



Original Article

Risk of Increased Post-Transfusion IL-8 Levels in Adult Patients with Malignancy Receiving Non-Leukodepleted Packed Red Cell Transfusions

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ABSTRACT

Background: Cancer is one of the primary causes of death, and almost > 40% of cancer patients experience anemia. Transfusion serves as one of the therapies given to anemic patients. Malignancy patients with decreased immune systems which are given blood component transfusion may cause transfusion reactions (Transfusion-related immunomodulation or TRIM), the mechanism of which is suspected to correlate to inflammatory cytokines, including IL-8. Blood products with a leukocyte count of $<5 \times 10^6$ per unit are called leukodepleted blood products. This study aimed to observe the incidence of increased IL-8 level in malignancy patients given leukodepleted (LD) and nonleukodepleted (NLD) Packed Red Cells (PRC) transfusions. The increased IL-8 level leads a possibility of recurrence and risk of infection.

Methods: This study was a quasi-experimental clinical trial conducted on 39 adult malignancy patients treated at Dr.Sardjito Hospital which were given leukodepleted and nonleukodepleted PRC transfusions, and the IL-8 levels were measured one hour before and after the transfusions.

Results: IL-8 levels in the NLD and LD groups increased significantly ($p < 0.05$), i.e. 13.25 (8.3-25.2) pg/mL and 9.1 (8.4-10.8) pg/mL before the transfusions, and 25.4 (9.9-72.6) pg/mL and 9.8 (9.1-11.6) pg/mL after the transfusions, respectively. It showed that the increase of IL-8 levels correlated to the given PRC transfusions. There was a significant difference in the median IL-8 levels of the LD and NLD groups before and after the transfusions. The relative risk of increased IL-8 levels in nonleukodepleted PRC transfusions was 4.0 (95% CI: 1.079-14.833).

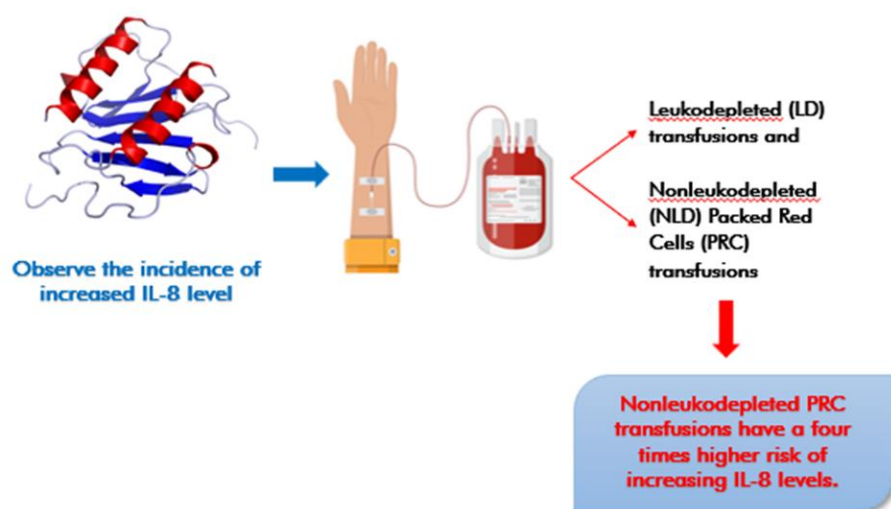
Conclusion: Nonleukodepleted PRC transfusions have a four times higher risk of increasing IL-8 levels than leukodepleted PRC transfusions.

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GRAPHICAL ABSTRACT



Introduction

Cancer is one of the primary causes of death worldwide. More than 60% of global new cancer cases and approximately 70% of deaths due to cancer occur annually in Africa, Asia, as well as Central and South Americas. According to Globocan data, in 2018, there were 18.1 million new cases of cancer, with a mortality rate of 9.6 million worldwide. One in five men and one in six women were affected by the disease, with a mortality rate of one in eight men and one in eleven women [1].

