ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
June 2025; 24(3):304-312.
DOI:10.18502/ijaai.v24i3.18681

Evaluation of T-cell Function after Blood Transfusion in Patients Undergoing Coronary Artery Bypass Grafting

Nasim Golafshani[1], Narges Golafshani[2], Ali Reza Mohseni[3], Mahboubeh Mohseni[4,5], Ahmad Najafi[1], Shervin Ziabakhsh-Tabari[5], Hossein Asgarian-Omran[2], Mohsen Tehrani[2], and Ali Akbar Pourfathollah[2]

[1] Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[2] Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
[3] Department of Laboratory Sciences, School of Allied Sciences, Mazandaran University of Medical Sciences, Sari, Iran
[4] Fatemeh Zahra Hospital, Mazandaran University of Medical Sciences, Sari, Iran
[5] Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Received: 24 January 2024; Received in revised form: 7 April 2024; Accepted: 12 May 2024

ABSTRACT

Blood transfusion is associated with increased mortality and morbidity. This study aimed to determine the effect of blood transfusion on T-helper 1 (T_H1), T_H2, and T_H17 function in patients undergoing coronary artery bypass grafting (CABG).

Two blood samples were obtained from patients undergoing CABG, before and 14 days after surgery. Production of interleukin (IL)-2, IL-4, interferon (IFN)-γ, IL-17A, and IL-10 by $CD4^+$ T cells was measured using enzyme-linked immunosorbent assay (ELISA). mRNA expression of T-box expressed in T cells (*T-bet)*, GATA binding protein 3 (*GATA3)*, RAR-related orphan receptor-γ (*ROR-γt),* signal transducer and activator of transcription 3 *(STAT3), STAT4*, and *STAT6* were measured using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR).

mRNA expression of T-bet and *STAT4* showed a significant decrease after blood transfusion. However, the concentration of IFN-γ in the culture supernatant showed no significant difference after blood transfusion. mRNA expression of *GATA3* and *STAT6* showed a significant decrease after blood transfusion. However, the concentration of IL-4 in the culture supernatant showed no significant difference after blood transfusion. mRNA expression of *ROR-γt* showed no significant decrease after blood transfusion; however, the expression of *STAT3* and the concentration of IL-4 in the culture supernatant did significantly decrease following blood transfusion. IL-10 production increased significantly postoperatively.

Decreased T_H1, T_H2, and T_H17 signaling pathway activity and increased IL-10 concentration indicate an immunomodulatory effect on the immune system after blood transfusion.

Keywords: Blood transfusion; Coronary artery bypass; Cytokines; Immunomodulation; T-lymphocytes; T_H1 cells; T_H2 cells T_H1; T_H2

Corresponding Authors: Ali Akbar Pourfatholah, PhD; Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran. Tel: (+98 912) 3548 650, Fax: (+98 21) 3738 330, Email: pourfa@modares.ac.ir

Mohsen Tehrani, MD, PhD; Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran. Tel: (+98 11) 3354 3764, Fax: (+98 11) 3354 3249, Email: drmtehrani@gmail.com

INTRODUCTION

Blood transfusion during coronary artery bypass grafting (CABG) is an unavoidable part of surgery protocol. However, blood transfusion has a lifesaving effect for patients undergoing CABG, but there are some adverse events including infection, increasing length of hospital stay, episodes of atrial fibrillation, acute renal failure, and stroke.[1,2] On the other side, the number of blood products negatively is associated with CABG outcome.[3] In this regard, attention has focused on the reduction the complications and mortality in CABG following blood transfusion.[4,5]

One of the main complications of blood transfusion is immune suppression. Red blood cell (RBC) products contain mediators that interact with immune cells and modulate the immune system.[6] Despite approaches to reducing the immunomodulatory effect of blood transfusions such as leukoreduction, and plasma depletion, modulating the immune system remains challenging, especially in CABG. The release of heme and hemoglobin during blood storage alters the macrophage from M1 to M2 phenotype. M2 phenotype is associated with interferon-γ (INF-γ) secretion and nitric oxide synthesis which causes the inhibition of the immune system.[8] On the other side, blood transfusion inhibition of the immune system during cardiac surgery causes alteration in the helper T cell (T_H)1/T_H2 balance.[9,10] The weakened immune system following CABG is accompanied by increasing infection, impairment of pulmonary function, and mortality.[11] Several factors were demonstrated for immunomodulation following CABG, however, the exact mechanism of immunomodulation following blood transfusion is not fully understood. Hence, understanding the exact mechanism of the immunomodulatory effect of blood transfusion in CABG can help improve surgical outcomes.

