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Impact of Storage Period on CD4⁺/CD8⁺ T Lymphocyte Ratio in Erythrocyte Suspensions

Eritrosit Süspansiyonlarında Depolanma Sürecinin CD4⁺/CD8⁺ T Lenfositleri Oranı Üzerine Etkisi

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Abstract

Introduction: Some immunologic changes in the recipient derived by allogeneic blood transfusion (ABT) are called Transfusion Related Immune Modulation (TRIM). Despite the exact mechanisms of these changes are not known, it is thought that ABT causes a decrease of CD4/CD8 ratio in the recipient. This study aimed to determine the CD4/CD8 ratio in stored erythrocyte suspensions (ES) and to obtain new information about TRIM mechanisms.

Materials and Methods: Whole blood components used in our study were collected from 10 healthy volunteers. ES' obtained from whole blood were divided into three equal aliquots. Test samples which were related to 0th, 21st and 42nd storage days were prepared from these aliquots. CD3, CD4 and CD8 surface markers in peripheral blood mononuclear cells were investigated with flow-cytometer in these test samples.

Results: Our data were evaluated according to storage days. Decrease of CD3 (p=0,001), CD4 (p<0,001), CD8 (p=0,012) expressing mononuclear blood cells and helper T cells in 21st-day samples, CD4 (p=0,035) and CD8 (p=0,017) expressing cells in 42nd-day samples compared to Day 0 samples and increase of CD3 (p=0,027) expressing cells in 42nd-day samples compared to 21st-day samples were found statistically significant.

Conclusion: In our study, we did not find any significant change in the ratio of CD4⁺/CD8⁺ cells in ES. Detailed studies can help us to obtain more comprehensive knowledge on this field.

Keywords: CD4-CD8 ratio, erythrocyte suspension, erythrocyte transfusion, immunomodulation, transfusion

Öz

Giriş: Allojeneik kan transfüzyonunun (AKT) alıcıda neden olduğu bazı immünolojik değişikliklere transfüzyonla ilişkili immunomodülasyon (TRIM) adı verilmektedir. Bu değişikliklerin mekanizması tam olarak bilinmese de, AKT'nin alıcının mononülear kan hücrelerindeki CD4/CD8 ifadesi oranında azalmaya yol açtığına inanılmaktadır. Bu çalışmada depolanan eritrosit süspansiyonlarından elde edilen mononükleer hücrelerdeki (ES) CD4/CD8 ifadesi oranını belirlemek ve TRIM mekanizmalarına yönelik yeni bilgiler edinmek amaçlanmıştır.

Gereç ve Yöntemler: Çalışmamızda kullanılan tam kanlar sağlıklı 10 gönüllüden elde edilmiştir. Bu tam kanlardan elde edilen ES'ler eşit üç parçaya bölünmüş ve ES'lerin 0, 21 ve 42. raf günlerine yönelik test örnekleri bu parçalardan elde edilmiştir. Test örneklerdeki CD3, CD4 ve CD8 yüzey belirteçleri akan hücre ölçer yardımıyla incelenmiştir.

Bulgular: Verilerimiz ES'lerin depolanma günlerine gore değerlendirilmiştir. Sıfırıncı gün örneklerine göre 21. gün örneklerinde CD3 (p=0,001), CD4 (p<0,001), CD8 (p=0,012) ifade eden hücrelerde ve yardımcı T hücrelerinde görülen azalma (p<0,001); 42. gün örneklerinde CD4 (p=0,035) ve CD8 (p=0,017) eksprese eden hücrelerde görülen azalma; 21. gün örneklerine göre 42. gün örneklerinde CD3 (p=0,027) ifade eden hücrelerde görülen artış istatistiksel olarak anlamlı bulunmuştur.