Hematological malignancy remains a global health issue. New cases of Leukemia, Hodgkin, and Non-Hodgkin Lymphoma (NHL), and Myeloma account for 8% of all new patient diagnoses in Europe, and an estimated 7% of these cancer patients die [2]. A study of 27,703 white patients and 2,059 black patients with Chronic Lymphocytic Leukemia (CLL) diagnosed in 1992-2007 in the USA found that in black patients, CLL appeared at a younger age and a more advanced stage. Furthermore, it shows that black patients have a lower life expectancy than white patients [3]. Case reports in Indonesia show that the prevalence of several types of leukemia, including AML, B-ALL, T-ALL, and pre-B-ALL, at the National Cancer Hospital reached 51.4%, 19.7%, 14.6%, and 4.5% [4].

Anemia is one of the most common hematological

disorders found in cancer patients in almost >40% of cases. It can also serve as an adverse prognostic factor [5] and affect patients with hematological malignancies, such as patients with Multiple Myeloma, Non-Hodgkin Lymphoma (NHL), Hodgkin Lymphoma, Chronic Lymphocytic Leukemia (CLL), and Myelodysplastic syndrome (MDS) [6].

Separating leukocytes with a special filter produces Packed Red Cell (PRC) products with a leukocyte count of $<5 \times 10^6$ per unit [7], referred to as leukodepleted PRC. Blood transfusion, as a measure that directly causes the recipient to be exposed to various kinds of antigens derived from blood products, cannot be separated from transfusion reactions, one of which is transfusion-related immunomodulation or TRIM. One of the clinical evidences of TRIM is post-transfusion infection [8]. In a meta-analysis study, hepatocellular cancer blood transfusion was associated with adverse clinical effects in patients undergoing surgery, including increased mortality, recurrence, and complications [9]. Transfer of allogeneic leukocytes by filtration on red blood cells and platelet products has been shown to significantly reduce FNHTR (Febrile Non-Hemolytic Transfusion Reactions), which can also cause refractory platelet transfusions [10].

Prestorage leukocyte depletion can reduce FNHTR frequency and severity. This study was conducted on 3,989 bags of whole blood stored for a maximum of 4 hours at 20 °C, which was then processed into erythrocyte and plasma-rich platelet cell fractions using a leukocyte filter [11]. A study, which was conducted from January 2001 to December 2009, divided 583 patients into three groups: group A who did not receive any transfusion, group B who received transfusions of two to three blood units, and group C who received transfusions of three blood units or more. It was found that group C had lower 5-year overall survival and higher recurrence rate than groups A and B [12]. Storage time of blood components plays a major role in the accumulation of cytokines that can cause transfusion reactions. Cytokines, such as IL-6, IL-8, and TNF-alpha, play an important role in FNHTR [13]. This study aimed to observe the incidence of increased IL-8 level in malignancy patients given leukodepleted (LD) and nonleukodepleted (NLD) Packed Red Cells (PRC) transfusions.

Materials and Methods

This study was conducted at Dr. Sardjito Hospital from June to August 2019 using a quasi-experimental design. The subjects were one-day care (ODC) adult patients who needed PRC transfusions, were treated at Dr. Sardjito Hospital, met the inclusion criteria, and were willing to participate in the study. Patients with active infection, chronic inflammatory disease, history of previous transfusion, history of taking immunosuppressive drugs, and chronic anticoagulants were excluded from this study.

The subjects were divided into two groups, those receiving leukodepleted PRC transfusions and those receiving nonleukodepleted PRC transfusions. Their blood samples were collected before the transfusions to measure the initial IL-8 levels, and then their blood samples were collected one hour after the transfusions to measure the second IL-8 levels. The results of IL-8 level measurements before and after the transfusions were analyzed to determine the increase in IL-8 levels and the differences

between the increase in IL-8 levels of the nonleukodepleted and the leukodepleted groups. Differences between the increase in IL-8 levels of the nonleukodepleted and the leukodepleted groups were tested using the independent sample t-test if the data were normally distributed or the Mann-Whitney test if the data was not normally distributed. Differences in IL-8 levels before and after the transfusions were tested with the paired sample t-test if the data were normally distributed or the Wilcoxon-Signed Rank test if the data was not normally distributed. Relative risk (RR) was calculated using data on the proportion of IL-8 levels that elevated in the nonleukodepleted and leukodepleted groups. Statistical significance was expressed at $p < 0.05$. Ethical clearance was issued by the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, through an ethical clearance letter no. KE/FK/0979/EC/2018.

