Pharmacogn J. 2022; 14(6): 778-781
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
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Original Article

Increased Risk of Tumor Necrosis Factor-Alpha Levels in Adult Patients with Malignancy Receiving Non-Leucodepleted Packed Red Cells Transfusion

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History

- Submission Date: 08-10-2022;
- Review completed: 16-11-2022;
- Accepted Date: 23-11-2022.

DOI : 10.5530/pj.2022.14.167

Article Available online

http://www.phcogj.com/v14/i6

ABSTRACT

Background and Objectives: Blood transfusion in patients with malignancy may evoke transfusion reactions. Leukocyte, as a major producer of cytokines, including Tumor Necrosis Factor-alpha (TNF-alpha), is considered to correlate to transfusion reactions. This study aims to determine the risk of increased TNF-alpha in adult patients with malignancy who received non-leucodepleted (nLD) erythrocyte transfusion compared to those receiving leucodepleted (LD) Packed Red Cells (PRC) transfusion. **Materials and Methods:** This quasi-experimental study was conducted on adult patients with malignancy who required PRC transfusion and underwent outpatient treatment. The patients were divided without randomization into nLD and LD groups, and then their pre-transfusion TNF-alpha levels and the post-transfusion changes were examined. **Results:** This study included thirty-one patients fulfilling the inclusion criteria. The TNF-alpha levels in nLD and LD groups after transfusion increased significantly ($p < 0.05$), i.e., from 0.81 (0.2 - 4.2) pg/mL and 1.7 (0.15 - 6.3) pg/mL to 10.1 (1.4 - 28.9) and 5.9 (0.95 - 12.9) pg/mL. There was no significant difference in the pre-transfusion median TNF-alpha levels between the nLD and LD groups ($p = 0.122$). However, the post-transfusion median TNF-alpha levels of the nLD group were significantly higher ($p = 0.024$). It indicated that the increase in TNF-alpha levels is associated with nLD blood products transfused. The Relative Risk of the increased TNF-alpha levels in nLD-PRC transfusion was 2.01 (95% Confidence Interval: 1,153-3,502). **Conclusion:** nLD-PRC transfusion poses a 2.01 times risk for increased TNF-alpha levels compared to LD-PRC transfusion.

Key words: Malignancy, Non-leucodepleted, PRC transfusion, Relative risk, TNF-alpha.

INTRODUCTION

Malignancy remains a major health problem. Malignancy cases are observable in many places with different geographical conditions, covering various ethnicities and ages of patients. The incidence and mortality rate of malignancy increases rapidly along with population growth.[1,2] Of all worldwide malignancy cases in 2012, hematologic malignancies accounted for 6.5% of cases and resulted in 2.4% deaths.[3,4] One of the malignancy complications is anemia. More than 40% of patients with malignancy are anemic, which can increase to 90% in patients undergoing chemotherapy.[5] Data from the European Cancer Anaemia Survey (ECAS) involving 15,000 patients from 24 cancer centers in 24 European countries showed 39% of patients were anemic at baseline. More than half (63%) of patients who were not anemic and subsequently underwent cancer therapy were anemic.[6,7]

Transfusion is one of the measures to treat anemia, with the global transfusion number reaching 80 million units per year.[8] However, transfusion is inseparable from the risk of transfusion reactions. Blood transfused results in the recipient being exposed to various antigens of the blood product. Such exposure creates conditions in blood circulation that evoke various immune responses.[9,10] Many cytokines play a role in transfusion reactions, one of which is TNF alpha.[11]

In relation to transfusion reactions, strict criteria for selecting the transfused blood components result in better outcomes.[12,13] Current technological developments make it possible to separate leukocytes, allowing the depreciation of the leukocytes in transfusion products. Leukocyte separation with a special filter produces PRC products with a leukocyte count of < 5 x 106 per unit.[14] Blood products with minimal leukocyte counts are referred to as leucodepleted PRC. It is important to observe the difference and the risk of increased TNF-alpha levels in adult patients with malignancy who received non-leucodepleted PRC transfusion compared to those receiving leucodepleted PRC transfusion.