Sonuç: Çalışmamızda ES'ler içerisinde CD4⁺/CD8⁺ hücre oranında istatistiksel olarak anlamlı bir değişiklik saptanmamıştır. Bu konuda daha kapsamlı bilgiye sahip olmak için daha detaylı çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: CD4-CD8 oranı, eritrosit süspansiyonu, eritrosit transfüzyonu, immunomodülasyon, transfüzyon

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Introduction

Allogeneic blood transfusion (ABT) has inherent complications. These complications can be classified as immune complications, non-immune complications and transfusion transmissible infections. Transfusion Related Immune Modulation (TRIM) is a rarely seen immune complication but can lead to serious consequences. It is defined as the clinical results of the immunological changes following ABT. It was first defined in 1973 and thought to have favourable effect on graft survival, as shown in renal transplant patients.[1]

In time some other conditions have been added to the effects of TRIM. According to these, ABT might lead to decrease in Crohn's disease recurrences and recurrent spontaneous abortion frequency, but increased cancer recurrences, postoperative bacterial infection rate, short-term mortality risk and reactivation of some latent infections in transfusion recipients.[2,3] TRIM is thought to be a result from changes in the recipients immune system after ABT. Some of these changes can be summarized as a decrease in T cell response, helper T cell (Th) count, antigen presentation, cytokine production, and CD4/CD8 ratio.[3–5] The trigger of these changes are yet to be known but mononuclear cells (MNC) in blood, biological response modifiers-immune mediators (BRM-IM) accumulated in the plasma during storage period, and the soluble HLAs (sHLA) are thought to be accountable.[2,3] In addition, there might be a relationship between TRIM and the storage time and the amount of transfused blood components.[6–10] However, studies to this day could not verify the existence and/or impact of the immunomodulatory effects of transfusion and also could not be sufficient to understand the underlying mechanism.

The aim of our study is to contribute the knowledge about TRIM. It has been shown that ABT leads to a decrease in $CD4^+/CD8^+$ cell ratio in transfusion recipients.[11–15] But unlike previous studies, we investigated $CD4^+/CD8^+$ ratio in blood mononuclear cells. Therefore, we focused on the $CD4^+/CD8^+$ mononuclear cell ratio in erythrocyte suspensions (ES) and evaluated TRIM related immunomodulatory factors in blood components and their possible association with storage period. For this purpose, $CD4^+$ and $CD8^+$ cells in ES' were analyzed.

Material and Methods

Blood Donation and Preparation of Blood Components for Tests

Our study was approved by Uludağ University Medical Faculty ethical committee (No: 2011-3/20). Whole blood donations for this study were made by 10 healthy voluntary donors admitted to Uludag University Medical Faculty Dr. Raşit Durusoy Blood Center. Donors were selected by our national blood donor eligibility criteria. "Pediatric blood bags" (PED/4 CPD/SAG-M 450/3x150 mL SAB; Kansuk, Turkey) were used for donation to divide the blood into portions. Leukoreduction were not performed. RBC with additive solution (CPD + SAG-M) and fresh plasma were prepared from whole blood units. Plasma components were not used in the study. ESs were divided equally into three pediatric blood bags. Those three units were used for the 0th, 21st and 42nd storage day. ES's were stored in blood bank refrigerators (Nüve, Turkey) at +4°C throughout the study period.

Flow Cytometry

$CD3^+$, $CD4^+$ and $CD8^+$ cells were analyzed in the 0th, 21st and 42nd storage day of samples by flow cytometer (FC) based on surface markers via fluorochrome-labelled monoclonal antibodies (mAb). MNC were isolated from each ES sample by density gradient using Ficoll (Histopaque-1077, Sigma-Aldrich, St. Louis, MO, USA). Monoclonal antibodies were CD4-FITC, CD3-APC (BioLegend, San Diego, CA, USA), CD8-PerCP (eBioscience, Waltham, MA, USA). For staining, 100µL MNC sample and 5 µL mAb (for each mAb) were incubated at room temperatures for fifteen minutes in the dark. 2 ml IsoFlow Sheath Fluid (Beckman/Coulter, Indianapolis, IN, USA) was added and washed with centrifugation at 1500 x rpm for five minutes. Finally 400 µL IsoFlow Sheath Fluid was added and then the stained cells were evaluated using FC (Navios, Beckman/Coulter, Indianapolis, IN, USA).