Results and Discussion

This study involved 39 subjects with the age range of 28-74 years old. The subjects were divided into two groups i.e. 26 adult malignancy patients who received nonleukodepleted (NLD) PRC transfusions and 13 patients who received leukodepleted (LD) PRC transfusions. There was no significant difference in patient age in the NLD and LD groups (Table 1).

There were 28 female patients (71.7%) and 11 male patients (28.3%) involved in this study. Hematological parameters revealed anemia in both the NLD and LD groups. The levels of hemoglobin (Hb), hematocrit (Ht), and leukocytes in the NLD and LD groups were not significantly different. A significant difference was found between the platelet levels of the NLD and LD groups ($p < 0.05$). It is similar to a study conducted by Ren Yi *et al.* (2003), who found a significant correlation between IL-8 levels and platelet counts ($r = 0.386$; $p = 0.009$). The IL-8 receptor is present in the platelets, and the platelets contain IL-8. It theoretically describes the important role of platelets in the storage of VEGF in circulation.

Table 1: Characteristics of subjects based on the NLD and LD groups

Variables		NLD (n = 26)	LD (n = 13)	P-value
Age (years old)	Median (min-max)	54.5 (37-74)	57 (28-68)	0.976*
Sex				
- Female	n (%)	19 (73.80)	9 (69.23)	0.801#
- Male	n (%)	7 (26.20)	4 (20.77)	
Hematology parameters				
- Hemoglobin (g/dL)	Median (min-max)	9.68 (6.6-11.1)	9.80 (8.2-11.2)	0.654*
- Hematocrit (%)	Median (min-max)	28.9 (24.2-33.2)	28.8 (19-35.2)	0.893*
- Leukocytes (x10 ³ /uL)	Median (min-max)	8.10 (2.6-32.7)	7.25 (3.69-19.1)	0.541*
- Platelets (x10 ³ /uL)	Median (min-max)	338.5 (81.9-649)	174 (72.5-674)	0.010*
Diagnosis				
Multiple Myeloma	n (%)	2 (7.7)		
MPD	n (%)		1 (7.7)	
NHL	n (%)	5 (19.2)	2 (15.4)	0.795#
Adeno ca esofagus	n (%)		1 (7.7)	
Basal cell ca	n (%)	1 (3.8)		
Ca caecum	n (%)	1 (3.8)		
Ca cervix	n (%)	1 (3.8)	2 (15.4)	
Ca colon	n (%)	1 (3.8)		
Ca corpus uteri	n (%)	1 (3.8)		
Ca gaster	n (%)		1 (7.7)	
Ca mammae	n (%)	10 (38.5)	2 (15.4)	
Ca mandibula	n (%)	1 (3.8)		
Ca ovarium	n (%)	1 (3.8)		
Ca pancreas	n (%)		1 (7.7)	
Ca sigmoid	n (%)		1 (7.7)	
Ca unknown primer origin	n (%)		1 (7.7)	
Leymiosarcoma	n (%)	1 (3.8)		
NPC	n (%)		1 (7.7)	
Signet ring cell ca	n (%)	1 (3.8)		
Number of transfusion				
- 1 unit	n (%)	23 (88.46)	10 (55.5)	0.05#
- 2 units	n (%)	3 (11.54)	8 (44.5)	
Storage time (days)	Median (min-max)	4 (1-9)	4 (3-6)	0.486*

*Mann-Whitney.

Chi-Square.

Table 2: Median IL-8 levels (pg/mL) in the nonleukodepleted and leukodepleted groups before and after the transfusions

Groups (n)	Before transfusion Median (min-max)	After transfusion Median (min-max)	p
Nonleukodepleted (26)	13.25 (8.3-25.2)	25.40 (9.9-72.6)	0.001*
Leukodepleted (13)	9.10 (8.4-10.8)	9.80 (9.1-11.6)	0.001*

*Mann-Whitney.

VEGF is vital for angiogenesis, and increased VEGF expression is associated with tumor progression and intrahepatic metastases in HCC. Platelets play an important role in the storage and transport of circulating IL-8 in cancer patients.

