RESEARCH ARTICLE
DOI: 10.4274/tjh.galenos.2025.2025.0106

Evaluation of Immune Functions in Transfusion-Dependent Thalassemia Patients with Alloimmunization

Nazlı Özge Özköteş[1], Tuba Hilkay Karapınar[1], Sultan Okur Acar[1], Yüce Ayhan[2], Nesrin Gülez[3], Yeşim Oymak[1], Ferah Genel[3]
[1]University of Health Sciences Türkiye, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital, Clinic of Pediatric Hematology and Oncology, İzmir, Türkiye
[2]University of Health Sciences Türkiye, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital, Blood Bank, İzmir, Türkiye
[3]University of Health Sciences Türkiye, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital, Clinic of Pediatric Immunology, İzmir, Türkiye

Nazlı Özge Özköteş, M.D., University of Health Sciences Türkiye, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital, Clinic of Pediatric Hematology and Oncology, İzmir, Türkiye
ozgegokce7@gmail.com

March 19, 2025
August 15, 2025

Abstract
Objective: Regular erythrocyte suspension transfusions are still performed in most patients to prevent anemia. In recent years, it has been mentioned that patients are exposed to multiple allogeneic antigens and this leads to changes in the immune system. Understanding the immune regulators responsible for alloantibody development in thalassemia patients will provide appropriate data for the reduction and/or prevention of alloimmunization rate. We aimed to evaluate the association of alloimmunization and immune functions in these patients.
Materials and Methods: Fifty-four patients with thalassemia between the ages of 1-24 were retrospectively analyzed. In these patients, the frequency and types of alloantibodies and the immune functions and demographic characteristics that affect their formation were examined.
Results: The rate of alloantibody detection was 29.6%. There was a median interval of 13.7 years from the start of transfusions to alloantibody development. The age of initiation of regular transfusion was significantly late in patients with alloantibody development. We found a strong relationship between alloantibody development and direct Coombs positivity, low C4 and low $CD19^+$ B cell numbers. However, no significant difference was found between the groups in terms of serum IgG, IgA, IgM and C3 levels, the total lymphocyte count, $CD3^+$, $CD4^+$, $CD8^+$, and NK cells counts.
Conclusion: We believe that studies at molecular level should be increased and studies should be conducted with a larger number of patients in order to elucidate the immune pathogenesis of alloimmunization and determine the markers that will enable early recognition.
Keywords: Beta thalassemia major, Alloimmunization, Alloantibody.

Özet:
Amaç: Düzenli eritrosit süspansiyon transfüzyonları, anemiyi önlemek için hala çoğu hastada uygulanmaktadır. Son yıllarda, hastaların çok sayıda allojenik antijene maruz kaldığı ve bunun immün sisteminde değişikliklere yol açtığı belirtilmiştir. Talasemi hastalarında alloantikor gelişiminden sorumlu immün düzenleyicilerini anlamak, alloimmunizasyon oranının azaltılması ve/veya önlenmesi için uygun veriler sağlayacaktır. Bu çalışmada, alloimmunizasyon ile immün fonksiyonları arasındaki ilişkiyi değerlendirmeyi amaçladık.
Gereç ve Yöntemler: 1-24 yaş arasındaki 54 talasemi hastası retrospektif olarak analiz edilmiştir. Bu hastalarda alloantikorların sıklığı ve türleri ile bunların oluşumunu etkileyen immün fonksiyonları ve demografik özellikler incelenmiştir.
Bulgular: Alloantikor tespit oranı %29,6 olarak bulundu. Transfüzyon başlangıcından alloantikor gelişimine kadar geçen süre median 13,7 yıl olarak bulundu. Alloantikor gelişen hastalarda düzenli transfüzyona başlama

yaşı belirgin olarak geç bulundu. Alloantikor gelişimi ile direkt Coombs pozitifliği, düşük C4 ve düşük CD19+ B hücre sayıları arasında güçlü bir ilişki bulunmuştur. Ancak, serum IgG, IgA, IgM ve C3 düzeyleri, toplam lenfosit sayısı, CD3+, CD4+, CD8+ ve NK hücre sayıları açısından gruplar arasında anlamlı bir fark bulunmamıştır.
Sonuç: Alloimmunizasyonun immün patogenezini aydınlatmak ve erken tanıyı sağlayacak belirteçleri belirlemek için moleküler düzeyde çalışmaların artırılması ve daha büyük hasta grupları ile çalışmalar yapılması gerektiğine inanıyoruz.