MATERIALS AND METHODS

Study Population

Patients who were referred to the Fatemeh Zahra Hospital for CABG were evaluated for the current study. The main inclusion criteria were receiving a blood transfusion during the CABG procedure. Patients with immune deficiency and CABG patients without blood transfusion were excluded. Additionally, the function of immune system cells can be evaluated by measuring cytokines. However, in the present study, we evaluated gene expression to maximize the reliability of results.

The inclusion criteria were age between 40 and 80 years, undergoing CABG with a history of coronary artery disease, use of heart and lung pumps during surgery, receiving 1 or 2 packed cell units, and 3 to 5 platelet units during or within 24 hours after surgery. Additionally, patients with a history of chronic viral infections, including hepatitis B virus, hepatitis C virus, human immunodeficiency viruses, autoimmune diseases, immune deficiencies, cancers, or the use of any immunosuppressive drugs were excluded from the study.

The study was approved by the local research ethics committee and written informed consent was obtained from all twenty patients. Two peripheral blood samples were taken from each subject, 1 before the surgery, and another 3 weeks after surgery.

Cell Culture and Stimulations

Peripheral blood mononuclear cells (PBMCs) were isolated from fresh peripheral blood using Lymphodex (Inno-Train, Germany) density centrifugation according to the manufacturer's instructions. Next, $CD4^+$ T cells were positively isolated from the PBMCs using a magnetic beads-conjugated anti-CD4 monoclonal antibodies (mAbs) kit (Miltenyi Biotec, Germany). The purity of magnetic-activated cell sorting (MACS)-separated $CD4^+$ T cells was measured using a dual-color flow cytometric analysis with anti-CD3-phycoerythrin (PE) and anti-CD4-fluorescein isothiocyanate (FITC) (both from BioLegend, USA) (Figure 1). To evaluate T-cell proliferation and cytokine production, isolated $CD4^+$ T cells (2×10^5/well) were cultured in 200 µL of Roswell Park Memorial Institute (RPMI)-1640 medium containing penicillin (100 IU/mL), streptomycin (100 µg/mL), and 10% (v/v) fetal calf serum (FCS) (Biosera, Nuaille, France) and stimulated with combination of phorbol 12-myristate 13-acetate (PMA)/ionomycin cocktail (final concentration of 0.081 µM PMA and 1.34 µM ionomycin, eBioscience, USA) for 6 hours at 37°C with 5% CO_2.

Gene Expression Analysis

Total RNA was isolated from 1×10^6 PBMCs using the FavorPrep Blood/Cultured Cell Total RNA Mini Kit (Favorgen, Taiwan) based on the manufacturer's protocol. The quantity and quality of isolated RNA were confirmed by a nano-spectrophotometer and

electrophoresis, respectively. Complementary DNA (cDNA) was synthesized using the Yekta Tajhiz Azma cDNA synthesis kit (Yekta Tajhiz Azma, Tehran, Iran). Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was performed using the Applied Biosystems 7500 Fast Real-Time PCR instrument (ABI, Thermo Fisher Scientific, USA) using Real Q Plus 2x Master Mix (High Rox, Ampliqon, Denmark) and specific primers for T-box expressed in T cells (*T-bet)*, GATA binding protein *3 (GATA3)*, RAR-related orphan receptor-γ *(ROR-γt)*, signal transducer and activator of transcription 3 *(STAT3)*, *STAT4*, and *STAT6*, as well as β-actin protein, as a housekeeping gene (Supplementary Table 1). PCR was carried out at 95°C for initial denaturation followed by 40 cycles at 95°C for 15 seconds, 60°C for 30 seconds, and extension at 72°C for 30 seconds. All samples were done in duplicate, and the relative expression levels of these genes were calculated using the $2^{-\Delta\Delta Ct}$ method, with β-actin as an internal housekeeping gene.[12]