METHODS

Study design

This quasi-experimental study was conducted at Clinical Laboratory Installation, "Tulip" Integrated Cancer Installation and Blood Transfusion Service Unit, Dr. Sardjito General Hospital and at the Department of Clinical Pathology and Laboratory Medicine, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada (FK-KMK UGM), involving adult patients with malignancy who required PRC transfusion and underwent outpatient treatment. Ethical clearance was issued by the Ethics Committee for Medical and Health Research, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. Informed consent was obtained from all participants. This study included adult patients with hematology malignancy and non-hematology malignancy who required PRC transfusion and underwent outpatient treatment. Those who were

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suffering from fever and bleeding manifestations were excluded. The blood component transfused was PRC. The patients were divided without randomization into nLD dan LD groups, and then their pre-transfusion TNF-alpha levels and the post-transfusion changes were examined.

Study procedures

Blood was available from donors, which was then processed into PRC. Some PRC units were then filtered using the Purecell® RC High-Efficiency Leukocyte Removal Filter for Blood Transfusion of 1 Unit (EU) kit. The blood was stored for 10 min at 4 °C before filtration. Leukodepletion in this study resulted in blood products with a leukocyte count of less than 2 x 10^5/unit.

Blood was drawn from patients consecutively, then prepared for crossmatch and TNF alpha tests. If the crossmatch test result was compatible, the blood transfusion was performed. The administration of the LD-PRC unit was decided according to the request of the referring doctor and the availability of the bloodstocks. If the appropriate bloodstock was unavailable, it would be communicated to the referring doctor for routine blood transfusion, i.e., nLD-PRC. One hour after the transfusion, a second blood draw was performed and then prepared for the TNF alpha test. The period of one hour was determined by considering the willingness of the participants, since they were outpatients, to follow the study.

TNF-alpha tests were performed using a kit from FineTest using the sandwich Enzyme-Linked Immune-Sorbent Assay (ELISA). In this technique, Anti-TNF-alpha antibodies were available and pre-coated on 96-well plates. Biotin-conjugated anti-TNF-alpha antibodies worked to detect antibodies bound to TNF-alpha. The addition of HRP-Conjugated Streptavidin (SABC) served to catalyze the substrate 3,3',5,5'-Tetramethylbenzidine (TMB), which caused a blue color and will turn yellow with the addition of a stop solution. Yellow Optical Density (OD) is proportional to TNF-alpha levels.

Statistical analysis

The difference in the pre- and post-transfusion TNF-alpha levels was tested with either Paired-Samples t Test if the data was normally distributed or Wilcoxon-Signed Rank Test if the data distribution was not normally distributed. The difference in the increase of TNF-alpha levels between the nLD group and the LD group was tested with either Independent Samples t Test if the data was normally distributed or Mann-Whitney Test if the data was not normally distributed. The Relative Risk was calculated using data on the proportion of TNF-alpha levels that increased in the nLD and LD groups. The statistical difference was considered significant at $p < 0.05$.

RESULTS

A total of thirty-one patients participated in this study, with an age range of 27 to 74 years old. The number of female patients was more than that of male patients, namely 21 (67.7%) and 10 (32.2%) people, respectively. The patients received non-leucodeplegic PRC (nLD) or leucodeplegic PRC (LD) transfusion. The number of patients in the two groups was almost the same, namely 16 patients receiving non-leucodeplegic PRC transfusions and 15 patients receiving leucodeplegic PRC transfusions. The proportion of female and male patients in the nLD group was not significantly different from that in the LD group. The mean age of the patients in the two groups was also not significantly different (Table 1).

The measurement of post-transfusion TNF-alpha levels in the nLD group showed a significant increase ($p<0.05$) compared to the pre-transfusion levels. Similarly, the LD group also showed a significant increase in TNF-alpha levels ($p < 0.05$) (Table 2).

The measurement results of the pre-transfusion TNF-alpha levels in the nLD group compared to those in the LD group indicated no significant difference ($p = 0.122$). However, the post-transfusion TNF-alpha levels in the nLD group were significantly higher ($p = 0.024$) than those in the LD group (Figure 1).

This study's Relative Risk (RR) calculation applied a cut-off value of 3, meaning that it was considered increasing if the post-transfusion TNF-alpha level was at least three times higher than the pre-transfusion level. The results indicated that nLD-PRC transfusion had a 2.01 times risk of increased TNF-alpha levels compared to LD-PRC transfusion, and the risk was significant (Table 3).