Statistical Analysis

Shapiro-Wilk test was used to evaluate distribution normality of the continuous variables. Descriptive statistics for continuous variables were presented as means ± SD. Paired Sample t-Test was used for in-group comparison. Statistical analysis was made by SPSS v.21 program and $p<0.05$ value accepted for statistical significance.

Results

The results and statistical analysis are summarized in Table 1 and exemplified in Figure 1. During the storage period of ES, $CD3^+$, $CD4^+$, $CD8^+$, $CD3^+CD4^+$ (helper T cell; Th) and $CD3^+CD8^+$ (cytotoxic T cell; Tc) cells decreased in the 21st and 42nd days compared with those of 0th day and increased in 42nd day compared with 21st day (Figure 2). The decline in CD3 ($p=0,001$), CD4 ($p<0,001$) and CD8 ($p=0,012$) cells and helper T cells ($p<0,001$) in 21st day in comparison with 0th day, CD4 ($p=0,035$) and CD8 ($p=0,017$) cells in 42nd day in comparison with 0th day and the increase in CD3 ($p=0,027$) cells 42nd day in comparison with 21st day were found statistically significant. During storage period of ES'CD4/CD8 and

Table 1. Results

	0th Days	21st Days	42th Days	0th-21st Days	21st-42nd Days	0th-42nd Days
	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)	p	p	p
CD3⁺	72% ± 10%	63% ± 10%	68% ± 6%	**0.001**	**0.027**	0.090
CD4⁺	46% ± 5%	40% ± 5%	42% ± 5%	**<0.001**	0.550	**0.035**
CD8⁺	29% ± 6%	22% ± 6%	23% ± 9%	**0.012**	0.517	**0.017**
Th	44% ± 6%	39% ± 5%	41% ± 5%	**<0.001**	0.539	0.204
Tc	24% ± 7%	20% ± 6%	23% ± 8%	0.068	0.231	0.249
CD4⁺/CD8⁺	1.71 ± 0.49	1.95 ± 0.53	2.03 ± 0.80	0.304	0.713	0.174
Th/Tc	1.92 ± 0.61	2.11± 0.57	2.06 ± 0.84	0.444	0.865	0.562

Results were demonstrated as rate of percentage, according to mean ± SD values. *p* values of <0.05 was considered as statistically significant and significant changes were written as bold.