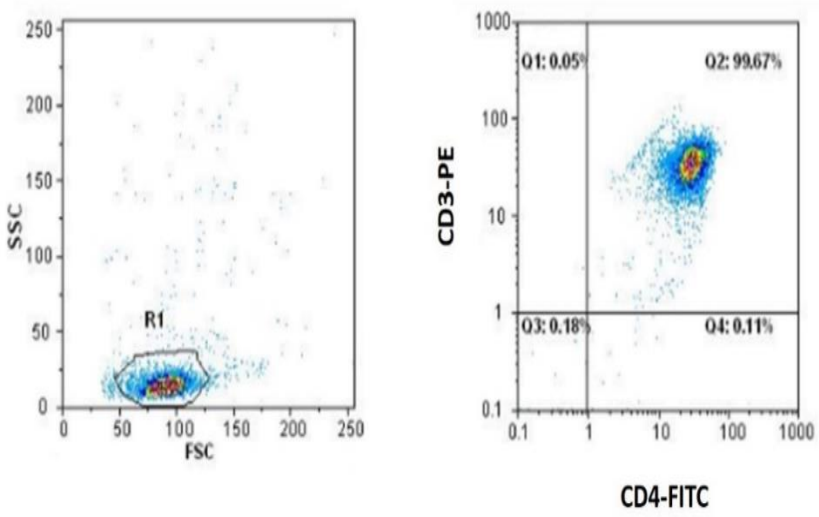


Figure 1. Purity of magnetic-activated cell sorting (MACS)-separated CD4⁺ T cells. A 2-color flow cytometry was performed on CD4⁺ T cells separated from the MACS column, using anti-CD3-phycoerythrin (PE) and anti-CD4-fluorescein isothiocyanate (FITC) antibodies. Here is a dot plot chart of a sample that shows a purity of more than 99%.

Cytokine Assay

After stimulation of $CD4^+$ T cells with PMA/ionomycin cocktail for 1 night, culture supernatants were collected and used to measure cytokines including IL-2, IL-10, IL-4, IFN-γ, and IL-17A using Human ELISA Ready Set Go Kits (Sanquin, Amsterdam, the Netherlands), according to the manufacturer's protocol. All samples were measured in duplicate.

Statistical Analysis

Data were expressed as mean±standard error of the mean. All statistical analyses were performed using SPSS 20 statistical package (SPSS, Chicago, USA). Results were evaluated by the Mann-Whitney U test and Spearman correlation tests as appropriate. *p* values<0.05 were considered statistically significant. All graphs were designed using GraphPad Prism 6 software.

RESULTS

T_H1 Pathway After Blood Transfusion

Considering the role of the T_H1 pathway in the immune response against viral infections and malignancies, in this study, we evaluated factors related to T_H1 function after blood transfusion. mRNA expression of 2 main transcription factors of the T_H1 pathway, *T-bet*, and *STAT4*, were evaluated using qRT-PCR and showed a significant decrease after blood transfusion ($p<0.0001$ for both) (Figure 2A and 2B). However, IFN-γ concentrations in culture supernatant showed no significant difference after blood transfusion ($p=0.374$) (Figure 2C).

T_H2 Pathway After Blood Transfusion

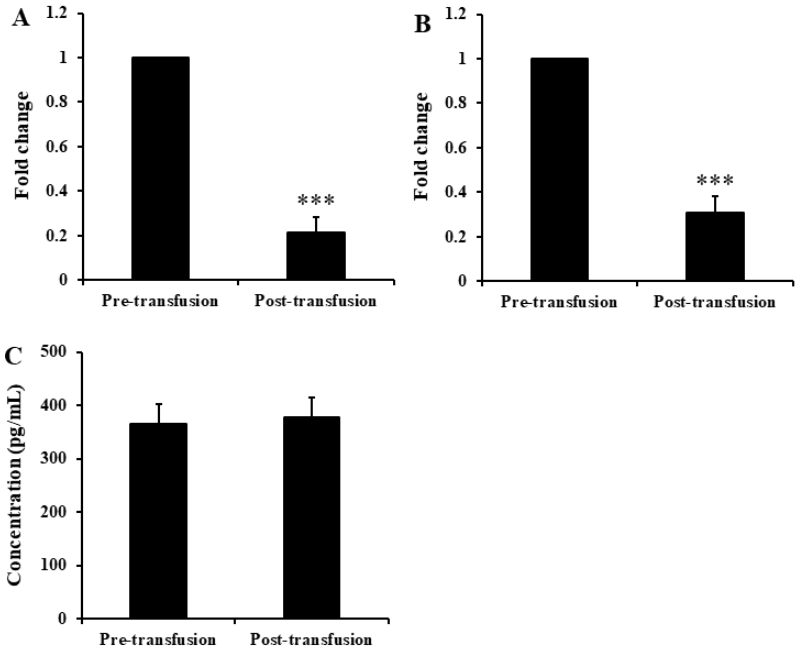
T_H2 cells can be a key factor in altering the immune response after blood transfusion by producing anti-inflammatory responses and suppressing several immune pathways, including T_H1. Therefore, we evaluated the mRNA expression of 2 main transcription factors of the T_H2 pathway, *GATA3* and *STAT6*, using qRT-PCR. The results showed that the mRNA expression of both *GATA3* ($p<0.001$) and *STAT6* ($p<0.001$) significantly decreased after blood transfusion (Figure 3A and 3B). However, IL-4 concentrations in the culture supernatant showed no significant change after blood transfusion ($p=0.774$) (Figure 3C).