Introduction:
Although allogeneic stem cell transplantation is an increasingly used treatment method in patients with beta thalassemia major (TM), regular erythrocyte suspension transfusions are still performed in most patients to prevent anemia. Complications due to both anemia and recurrent transfusions are also tried to be prevented, thus targeting the maintenance of normal growth and development. In recent years, it has been noted that patients are exposed to multiple allogeneic antigens, leading to changes in the immune system and iron accumulation with repeated transfusions. Alloimmunization resulting from the development of one or more specific erythrocyte antibodies is one of these complications. From a clinical perspective, erythrocyte alloantibodies cause haemolysis, difficulty cross-matching of blood, and an increased frequency of transfusions [1,2]. Factors causing alloimmunization to appear are complex and still poorly understood. However, erythrocyte antigenic differences between the blood donor and recipient, the recipient's immunity status, and the immune modulator effect of repeated allogenic blood transfusions on the recipient's immune system are thought to play a role in its pathophysiology [1]. There are some reports evaluating to red blood cell alloimmunization in thalassemia patients [3,4]. However, there is a little study evaluating the immune function of these alloimmunized patients. It has been proposed that alloimmunization develops as a result of erythrocyte antigenic differences between the blood donor and the recipient, the recipient's immunity status, and the immune modulator effect of repeated allogenic blood transfusions on the recipient's immune system. An understanding of immune mechanisms responsible for the development of alloantibodies in thalassemia patients will provide necessary data to limit and/or prevent the rate of alloimmunization. In the present study, we aimed to evaluate the association of alloimmunization and immune mechanisms in transfusion-dependent patients with thalassemia.

Material and Methods
The study included 54 β-TM patients aged 1-24 years who were regularly followed at Dr. Behçet Uz Children's Research and Training Hospital Thalassemia Unit between January 2010 and December 2019. Patients who had missing medical records and who were unwilling to participate in the study were excluded. The study data were obtained from the hospital's electronic database and the Thalassemia Unit's patient follow-up files, and retrospectively examined. The patients' demographic information (sex, age, date of start and duration of regular transfusions, transfusion frequency, and splenectomy status), iron chelator use and dosage, laboratory results, development of autoantibodies, and time from the start of regular transfusions to the development of alloantibodies were recorded.
Laboratory studies included complete blood count, serum C3, C4, IgG, IgA, IgM, and serum ferritin levels, and lymphocyte panel. Immunoglobulin levels were evaluated according to the age groups, by the results obtained from the study of Aksu et al. [5] Lymphocyte subgroups (CD3, CD4, CD8, CD16, CD19, CD45, CD56, and Anti-HLA-DR) were studied using the flow cytometry (FACS CANTO II model of Becton Dickinson). Lymphocytes subpopulation levels were evaluated according to the age groups, by the results obtained from the study of Comans-Bitter et al. [6]. According to the percentile values for age, those <5 were considered low, those between 5 and 95 percentiles were considered normal, and those >95 were considered high. In addition, the absolute values of lymphocytes subgroups were recorded.
In our hospital, blood products (with pre-storage leukocyte reduced) are supplied by Turkish Red Crescent Society. When our patients are to be transfused, appropriate blood products are determined in our hospital's transfusion centre by checking ABO blood group, Rh factor, and subgroup compatibilities and cross-matching of blood. Antibody screens and identification tests are carried out in every patient before each transfusion. When alloantibodies are detected, transfusions are continued with blood products that do not contain the detected antigen.
Antibody scanning and identification tests were carried out with the microcolumn agglutination method to search for alloantibodies. Commercial cell sets prepared from erythrocytes obtained from O group donors with known antigenic properties were studied in gel-containing microcolumns (Across Gel, Diapro, Turkiye). Donor erythrocytes used in antibody identification test panels with different antigen combinations in various cell lines were used to observe reactions occurring with antibodies against blood group RHD/RHCE, Kell, Kidd, Lewis, Lutheran, Duffy, MNS, and P erythrocyte antigens. In patients with indirect Coombs positivity, if the antigen equivalent to the identified antibody was absent, it was considered an alloantibody. For the investigation of C, c, E, e, Cw, and K antigens, specific antisera containing microcolumns were used (Across Gel, DiaPro Turkiye).