The subjects in this study included 10 adult patients diagnosed with hematological malignancy and 29 patients diagnosed with non-hematological malignancy. Subjects with the highest prevalence of non-hematological malignancies were breast cancer patients, i.e. 12 (30.7%). According to The World Health Organization (WHO) (2018), breast malignancy constitutes 30.9% of all malignancies in women. According to GLOBOCAN data, the most common malignancy in both sexes in Indonesia is breast malignancy, accounting for 16.7% of the total cases. Subjects with the highest prevalence of hematological malignancies were Non-Hodgkin's Lymphoma (NHL) patients, amounting to 7 people (17.94%).

In this study, the subjects received 1-2 units of nonleukodepleted or leukodepleted PRC transfusions, and there was no significant difference in the number of PRC transfused. Furthermore, there was no significant difference between the storage time of the transfused PRC products for NLD and LD groups (>0.05).

The IL-8 levels of NLD and LD groups were measured before and after the transfusions, and the increase in IL-8 levels was assessed. The measurement results showed that there was an increase in IL-8 levels in the NLD and the LD groups after the transfusions, as listed in [Table 2](#). This study also found a significant difference in IL-8 levels in both groups after the transfusions, which was higher than those before the transfusions ($p < 0.05$) ([Figure 1](#)).

Increased IL-8 levels have been studied and are associated with an increased stage of malignancy. In hepatocellular carcinoma (HCC), increased IL-8 levels were significantly associated with tumor size and tumor malignancy stage, namely in patients with tumor size of >5 cm ($p = 0.016$), the presence of tumor capsule ($p = 0.0035$), the involvement of venous invasion, and the advanced stage of the disease ($p = 0.037$). The IL-8 serum levels of grade III or IV patients were

found to be significantly higher than those of grade I or II patients ($p = 0.037$), with a risk ratio of 4.09. However, the IL-8 serum levels of grade I or II patients were found to be significantly different from those of healthy people. There was no significant difference between the IL-8 levels of male and female hepatocellular carcinoma patients in terms of clinicopathological features [14].

Increased IL-8 levels in breast cancer patients are associated with a higher grade of breast cancer. According to the measurement of IL-8 levels in breast cancer patients according to the clinical stage (based on the TNM classification), it was found that the median IL-8 levels of stage III patients were 1.4 times higher than those of grade II patients. It can be utilized for patient prognosis so that more aggressive management can be performed. In addition, this study compared breast cancer patients with healthy controls, which found a statistically significant difference ($p < 0.05$). The IL-8 levels of the breast cancer patients and the control group reached 40.1 (7.8-76.0) and 5.2 (3.9-8.0), respectively [15].

The IL-8 levels of the NLD group did not differ statistically before the transfusions, but were significantly higher after transfusion than those of the LD groups. It showed that the increase of IL-8 levels correlated to PRC transfusions.

Leukodepleted blood products have a lower number of leukocytes than nonleukodepleted blood products. Leukocytes are a source of cytokines, so blood products with reduced leukocytes have reduced cytokine levels. A study on red cell concentrates from 30 healthy donors, consisting of 29 male and 1 female donors, found an average leukocyte count of $3.68 \pm 1.1 \times 10^9$ /unit in PRC. In contrast, in products with further processing, buffy-coat depleted PRC, the average leukocyte count was lower i.e. $5.5 \pm 1.6 \times 10^8$ /unit. IL-8 cytokine levels in PRC increased progressively during storage. On day 0, the levels ranged from 2-86 pg/mL, increasing to 480 pg/mL on day 28. There were no PRC samples with normal cytokine levels after day 14. IL-8 cytokine levels increased significantly at 0-28 days of storage ($p = 0.000$) [13].