T_H17 Pathway after Blood Transfusion

T_H17 cells, characterized by the secretion of IL-17A, are now identified as essential cells in the clearance of extracellular pathogens and the development of many autoimmune responses. Therefore, changes in their function after blood transfusion can be used to predict immune responses. qRT-PCR results showed that mRNA expression of *ROR-γt*, the main transcription factor of the T_H17 pathway, did not change significantly after blood transfusion ($p=0.054$) (Figure 4A); however, expression of *STAT3*, another transcription factor of the T_H17 pathway, decreased significantly ($p=0.025$) (Figure 4B). IL-17A concentrations in culture supernatant also decreased significantly after blood transfusion ($p=0.007$) (Figure 4C).

Immunomodulatory Pathway after Blood Transfusion

Since IL-10, as an anti-inflammatory cytokine, enhances the immune response of T_H2 cells and has been described as a major inhibitor of T_H1 cell function, we measured the concentration of this cytokine in culture supernatant before and after blood transfusion. The results showed that IL-10 production significantly increased after blood transfusion (Figure 5)..



Figures 2. (A and B) T-box expressed in T cells (T-bet) and signal transducer and activator of transcription 4 (*STAT4)* gene expression. After the isolation of $CD4^+$ T cells, total RNA was extracted, and cDNA was synthesized. Quantitative reverse transcriptase polymerase chain reaction was performed with specific primers for T-bet and *STAT4*. The gene expression results are shown as the mean, standard deviation of $2^{-\Delta\Delta Ct}$ after normalization with the control gene. (C) Evaluation of interferon-γ concentration. $CD4^+$ T cells were stimulated with PMA/ionomycin for 6 hours, and the culture supernatant was collected to measure the concentration of IFN-γ using enzyme-linked immunosorbent assay. * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

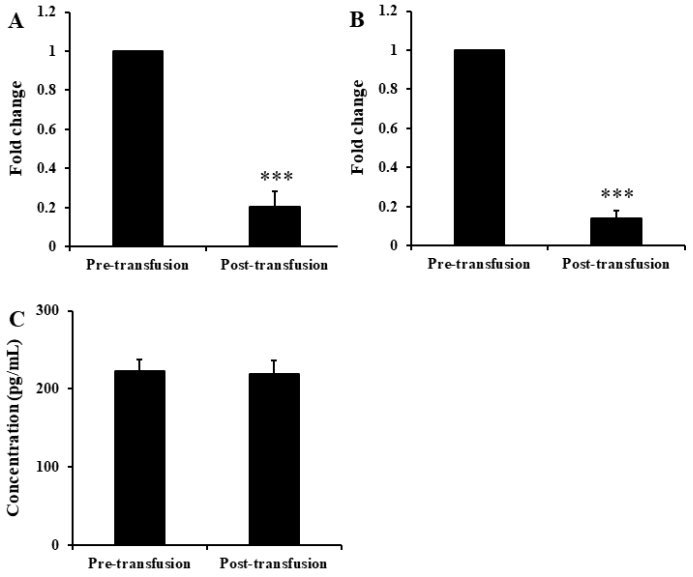


Figure 3. (A and B) GATA binding protein 3 (*GATA3)* and signal transducer and activator of transcription 6 (*STAT6)* gene expression. After the isolation of CD4$^+$ T cells, total RNA was extracted, and cDNA was synthesized. Quantitative reverse transcriptase polymerase chain reaction was performed with specific primers for *GATA3* and STAT6. The gene expression results are shown as the mean, standard deviation of $2^{-\Delta\Delta Ct}$ after normalization with the control gene. (C) Evaluation of IL-4 concentration. CD4$^+$ T cells were stimulated with a combination of phorbol 12-myristate 13-acetate (PMA)/ionomycin for 6 hours, and the culture supernatant was collected to measure the concentration of IL-4 using ELISA. * p<0.05; ** p<0.01; *** p<0.001.