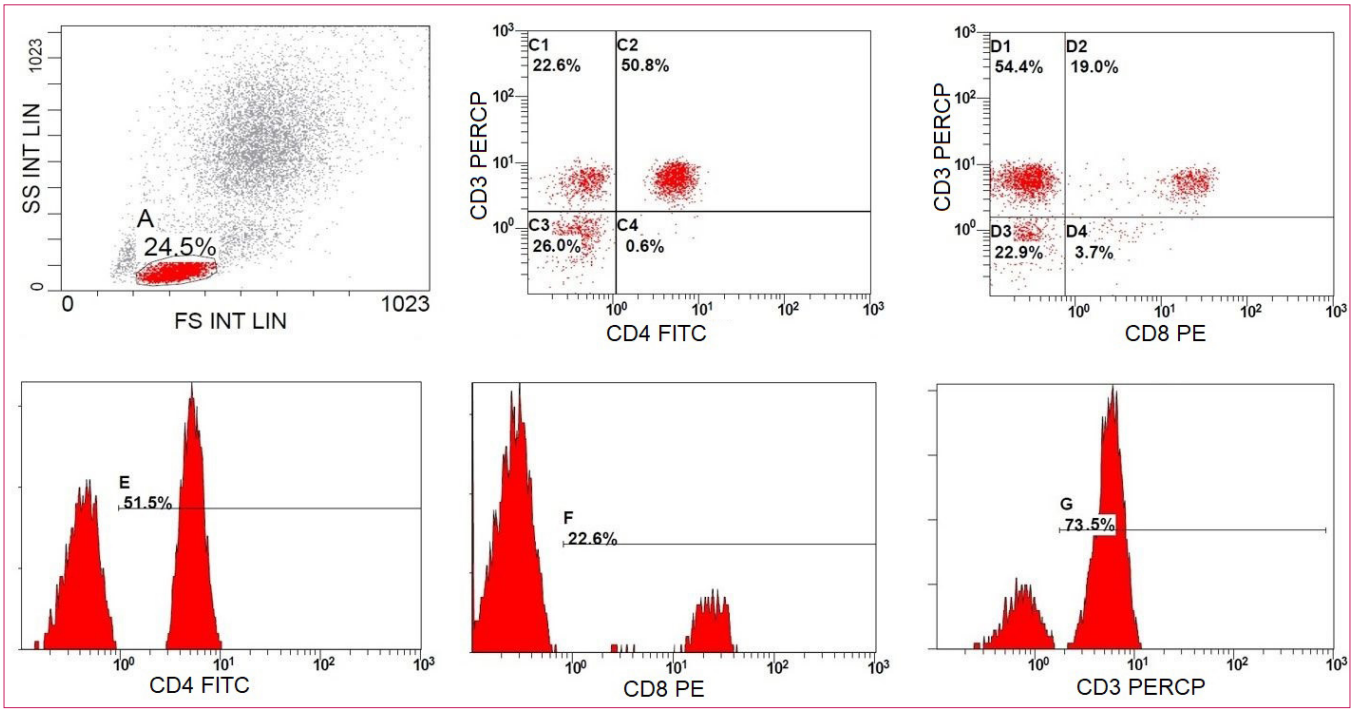

Figure 1. Representative FC dotplots.

Erythrocyte suspensions (ES) were obtained from whole bloods that were donated by voluntary donors. ESs were divided equally into three pediatric blood bags. Those three units were used for the 0th, 21st and 42nd storage day samples. Flow-cytometric analysis was performed in those samples. $CD4^+$, $CD8^+$, $CD3^+CD4^+$ (Th) and $CD3^+CD8^+$ (Tc) lymphocytes have been shown in representative dotplots that were related to 0th storage day samples.

Th/Tc ratios increased both in the 21st and 42nd days in comparison with 0th day (Figure 2). But those increases were not significant.

Discussion

There are many studies that investigate TRIM triggering factors and immunomodulatory changes caused by those factors.[2-5] Some studies described blood components, while some others targeted ABT recipients to determine immunomodulatory changes.[2-5] Even though some explanations were made for TRIM causing factors and changes in the immune system of the recipient, causes and consequences of TRIM can still not be explained exactly.

Some studies are focused on the changes of $CD4^+/CD8^+$ cell ratios in the recipient. Decrease $CD4^+/CD8^+$ cell ratio