DISCUSSION

This study measured TNF-alpha levels in the nLD and LD groups. The pre-transfusion TNF-alpha levels of the nLD group and the LD group

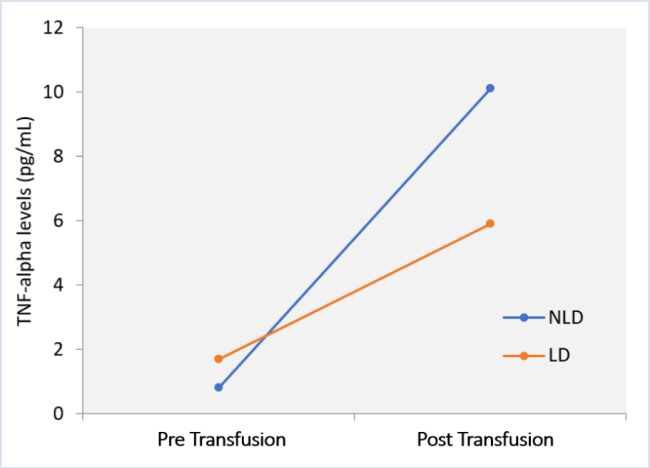

Figure 1: The difference in the increase of TNF-alpha levels in the nLD and LD groups.

Table 1: Characteristics of the study subjects based on the leukodepletion groups.

Variable	nLD (n=16)	LD (n=15)	p-value
Age (year)(median)	62 (27-74)	54(28-70)	0.271
Sex			
−Female	9	12	
−Male	7	3	
Hematological parameters			
−Hemoglobin (g/dL)	9.7	9.1	0.166
−Hematocrit (%)	28.9	28.3	0.374
−Leukocytes (x10^3/uL)	8.66	5.89	0.010
−Platelets (x10^3/uL)	339	211	0.010
Diagnosis			
−Hematologic malignancy	4 (25%)	4 (26%)	
−Non-hematologic malignancy	12 (75%)	11 (74%)	
Number of transfusions			
−1 unit	15	5	
−2 units	1	10	
Length of storage (day) (median)	3 (1-9)	4(3-8)	0.18

Table 2: Pre- and post-transfusion median TNF-alpha levels.

Groups (n)	Pre-transfusion (pg/mL)	Post-transfusion (pg/mL)	p-value
nLD (16)	0.81 (0.2 – 4.2)	10.1 (1.4 – 28.9)	0.000
LD (15)	1.7 (0.15 – 6.3)	5.9 (0.95 – 12.9)	0.001

in this study were not significantly different (p=0.122). However, the post-transfusion TNF-alpha levels in the nLD group were significantly higher (p=0.024) than those in the LD group. The post-transfusion TNF-alpha levels increased significantly in both groups, indicating that the increase in TNF-alpha levels correlated to transfusion. The post-transfusion TNF-alpha levels in the nLD group were significantly higher than those in the LD group. Meanwhile, the baseline or pre-transfusion TNF-alpha levels in the nLD group and those in the LD group were not different, indicating that the increase in TNF-alpha levels was also associated with filtration or non-filtrarion on the blood products.

The increase in TNF-alpha levels has been studied, and the results indicated it was associated with increased malignancy. A study on breast cancer patients reported that increased TNF-alpha levels were associated with higher malignancy grades. Measurements based on histological grade indicated higher TNF-alpha levels at grade 3, which were 1.56 times higher than those in grade 1. Measurements based on TNM staging also revealed higher TNF-alpha levels at stage T4dN2M0, i.e., 2 times higher than those at stage T3N1M0.[15]

Increased TNF-alpha levels have also been reported in a study on oral squamous cell carcinoma patients. The TNF-alpha levels in the carcinoma condition were 1.4 times higher than those in the premalignant condition. Analysis of the carcinoma group based on clinical stage showed that TNF-alpha levels at stage IV were 2.74 times that those at stage I.[16]

The increased stage of malignancy indicates the progression of the disease, i.e. the growth and spread of tumors. Cancer progression can occur in both untreated and treated patients. Even patients who have experienced remission remain at risk of recurrence; cancer cells that have not been detected and now have grown to a detectable stage. The association between an increase in TNF-alpha levels and an increase in the stage of malignancy is explained, among others, by the fact that cancer cells produce TNF-alpha continuously in malignancy.[17] We also found the possible association of TNF-alpha with malignancy from studies on endometriosis patients, reporting excessive angiogenic activity in endometriosis patients,[18] and peritoneal fluid TNF-alpha levels were higher than those in patients without endometriosis.[19] Angiogenesis is essential for cancer cell growth, and *in vitro* studies revealed that allogeneic leukocytes were associated with increased angiogenesis. The results of this study showed an increase in the post-transfusion TNF-alpha levels; thus, it can be considered to have a prognosis of recurrence.

