [2]. Case reports in Europe in 2005 recorded an estimated 230,000 new malignancy cases, consisting of leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, and myeloma, with an estimated death from the malignancy of 7% [3]. Meanwhile, case reports in Indonesia recorded that the prevalence of several types of leukemia, including AML, B-ALL, T-ALL and pre-B-ALL, at the National Cancer Hospital accounted for 51.4%, 19.7%, 14.6%, and 4.5%, respectively [4].

More than 40% of cancer patients have anemia. The number can increase to 90% in patients undergoing chemotherapy [5]. Data from the European Cancer Anemia Survey (ECAS), involving 15,000 patients from 24 cancer centers in 24 European countries, revealed that 39% of patients had anemia at initial observation. Patients who initially had no anemia developed anemia after undergoing cancer therapy with an incidence of 63% [6]. Studies at the Cipto Mangunkusumo national referral hospital reported anemia caused by iron deficiency, thalassemia, aplastic, hemolytic, and other causes with successive proportions of 14%, 54%, 16% and 16% [4].

Transfusion is one of the therapies administered to anemia patients. Currently, blood transfusions using blood products in the form of components are more common rather than whole blood. Strict criteria for selecting transfused blood components affect better outcomes [7]. Blood component products are preferred to reduce transfusion reactions. Current technological developments allow the separation of leukocytes from erythrocytes so that the number of leukocytes can be minimized. Separating leukocytes with a special filter produces Packed Red Cells (PRC) products with a leukocyte count of $< 5 \times 10^6$ per unit [8]. A blood product with minimal leukocyte counts is referred to as leucodepleted PRC. Leucocytes are a source of IL-6 so leucodepletion affects the amount of IL-6 in blood products [9]. Donor blood processing has been able to produce leucodepleted PRC, however, many blood transfusion services still use nonleucodepleted PRC.

Blood transfusion as a procedure that directly causes the recipient to be exposed to various kinds of antigens derived from blood products is inseparable from transfusion reactions, one of which is transfusion-related immunomodulation or TRIM [10,11]. Clinical evidence of TRIM in the form of cancer recurrence and the incidence of post-transfusion infection has been studied. Clinical evidence of cancer recurrence was reported in a study involving patients with high-grade soft tissue sarcoma undergoing chemotherapy, indicating that blood transfusion in this study was associated with increased recurrence [12]. Infection incidences were also found in patients undergoing spinal surgery and requiring transfusion. The rate reached 21% in patients who received allogeneic blood product transfusions [13].

Transfusion reactions, in this case, TRIM, are considered to be mediated by allogeneic mononuclear cells, mediators in plasma derived from leukocytes, and HLA peptides circulating in allogeneic plasma [14]. The mechanism of immunomodulation in transfusion remains widely incomprehensible [15], and the literature on TRIM remain limited. One of the important mediators in the immune system is IL-6 [16]. The mechanism of IL-6 related to post-transfusion cancer recurrence is considered, among others, through the angiogenesis stage in tumor growth. Studies on animals have shown that IL-6 stimulates neovascularization. The production of IL-6 observed in this study is considered part of the inflammatory response, as a consequence of leukocyte infiltration [17]. In addition, the role of IL-6 is also considered through anemia. IL-6 cytokines have an inhibitory effect on erythropoiesis in the bone marrow [18].

The effect of transfusion on elevated IL-6 levels in malignancy was reported in a study involving patients with colorectal cancer who were transfused during surgery. IL-6 levels increased both on the 7th day and the 14th day after surgery [18]. Other studies on IL-6 also showed that increased expression of IL-6 correlated with the stage of malignancy. A study on colorectal cancer revealed a significant (three times) increase in IL-6 mRNA expression at the late stage compared to that in the early stage ($p = 0.004$) [19].

2. Materials and Methods

A quasi-experimental design was applied in this study. The population in this study included 64 adult patients with hematological and nonhematological malignancies who required PRC transfusions and visited the outpatient care at the "Tulip" Integrated Cancer Department of Dr. Sardjito Central Public Hospital, Yogyakarta, Indonesia from June to August 2019. The inclusion criteria were adult patients, who were planned to receive a maximum of two PRC transfusions by the doctor in charge. Patients with fever and bleeding manifestations were excluded.

Malignancy patients were divided into two groups without randomization, i.e the leucodepleted (LD) group as the treatment group and the nonleucodepleted (NLD) group as the control group. The intervention was performed by administering blood units with leucodepletion compared to that with regular blood units. The same IL-6 level measurements were performed in both groups. Sampling was carried out consecutively. Those who were willing to participate signed an informed consent form, their blood sample was collected, then transfusions were performed. The treatment group received leucodepleted PRC transfusions while the control group received nonleucodepleted PRC transfusions. One hour after the transfusion, another blood sample was taken.

Leucodepletion was conducted using the Purecell® RC High Efficiency Leucocyte Removal Filter for Blood Transfusion of 1 Unit (EU) kit. Blood was stored for 10 minutes at 4°C before filtration. The leucodepletion in this study resulted in blood products with a leukocyte count of less than 2 x 10^5/unit. Furthermore, IL-6 tests were carried out using the kit from FineTest with the Enzyme-Linked Immune-Sorbent Assay (ELISA) sandwich technique.

The differences in IL-6 levels of before and after transfusion were tested with Wilcoxon-Signed Rank Test. Differences in increased IL-6 levels between the exposed and nonexposed groups were tested by Mann-Whitney Test. Statistical significance was expressed at $p < 0.05$.