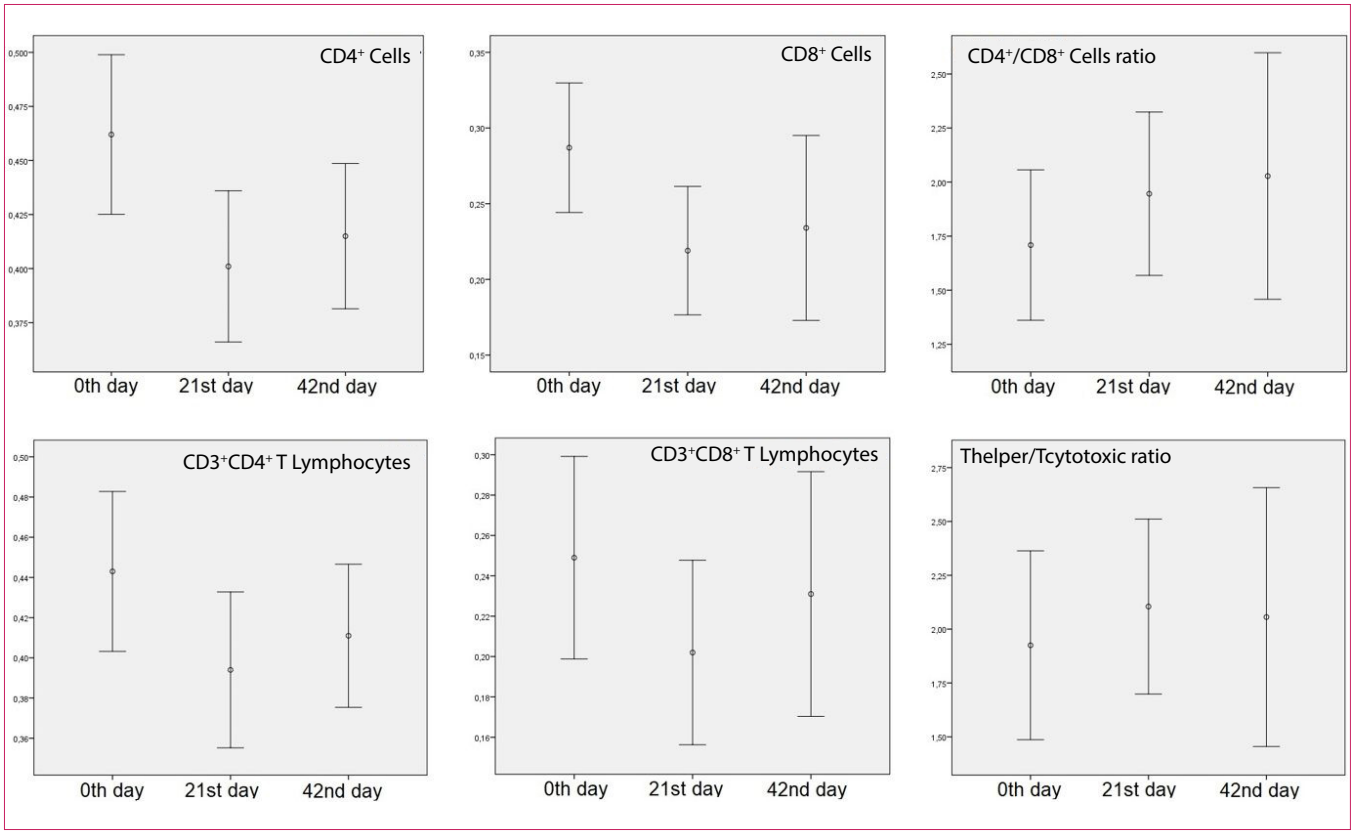

Figure 2. Changes in cells and ratios during storage period.
Boxplots are showing lymphocytes, $CD4^+$, $CD8^+$, $CD3^+CD4^+$ (Th), $CD3^+CD8^+$ (Tc) cells and $CD4^+/CD8^+$ and Th/Tc ratios.

in ABT recipient was shown in some studies.[11–15] Our study aimed to clarify whether this changes start also in the blood component. Blood components are stored in special storage conditions for specific periods by the use of special solutions. Storage conditions may have an impact on the $CD4^+/CD8^+$ ratio in the ES and the storage process may contribute to this changes. These factors, which may affect the donor cells, may be reflected in the recipient after ABT and causing a decrease in $CD4^+/CD8^+$ ratio also in the recipient.

In our study, $CD3^+$, $CD4^+$, $CD8^+$, Th and Tc cells in ES decreased on the 21st and 42nd days compared with the cells at 0th day and increased in 42nd day compared with the ones in 21st storage day. A similar result was published by Fernandez et al.[16] who investigated the effect of ABT on patients who undwent cardiovascular surgery. In our study, CD4/CD8 and Th/Tc ratios were increased during the storage period. However, these results were not statistically significant and do not seem to be in line with the published results.[11–15] Kaplan et al.[11] evaluated the $CD4^+/CD8^+$ cells ratio in Sickle Cell Anemia patients with and without blood transfusion. In this study, they found that $CD4^+/CD8^+$ cells ratio decreased significantly in ABT recipients. Kaplan attributed this to the relatively faster and stronger response of the $CD8^+$ cells to the intense antigenic stimulation encountered in the ABT recipient. They supported this effect by the fact that the $CD4^+/CD8^+$ cell ratio in the recipient remained low even at 12 months after bone marrow transplantation, since regeneration of $CD8^+$ cells after bone marrow transplantation was faster than that of $CD4^+$ cells.[17] The increase in $CD4^+/CD8^+$ cell and Th/Tc ratios that we found shows the opposite. Although these results are not significant, it can be interpreted that $CD8^+$ cells are more affected by the storage process than $CD4^+$ cells. However, while there was no significant change in number of Tc cells in the 21st day samples. The decrease in Th cells was reported before While Tc cells in ES may react more rapidly to the storage condition and environment, this reaction may be delayed in Th cells. The absence of a significant change in both Tc and Th cells in 42nd day samples, may indicate that the delayed response of Th cells to environmental conditions might be more emphasized Chen et al.[12] investigated the ratio of $CD3^+$, $CD4^+$, $CD8^+$ cells and $CD4^+/CD8^+$ cells ratio in pre-anesthesia, postoperative and postoperative