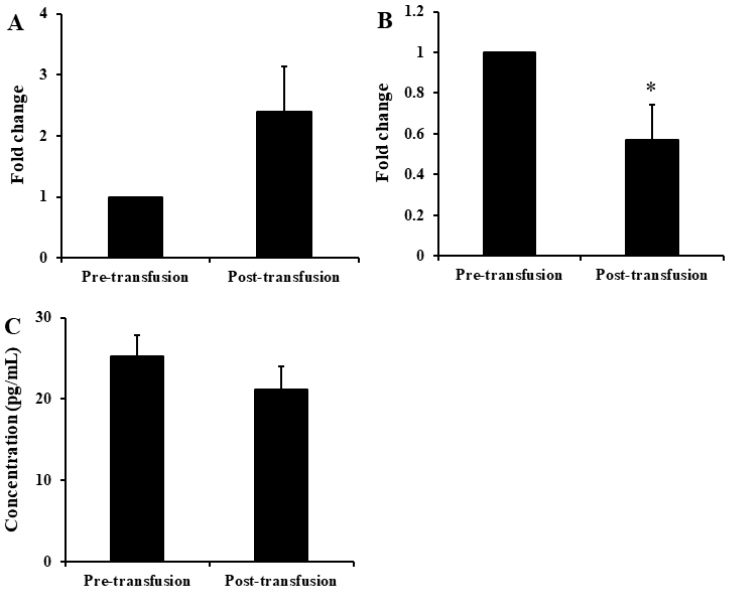


Figure 4. (A and B) RAR-related orphan receptor-γ (*ROR-gt)* and signal transducer and activator of transcription 3 (*STAT3)* gene expression. After the isolation of CD4$^+$ T cells, total RNA was extracted, and cDNA was synthesized. Quantitative reverse transcriptase polymerase chain reaction was performed with specific primers for *ROR-γt* and *STAT3*. The gene expression results are shown as the mean, standard deviation of $2^{-\Delta\Delta Ct}$ after normalization with the control gene. (C) Evaluation of interleukin-17A concentration. CD4$^+$ T cells were stimulated with a combination of phorbol 12-myristate 13-acetate (PMA)/ionomycin for 6 hours, and the culture supernatant was collected to measure the concentration of interleukin-17A using enzyme-linked immunosorbent assay. * p<0.05; ** p<0.01; *** p<0.001.

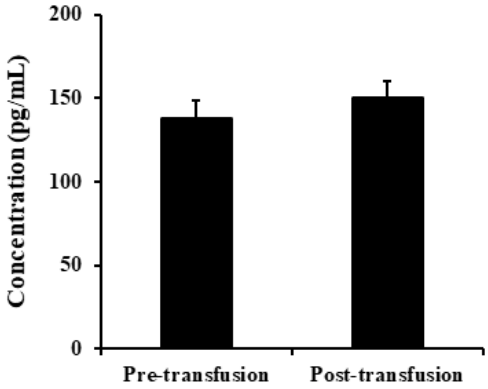


Figure 5. Evaluation of interleukin-10 concentration. CD4$^+$ T cells were stimulated with a combination of phorbol 12-myristate 13-acetate (PMA) /ionomycin for 6 hours, and the culture supernatant was collected to measure the concentration of interleukin-10 using enzyme-linked immunosorbent assay. * p<0.05; ** p<0.01; *** p<0.001.

DISCUSSION

Blood transfusions during and after surgery are associated with changes in the patient's immune system. These changes can be detected by evaluation of immune cell frequencies, cytokine concentrations, changes in the expression of surface markers, and transcription factors.[13] CD4$^+$ T cells, together with cell-mediated immunity, play a pivotal role in the immune response after surgery.[14]

Although several studies have evaluated the influence of blood transfusion on immune system function, they investigated immune response within 48 to 72 hours following surgery.[15-17] Since the inflammatory cascade of cytokines is highly complicated following CABG, determining the exact shift of the T_H1/T_H2 balance requires more time. To reduce this interpretation factor, we evaluated patients 2 weeks after CABG.