The calculation of the Relative Risk (RR) in this study showed that non-leucodepleted transfusion had a 2.01 times risk of increased TNF-alpha levels compared to leucodepleted transfusion, and the risk was significant. This result was similar to those of previous studies. A study involving 520 patients with malignancy reported that transfusion was associated with a higher recurrence rate of malignancy with an Odds Ratio of 1.6. The difference in the recurrence rate of malignancy in the transfused and non-transfused groups was statistically significant.[20] Another study on 194 patients with malignancy with a 5-year follow-up also concluded that transfusion was associated with a higher recurrence rate of malignancy. The recurrence rate of malignancy in the study was 53.09% in the transfused group compared to 19.59% in the non-transfused group, and the difference was significant (95% CI: 2.954-7.309).[21] In terms of recurrence period, a study on ovarian cancer also concluded that transfusion was associated with cancer progression and cancer recurrence. The study involved 136 stage III ovarian cancer patients; it was found that the recurrence period in the transfused group was significantly shorter (p = 0.03) than that in the non-transfused group; 11 (18-14) months compared to 17 (6-27) months.[12]

We have studied the post-transfusion risk of increased TNF-alpha levels using leucodepleted PRC compared to that using non-leucodepleted PRC. Nevertheless, we had limited time and funds. The available leukocyte filters and TNF-alpha reagents were limited, as well as access to data on the length of illness and duration of therapy. For this reason, the data obtained and the interpretation of the data in this study are inseparable from these limitations.

CONFLICTS OF INTEREST

The authors declare that they do not have any conflicts of interest with regard to this work.

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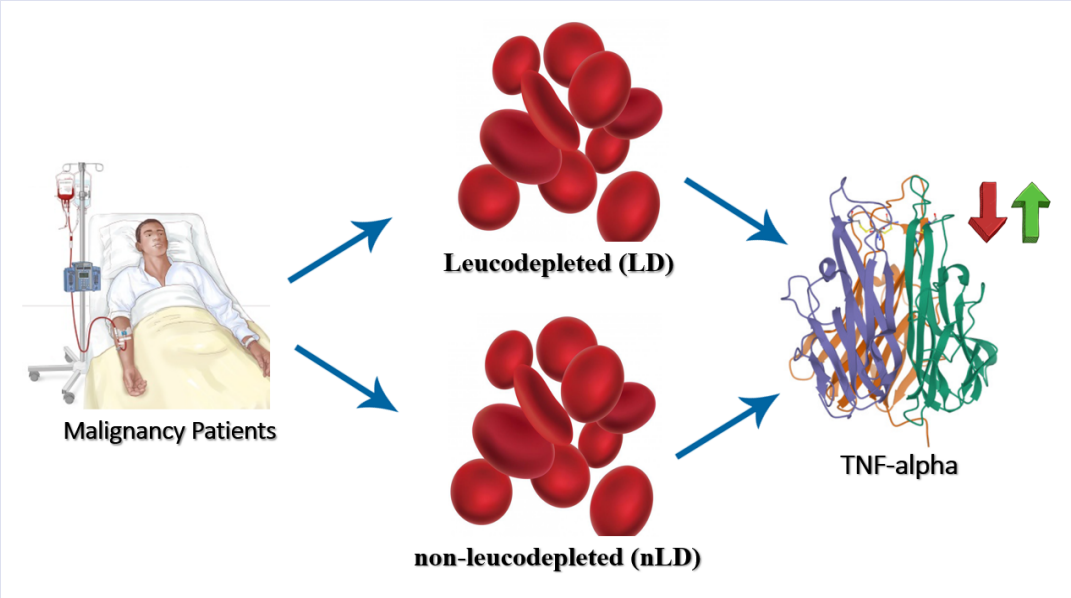
GRAPHICAL ABSTRACT



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Cite this article: ATriyono T, Jati BHP, Sukorinic U. Increased Risk of Tumor Necrosis Factor-Alpha Levels in Adult Patients with Malignancy Receiving Non-Leucodepleted Packed Red Cells Transfusion. Pharmacogn J. 2022;14(6): 778-781.