5th day samples in patients undergoing gastric cancer surgery. One group of patients received ABT while the other group received autologous blood transfusion. $CD3^+$, $CD4^+$ cell counts and $CD4^+/CD8^+$ cell ratio were significantly decreased in postoperative samples of both groups. This result which is comparable with our findings, suggested that Tc cells react faster to environmental conditions than Th cells. In this study, the normalization of the values on the 5th day in the autologous transfusion group suggests that the decrease in the postoperative samples might be due to surgical intervention. Absence of an improvement in postoperative 5th day in the ABT group could be considered as an indicator of the immunomodulatory effect of ABT. The fact that $CD3^+$, $CD4^+$, $CD8^+$ cell counts were significantly lower in cardiovascular surgery patients which received ABT when compared with patients that not received allogenic blood, and that $CD4^+$ and $CD8^+$ cell counts supports the possible immunomodulatory effects of ABT.[16,18] The significant decrease of $CD4^+$ and $CD8^+$ cell counts on the 21st and 42nd storage days in our study may also support this. The possible effect of storage conditions and duration on these cells may affect the ABT recipient. However, there are also opposing arguments. The study by Sun et al.[19] suggested that changes in the $CD4^+/CD8^+$ cell ratio are caused by the effect of the actual disease or treatment, regardless of transfusion. In their study they compared ABT performed and not performed gastric cancer patients which underwent gastrectomy. $CD4^+/CD8^+$ cell ratio increased in both groups on postoperative 4th and 30th days.[19] Their results showed that this increase was not associated with transfusion.[19] Similarly, it has been found that ABT alone can not stimulate specific Tc cells in the recipient, but can show this effect when administered in combination with allogeneic transplantation.[20] These studies support the view that diseases and treatments might affect immunomodulation.

Some authors have shown that the negative consequences of ABT are related to the storage time and conditions of blood components.[7–10] However, Hayek et al. suggested that the clinical condition of the patient was the main factor.[21] They have shown that decrease was seen in hospitalized patients but not in healthy individuals.[21,22] There are also animal studies supporting these findings.[23–26] However, our findings suggest that storage time and conditions also affect donated cells from healthy blood donors. Factors that lead to a significant reduction in $CD4^+$ and $CD8^+$ cells and a moderate increase in the ratio of $CD4^+/CD8^+$ and Th/Tc cells during storage within ES may lead to similar results in the ABT recipient after transfusion. Addition of different clinical parameters (such as disease, clinical manifestation, treatment in the recipient) to the changing characteristics of ES during storage period may cause a decrease in $CD4^+/CD8^+$ cell and Th/Tc cell ratios in ABT recipients. The results of our previous study also showed that changes in ES during storage might be associated with TRIM.[27]

Studies to date suggest that many variables originating from blood components and patients may have a role in the development of TRIM.[2-10] Treatments, diseases, amount and type of transfusions, molecules or cells or components of cells shelf life of the blood component and many other factors may have a role in the development of TRIM.[2-10] For instance, HLA haplotype similarity between the recipient and donor has also been implicated in the development of TRIM.[28] Alloimmunization or immunosuppression may occur after ABT, depending on their similarity between HLA-DR antigens.[28] It has been suggested that an immune tolerance is triggered if at least one antigen is shared, and alloimmunization occurs in the absence of similarity.[29] Similarity has been shown to cause an immune non-responsiveness in the recipient to donor Tcs.[30] Further studies are needed with TRIM.