Stored whole blood has an immunosuppressive effect attributed mainly to storage-related factors. The inhibitory effects of blood transfusions are reduced but not eliminated by prestorage leukofiltration. However, the severity of immunomodulation from blood transfusion is highly dependent on the leukocyte count. Khan et al investigated the consequences of different amounts of leukocyte transfusion on the outcome of patients undergoing cardiac surgery. They found that patients who received leukofiltered blood had shorter intensive care unit and hospital stays, and shorter duration of mechanical ventilation duration compared with patients who received buffy coat-depleted, and non-leukoreduced packed cells. They also indicated that 7 days after surgery, IL-10 was significantly higher in the buffy coat-depleted, and non-leukoreduced groups.[18] In our study, we observed high IL-10, 14 days postoperatively. These findings demonstrate that the immune systems of patients who underwent CABG are at their weakest 7 to 14 days after the operation. In this regard, Martinez et al revealed that intraoperative cell-saver blood transfusion is accompanied by IL-10 reduction postoperatively.[19] Blood transfusion alters the function of helper T cells, including a decreased ratio of helper to suppressor lymphocytes, and reduced production of inflammatory cytokines,[20] while autologous blood transfusion activates the T_H1 pathway by decreasing IL-10 and increasing IL-2.[21] Additionally, the immune response to major surgery is anti-inflammatory.[22] The results of our study showed a decrease in the expression of *T-bet* and *STAT4*, both involved in the T_H1 pathway and IFN-γ production, after blood transfusion. Also, the expression of *GATA3* and *STAT6*, both involved in the T_H2 pathway and IL-4 production, decreased. However, production of IFN-γ and IL-4 by CD4$^+$ T cells did not show significant changes. Moreover, both expression of *STAT3* and production of IL-17A by CD4$^+$ T cells significantly decreased following blood transfusion, indicating a suppressed T_H17 function following blood transfusion. Based on these findings, it can be said that allogeneic blood transfusion in patients undergoing surgery may lead to suppression of the immune system.

In contrast with our findings Spinella et al., have shown that the levels of IL-2, IL-17, IL-10, and IFN-γ did not increase significantly after cardiac surgery. They also demonstrated that fresh transfusion versus aged RBCs did not result in substantial changes in hemostasis, immune, or nitric oxide parameters.[23] Additionally, the

type of cardiac surgery influences the cytokines secretion postoperative. Saracevic et al. indicated that off-pump surgery is accompanied by having higher IL-6 and IL-10 levels than on-pump surgery,[24] this finding can explain the heterogeneity between kinds of literature.

In the study by Tárnok et al,[14] it was reported that increased IL-10 secretion postoperatively is a negative risk factor for outcome. In the present study, a significant increase in IL-10 production by $CD4^+$ T cells indicate suppression of the immune response. It can be hypothesized that an increase in IL-10 might be associated with increased regulatory T-cell function and its transcription factors, which requires investigation in future studies.

The results of our study also showed a decline in the T_H17 pathway. Decreasing IL-17A, a pleiotropic inflammatory cytokine that is considered a multiple-target cytokine in inflammation [25] after blood transfusion can enhance the imbalance of immune factors postoperative and weaken the patient's immune system. The published guidelines for the management of cardiac patients requiring surgery and without surgery are being updated to reduce the mortality rate, postoperative complications, and length of hospitalization, and improve surgical outcomes.[5,26] In this regard, the published information from patients with the same goals as the present study can be useful.

The disruption in the molecular pathways observed in our study is probably due to the presence of leukocyte DNA. In general, the intracellular response of blood receptor T cells to donor DNA can cause this reversal of molecular pathways and be a defense mechanism to prevent the immune system from overreacting. However, some studies showed that fresh allogeneic blood counts with normal leukocyte counts did not significantly affect IL-10 and tumor necrosis factor-α production and leukocytes do not appear to be responsible for that. Leukocytes degenerate and become apoptotic during storage in the refrigerator, and apoptotic cells in transfused blood cannot suppress the immune system by producing immunologically active substances.[27] Major histocompatibility complex-peptide complexes that remain on apoptotic cells have been suggested to act as T-cell receptor antagonists and inhibit T-cell function.[28] On the other hand, evidence suggests that by degrading leukocytes, leukocyte-derived bioactive substances such as histamine, eosinophil cationic protein, eosinophil protein X, and myeloperoxidase accumulate extracellularly, all of which play an essential role in suppressing the immune system.[29,30] It has been shown that RBCs may also be involved in suppressing storage-dependent immune systems because erythrocyte membrane phospholipids activate macrophage-derived prostaglandins, which are potent immune regulators.[31,32]

In summary, the results of this study showed that blood transfusions alter the balance of helper T cells and reduce their activity. A decline in the expression of T_H1, T_H2, and T_H17 transcription factors and an increase in IL-10 production indicate the immunomodulatory effect of blood transfusion. Our findings demonstrated that 14 days postoperatively the immune systems of patients are weak and susceptible to further complications such as infection and impaired pulmonary function. Further studies are needed to determine the duration of the immunomodulated immune system following CABG.