3. Results and Discussion

Subject Characteristics

A total of 64 subjects were involved in this study with an age range of 26 to 93 years. The number of female was higher than male subjects, i.e 46 (71.87%) and 18 (28.13%), respectively. The subjects consisted of hematological and nonhematological malignancy patients. Hematological parameters showed anemia in both the NLD and LD groups. The subjects received transfusions of 1-2 units of nonleucodepleted or leucodepleted PRC. There was no significant difference in the storage period of the transfused PRC products (Table 1).

Table 1. Characteristics of the subjects based on leucodepletion group

Variables		NLD (n = 30)	LD (n = 34)	*p-value*
Age (years)	Median (min-max)	57 (27-74)	57 (26-93)	0.809*
Sex				
Female	n	20	26	0.384#
Male	n	10	8	
Hematology parameters				
Hemoglobin (g/dL)	Median (min-max)	9.8 (8.1-11.7)	9.2 (3.0-11.1)	0.004*
Hematocrit (%)	Median (min-max)	29.0 (23.6-34.2)	28.1 (9.4-35.2)	0.029*
Leukocytes (x10^3/uL)	Median (min-max)	6.9 (2.6-32.7)	5.9 (1.1-19.9)	0.135*
Platelets (x10^3/uL)	Median (min-max)	326 (88.1-649)	176 (10.4-674)	0.003*
Number of PRC transfused				
1 unit	n (%)	28 (93)	18 (53)	0.001#
2 units	n (%)	2 (7)	16 (47)	
Storage time (days)		4 (1-9)	4.5 (1-13)	0.230*

*Mann Whitney
#Chi-Square

IL-6 levels after transfusion

There was no significant difference between the median before transfusion IL-6 levels in the NLD and LD groups, i.e 2.40 (0.90 to 8.60) pg/mL and 2.75 (1.00 to 5.20) pg/mL, respectively(p = 0.25). 7.80) pg/mL and 1.90 (0.90 - 5.00) pg/mL, respectively. There was no significant difference of IL-6 levels in two groups (p = 0.18). In the NLD group, a slight increase of after transfusion IL-6 level was identified, but a decrease of after transfusion IL-6 level was showed in the LD group (Table 2).

Table 2 Before and after-transfusion IL-6 levels

	Median IL-6 levels in NLD group (pg/mL)	Median IL-6 levels in LD group (pg/mL)	P-value
Before transfusion	2.40 (0.90-8.60)	2.75 (1.00-5.20)	0.25
After transfusion	2.45 (0.60-7.80)	1.90 (0.90-5.00)	0.18

This study indicates that the PRC transfusions have no effect on IL-6 levels measured 1 hour after transfusion. Zhao et.al reported a study on 91 pediatric patients with acute lymphoblastic leukemia (ALL), which showed higher IL-6 levels in the transfusion group than that in the non transfusion group [20]. Another study of 45 transfusions using blood products with reduced leukocyte count showed a febrile reaction in six of the transfusions. High IL-6 levels were found in these patients after transfusion. However, in stored blood products, both thrombocyte concentrate and PRC, IL-6 levels were also found to increase [21].

The median IL-6 levels after transfusion in the NLD and LD groups reached 2.45 (0.60 to 7.80) pg/mL and 1.90 (0.90 to 5.00) pg/mL, respectively. There was no significant difference between IL-6 levels in the two groups (p = 0.18). It means that, in this study, leucodepletion did no effect on IL-6 levels. It is different from the results of a previous study on 64 patients who received PRP and Buffy Coat (BC) transfusions (the number of leukocytes in PRP products was higher than that in BC). Febrile reactions were more common in PRP transfusions than that in BC transfusions, i.e 9.3% and 2.7%, respectively (p = 0.007). IL-6 levels and the leukocyte counts correlated with a febrile reaction in the PRP group, while IL-6 levels were not detected in BC transfusions, which correlated with a very low number of leukocytes [22].

A study conducted by Hui Zhao et al. indicated that allogeneic blood transfusions can increase IL-6 and sIL-2R serum levels in ALL patients. In this study, IL-6 and sIL-2R serum levels in the study group were lower than those in the control group, which is consistent with the fact that children with ALL have decreased immunity. The study results showed that IL-6 serum levels increased significantly after allogeneic blood transfusion. IL-6 serum levels decreased gradually 12 weeks after the transfusions but remained higher than the pre-transfusion levels. It is similar to previous studies which showed that IL-6 serum levels increased after transfusion, indicating a decrease in the ability of the immune system to produce antibodies. Venous blood sampling was performed before transfusion & 4, 8, and 12 weeks after transfusion [23]. Another study, in a group of pediatric patients with allergic transfusion reactions, showed that IL-6 strongly correlated with the patients' status in the presence of infection, inflammation, and sensitivity [24].

Another further study revealed that IL-6 plays an important role in cancer pathogenesis, and components of the IL-6 signaling pathway, including IL-6, IL-6R, gp130, JAK and STAT3, have promising prospects as targets for cancer therapy.

Previous studies have reported the role of IL-6 as an endogenous pyrogen so that a febrile reaction can occur in relation to high levels of IL-6 in blood products, whereas in this study, there was no significant difference between the storage time of transfused PRCs in the NLD and LD groups. No differences in IL-6 levels were found in this study, possibly due to the relatively short observation

time, i.e 1 hour after transfusion. Therefore, further studies are required with a longer observation period to determine changes in IL-6 levels after transfusion.

4. Conclusion

PRC transfusions have no significant effect on IL-6 levels measured 1 hour after transfusion. Nonleucodepleted PRC transfusions resulted in a non-statistically significant increase in IL-6 levels compared to leucodepleted PRC transfusions.

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