Our study was performed on ES donated by healthy blood donors showing that $CD4^+$ and $CD8^+$ cells can respond to different conditions to different extents.

Finally, the function and viability of lymphocytes were not evaluated in our study. These issues will be investigated in future studies.

Ethics Committee Approval: Uludağ University School of Medicine Ethical Board (No 2011-3/20)

Conflict of interest: The authors declared that there were no conflicts of interest.

Contribution of authors: Concept: SHB, HBO, YH; Design: SHB, LTK; Data Collection or Processing: SHB, FB, FEC; Analysis or Interpretation: SHB, FB, FEC; Literature Search: SHB, LTK; Writing: SHB, LTK; Critical Review: YH, HBO, GG.

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References

1. Opelz G, Sengar DP, Mickey MR, Terasaki PI. Effect of blood transfusions on subsequent kidney transplants. Transplant Proc 1973;5:253–9. [PMID]
2. Vamvakas EC, Blajchman MA. Transfusion-related immunomodulation (TRIM): an update. Blood Rev 2007;21:327–48. [Crossref]

3. Bal SH, Oral HB. Transfusion Related Immunomodulation. Turk J Immunol 2016;4:37–46. [Crossref]
4. Blajchman MA, Bordin JO. Mechanisms of transfusion-associated immunosuppression. Curr Opin Hematol 1994;1:457–61. [PMID]
5. Brunson ME, Alexander JW. Mechanisms of transfusion-induced immunosuppression. Transfusion 1990;30:651–8. [Crossref]
6. Bilgin YM, van de Watering LM, Eijsman L, Versteegh MI, Brand R, van Oers MHJ, Brand A. Double-blind, randomized controlled trial on the effect of leukocyte-depleted erythrocyte transfusions in cardiac valve surgery. Circulation 2004;109:2755–60. [Crossref]
7. Koch CG, Li L, Sessler DI, Figueroa P, Hoeltge GA, Mihaljevic T, Blackstone EH. Duration of red-cell storage and complications after cardiac surgery. N Engl J Med 2008;358:1229–39. [Crossref]
8. Goel R, Johnson DJ, Scott AV, Tobian AA, Ness PM, Nagababu E, Frank SM. Red blood cells stored 35 days or more are associated with adverse outcomes in high-risk patients. Transfusion 2016;56:1690–8. [Crossref]
9. Rapido F, Brittenham GM, Bandyopadhyay S, La Carpia F, L'Acqua C, McMahon DJ, et al. Prolonged red cell storage before transfusion increases extravascular hemolysis. J Clin Invest 2017;127:375–82. [Crossref]
10. Offner PJ, Moore EE, Biffl WL, Johnson JL, Silliman CC. Increased rate of infection associated with transfusion of old blood after severe injury. Arch Surg 2002;137:711–6; discussion 716–7. [Crossref]
11. Kaplan J, Sarnaik S, Gitlin J, Lusher J. Diminished helper/suppressor lymphocyte ratios and natural killer activity in recipients of repeated blood transfusions. Blood 1984;64:308–10. [Crossref]
12. Chen G, Zhang FJ, Gong M, Yan M. Effect of perioperative autologous versus allogeneic blood transfusion on the immune system in gastric cancer patients. J Zhejiang Univ Sci B 2007;8:560–5. [Crossref]
13. Gafter U, Kalechman Y, Sredni B. Induction of a subpopulation of suppressor cells by a single blood transfusion. Kidney Int 1992;41:143–8. [Crossref]
14. Kessler CM, Schulof RS, Goldstein AL, Naylor PH, Luban NL, Kelleher JF, Reaman GH. Abnormal T-lymphocyte subpopulations associated with transfusion of blood-derived products. Lancet 1983;321:991–2. [Crossref]