STATEMENT OF ETHICS

All study subjects gave their written informed consent. The study protocol was approved by the Ethics Committee of Mazandaran University of Medical Sciences.

FUNDING

This study was financially supported by Mazandaran University of Medical Sciences; grant number MCBRC-MAZUMS-5592.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

The authors thank the patients and their families for their support, cooperation, and patience. We would like to thank the staff of the departments associated with the care and management of the patients.

REFERENCES

1. Dorneles CdC, Bodanese LC, Guaragna JCVdC, Macagnan FE, Coelho JC, Borges AP, et al. The impact of blood transfusion on morbidity and mortality after cardiac surgery. Comparative Study. 2011;26(2):222-9.
2. Ming Y, Liu J, Zhang F, Chen C, Zhou L, Du L, et al. Transfusion of red blood cells, fresh frozen plasma, or

platelets is associated with mortality and infection after cardiac surgery in a dose-dependent manner. Anesth Analg. 2020;130(2):488-97.
3. Ad N, Massimiano PS, Rongione AJ, Taylor B, Schena S, Alejo D, et al. Number and type of blood products are negatively associated with outcomes after cardiac surgery. Ann Thorac Surg. 2022;113(3):748-56.
4. Brevig J, McDonald J, Zelinka ES, Gallagher T, Jin R, Grunkemeier GL. Blood transfusion reduction in cardiac surgery: multidisciplinary approach at a community hospital. Ann Thorac Surg. 2009;87(2):532-9.
5. Irving AH, Harris A, Petrie D, Higgins A, Smith J, McQuilten ZK. Impact of patient blood management guidelines on blood transfusions and patient outcomes during cardiac surgery. J Thorac Cardiovasc Surg. 2020;160(2):437-45. e20.
6. Remy KE, Hall MW, Cholette J, Juffermans NP, Nicol K, Doctor A, et al. Mechanisms of red blood cell transfusion-related immunomodulation. Transfusion. 2018;58(3):804-15.
7. Refaai MA, Blumberg N. Transfusion immunomodulation from a clinical perspective: an update. Expert Rev Hematol. 2013;6(6):653-63.
8. Yazdanbakhsh K, Bao W, Zhong H. Immunoregulatory effects of stored red blood cells. Hematology Am Soc Hematol Educ Program. 2011;2011(1):466-9.
9. Franke A, Lante W, Kurig E, Zöller LG, Weinhold C, Markewitz A. Hyporesponsiveness of T cell subsets after cardiac surgery: a product of altered cell function or merely a result of absolute cell count changes in peripheral blood? Eur J Cardiothorac Surg. 2006;30(1):64-71.
10. Wang X, Fan H, Wang Y, Yin X, Liu G, Gao C, et al. Elevated peripheral T helper cells are associated with atrial fibrillation in patients with rheumatoid arthritis. Front Immunol. 2021;12:744254.
11. Gaudriot B, Uhel F, Gregoire M, Gacouin A, Biedermann S, Roisne A, et al. Immune dysfunction after cardiac surgery with cardiopulmonary bypass: beneficial effects of maintaining mechanical ventilation. Shock. 2015;44(3):228-33.
12. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 2001;25(4):402-8.
13. Leal-Noval SR, Muñoz-Gómez M, Arellano V, Adsuar A, Jiménez-Sánchez M, Corcia Y, et al. Influence of red blood cell transfusion on CD4+ T-helper cells immune response in patients undergoing cardiac surgery. J Surgical Res. 2010;164(1):43-9.
14. Tárnok A, Schneider P. Induction of transient immune suppression and T_H1/T_H2 disbalance by pediatric cardiac surgery with cardiopulmonary bypass. Clin Applied Immunol Rev. 2001;1(5):291-313.
15. Paruk F, Chausse JM. Monitoring the post surgery inflammatory host response. J Emergency Critical Care Med. 2019;3.
16. Rogozynski NP, Dixon B. The T_H1/T_H2 paradigm: A misrepresentation of helper T cell plasticity. Immunol Lett. 2024 Aug;268:106870. T_H1T_H2.
17. Squiccimarro E, Labriola C, Malvindi PG, Margari V, Guida P, Visicchio G, et al. Prevalence and clinical impact of systemic inflammatory reaction after cardiac surgery. J Cardiothorac Vasc Anesth. 2019;33(6):1682-90.
18. Khan AI, Patidar GK, Lakshmy R, Makhija N, Talwar S, Hazarika A. Effect of leukoreduction on transfusion-related immunomodulation in patients undergoing cardiac surgery. Transfusion Med. 2020;30(6):497-504.
19. Martinez MJ, Schwingshackl A, Romero T, Roach GD, Belperio JA, Federman MD. Cell saver blood transfusions may be associated with a decrease in inflammation and improved outcome measures in pediatric cardiac surgery patients. Perfusion. 2023;38(4):717-24.
20. Valizadeh S, Chegini A, Behnaz F, Pourfatollah AA, Karbalaeifar R. Unraveling the Impact of Blood Transfusion on Transcription Factors Regulating T Helper 1, 2, 17 and Regulatory T cells. Iranian J Allergy Asthma Immunol. 2024:1-8.
21. Li C, Zhu D, Zhao Y, Guo Q, Sun W, Li L, et al. Dendritic cells therapy with cytokine-induced killer cells and activated cytotoxic T cells attenuated T_H2 bias immune response. Immunol Invest. 2020;49(5):522-34.
22. Ackerman RS, Luddy KA, Icard BE, Fernández JP, Gatenby RA, Muncey AR. The effects of anesthetics and perioperative medications on immune function: a narrative review. Anesth Analg. 2021;133(3):676-89.
23. Spinella PC, Sniecinski RM, Trachtenberg F, Inglis HC, Ranganathan G, Heitman JW, et al. Effects of blood storage age on immune, coagulation, and nitric oxide parameters in transfused patients undergoing cardiac surgery. Transfusion. 2019;59(4):1209-22.
24. Saracevic A, Medved I, VLAH SH, Kozmar A, Bilic-Zulle L, Simundic A-M. The association of systemic inflammatory markers with indicators of stress and cardiac necrosis in patients undergoing aortic valve replacement and revascularization surgeries. Physiol Res. 2020;69(2):261.
25. Najafi S, Ghanavat M, Shahrabi S, Gatavizadeh Z, Saki N. The effect of inflammatory factors and their inhibitors on the hematopoietic stem cells fate. Cell Biol Int. 2021;45(5):900-12.