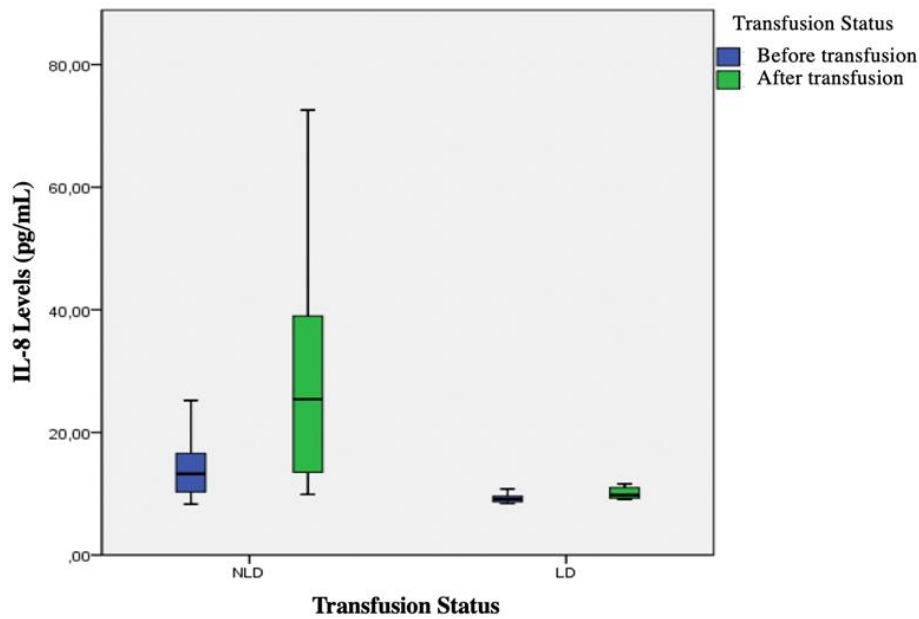


Figure 1: Differences between the increase in the IL-8 levels of NLD and LD groups before and after PRC transfusions

Table 3: Relative risk (RR) of increased IL-8 levels in nonleukodepleted (NLD) PRC transfusions

Groups (n)	IL-8 Levels Increased	IL-8 Levels not Increased	RR (95% CI)
NLD (26)	16	10	4.00 (1.079-14.833)#
LD (13)	2	11	

#Chi-Square; $p < 0.05$

Cytokines accumulate in stored whole blood. Pre-storage leukocyte depletion has been shown to reduce cytokine levels, which can reduce the incidence of febrile non-hemolytic transfusion reaction (FNHTR). As a leukocyte filter, the component separation process can be performed up to 24 hours after donation. It is suspected that inflammatory cytokines accumulate during this period. In this study, plasma samples were taken at 4 hours, 10 hours, and 20 hours after donation, and then cytokine measurements found an increase in IL-8 levels of >20 times. Pre-storage leukocyte filters within 10 hours after donation will reduce the concentration of pyrogen mediators [11].

Cytokines, such as IL-8, are actively synthesized and released along the storage of platelets and red blood cells. A linear correlation occurred between cytokine levels, leukocyte content, and storage time. Cytokines accumulated faster at 22 °C than that at 4 °C. Pre-storage leukocyte reduction prevents cytokine accumulation and is associated with significantly less FNHTR. *In vitro*,

when plasma is exposed to a blood plastic bag on thrombocyte concentrate products, the complement will be activated, stimulating monocytes on platelets to produce cytokines. Several groups showed that reducing leukocytes in PRC and platelet components to 5×10^6 per blood bag component before storage (pre-storage leukoreduction) prevented the IL-8 accumulation and proinflammatory cytokines, such as IL-1 β , IL-6, and TNF [16].

Up to now, there have been no published normal IL-8 levels. A study in healthy individuals reported normal IL-8 levels of 0-12 pg/mL [13]. If the IL-8 level range is applied in this study, 17 (43.5%) patients in this study are found to be in the normal range. After the transfusions, 18 (51.2%) patients, consisting of 16 patients from the NLD group and 2 patients from the LD group, had increased IL-8 levels. This study observed patients with malignancy and found that the median IL-8 level of the NLD group was significantly higher than that of the LD group.

The IL-8 levels of LD and NLD groups were assessed. The relative risk of increased IL-8 levels in nonleukodepleted PRC transfusions was 4.0 (95% CI: 1.079-14.833), and the risk is significant (Table 3). These results support the suggested practices to use LD PRC transfusion in adult malignancy patients.

Conclusion

Non-leukodepleted PRC transfusions in adult malignancy patients had four times higher risks of increased IL-8 levels than leukodepleted PRC transfusions, thereby increasing the risk of malignancy recurrence and transfusion reactions.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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