15. Sirchia G, Mercuriali F, Pizzi C, Rosso di San Secondo VE, Borzini P, Aniasi A. Blood transfusion and kidney transplantation: effect of small doses of blood on kidney graft function and survival. Transplant Proc 1982;14:263–71. [PMID]
16. Fernandez LA, MacSween JM, You CK, Gorelick M. Immunologic changes after blood transfusion in patients undergoing vascular surgery. Am J Surg 1992;163:263–9. [Crossref]
17. Schroff RW, Gale RP, Fahey JL. Regeneration of T cell subpopulations after bone marrow transplantation: cytomegalovirus infection and lymphoid subset imbalance. J Immunol 1982;129:1926–30. https://www.jimmunol.org/content/129/5/1926.long
18. Kadam PP, Manglani MV, Sharma SM, Sharma RA, Setia MS. Immunoglobulin levels and CD4/CD8 counts in beta- thalassemia major. Indian Pediatr 2014;51:1000–2. [Crossref]
19. Sun CF, Hsieh YY, Ngan KW, Wang WT. Search for immunomodulatory effects of blood transfusion in gastric cancer patients: flow cytometry of Th1/Th2 cells in peripheral blood. Ann Clin Lab Sci 2001;31:171–8. http://www.annclinlabsci.org/content/31/2/171.long
20. Wood KJ, Dallman MJ, Morris PJ. Blood transfusion induces specific cytotoxic cells. Transplant Proc 1987;19:1458–60. [PMID]
21. Hayek SS, Neuman R, Ashraf K, Sher S, Newman JL, Karatela S, et al. Effect of storage-aged red blood cell transfusions on endothelial function in healthy subjects. Transfusion 2015;55:2768–70. [Crossref]
22. Neuman R, Hayek S, Rahman A, Poole JC, Menon V, Sher S, et al. Effects of storage-aged red blood cell transfusions on endothelial function in hospitalized patients. Transfusion 2015;55:782–90. [Crossref]
23. Lei C, Yu B, Shahid M, Beloiartsev A, Bloch KD, Zapol WM. Inhaled nitric oxide attenuates the adverse effects of transfusing stored syngeneic erythrocytes in mice with endothelial dysfunction after hemorrhagic shock. Anesthesiology 2012;117:1190–202. [Crossref]
24. Yu B, Lei C, Baron DM, Steinbicker AU, Bloch KD, Zapol WM. Diabetes augments and inhaled nitric oxide prevents the adverse hemodynamic effects of transfusing syngeneic stored blood in mice. Transfusion 2012;52:1410–22. [Crossref]
25. Donadee C, Raat NJH, Kanias T, Tejero J, Lee JS, Kelley EE, et al. Nitric oxide scavenging by red blood cell microparticles and cell-free hemoglobin as a mechanism for the red cell storage lesion. Circulation 2011;124:465–76. [Crossref]
26. Solomon SB, Wang D, Sun J, Kanias T, Feng J, Helms CC, et al. Mortality increases after massive exchange transfusion with older stored blood in canines with experimental pneumonia. Blood 2013;121:1663–72. [Crossref]
27. Bal SH, Heper Y, Kumaş LT, Guvenc F, Budak F, Göral G, Oral HB. Effect of storage period of red blood cell suspensions on helper T-cell subpopulations. Blood Transfus 2018;16:262–72. [Crossref]
28. Lagaaij EL, Ruigrok MB, van Rood JJ, Hendriks GF, van der Woude F, Weimar W, et al. Blood transfusion induced changes in cell-mediated lympholysis: to immunize or not to immunize. J Immunol 1991;147:3348–52. https://www.jimmunol.org/content/147/10/3348.long
29. Bordin JO, Heddle NM, Blajchman MA. Biologic effects of leukocytes present in transfused cellular blood products. Blood 1994;84:1703–21. [Crossref]
30. Munson JL, van Twuyver E, Mooijaart RJ, de Waal LP. Selective changes in human T-cell receptor repertoire after blood transfusion. Transplant Proc 1993;25:492–3. [PMID]