26. Halvorsen S, Mehilli J, Cassese S, Hall TS, Abdelhamid M, Barbato E, et al. 2022 ESC Guidelines on cardiovascular assessment and management of patients undergoing non-cardiac surgery: Developed by the task force for cardiovascular assessment and management of patients undergoing non-cardiac surgery of the European Society of Cardiology (ESC) Endorsed by the European Society of Anaesthesiology and Intensive Care (ESAIC). Eur Heart J. 2022;43(39):3826-924.
27. Mincheff M, Meryman H. Blood transfusion, blood storage and immunomodulation. Immunol Invest. 1995;24(1-2):303-9.
28. Morikawa Y, Furotani M, Matsuura N, Kakudo K. The Role of Antigen-Presenting Cells in the Regulation of Delayed-Type Hypersensitivity: II. Epidermal Langerhans' Cells and Peritoneal Exudate Macrophages. Cell Immunol. 1993;152(1):200-10.
29. Fredens K, Dybdahl H, Dahl R, Baandrup U. Extracellular deposit of the cationic proteins ECP and EPX in tissue infiltrations of eosinophils related to tissue damage. Apmis. 1988;96(7-12):711-9.
30. Nielsen H, Reimert C, Pedersen A, Brünner N, Edvardsen L, Dybkjaer E, et al. Time-dependent, spontaneous release of white cell-and platelet-derived bioactive substances from stored human blood. Transfusion. 1996;36(11-12):960-5.
31. Feola M, Simoni J, Tran R, Lox CD, Canizaro PC. Toxic factors in the red blood cell membrane. J Trauma. 1989;29(8):1065-75.
32. Grover GJ, Loegering DJ. Effect of splenic sequestration of erythrocytes on splenic clearance function and susceptibility to septic peritonitis. Infec Immun. 1982;36(1):96-102.