The detection of Lua and Kpa antigens was performed by using monoclonal antisera (Lorne Laboratories, UK) in neutral gel microcolumns (Across Gel, DiaPro Turkiye).
Data were analysed using IBM SPSS Statistics for Windows, version 25.0. The results are presented as mean, standard deviation, median, absolute number, or percentile. Categorical variables were compared with the Chi-square test and the Fisher test. Mann-Whitney U test and Kruskal-Wallis test were used to compare two and three groups, respectively, to evaluate inter-group differences of continuous variables. Statistical significance was set at $p<0.05$.
This Study protocol was reviewed and approved by the local ethics committee of the Dr. Behçet Uz Children's Research and Training Hospital, approval number 2019/17-13.

Results:
Twenty-seven patients (50%) were male and 27 (50%) were female. The median follow-up duration was 14.6 (minimum 6 months-maximum 23.1) years. Their median age was 15.8 (minimum 1.1-maximum 24) years. The distribution of patients' age groups is shown in Table 1. The median age of starting regular transfusions was 1 year old (minimum 4 months-maximum 4.5 years old). The median time from the start of regular transfusions to the study day was 14.3 (minimum 4 months-maximum 22.4) years. Five of 54 patients had undergone splenectomy at ages 3, 4, 11, 13, and 15. None of them developed alloantibodies during the study. The absolute numbers of lymphocyte subgroups in patients with and without splenectomy were compared. We found that absolute total lymphocyte, CD8, CD19, and NK cells numbers were significantly higher in patients who underwent splenectomy ($p=0.047$, $p=0.020$, $p=0.013$, and $p=0.034$, respectively). These findings are shown in Table 1.
Forty-eight (88.8%) of 54 patients were using deferasirox as an iron chelator, and 6 (11.2%) were using the combination of deferasirox and deferiprone. When the patients were grouped by blood group, 20 (37%) patients had blood group A; 8 (14.8%) had blood group B, 21 (38.9%) had blood group O, and 5 (9.3%) had blood group AB.

Findings in patients with alloantibodies
While 38 (70.4%) patients had no alloantibodies, 16 (29.6%) patients had alloantibodies; of the latter group, 7 (13%) patients had a single alloantibody and 9 (16.6%) patients had 2 or more antibodies. The median had a time from starting transfusions to alloantibody development of 13.7 (minimum 1 month-maximum 22) years. Eleven (68.8%) of 16 patients with alloantibodies were female, and 5 (31.2%) were male. Although there was no significant difference between both genders regarding the presence of alloantibodies ($p=0.074$), the latter was three times greater in females than males. The patients without alloantibodies had a median age of 14.8 (minimum 1.6-maximum 24) years, and those with alloantibodies had a median age of 16.6 (minimum 1.1-maximum 23.9) years. Distribution of the presence of alloantibodies by age groups is shown in Table 2.
A total of 30 alloantibodies were detected in 16 patients. The most common alloantibody was anti-E (8 patients), followed by anti-K (5 patients). Anti-C and anti-CW (4 patients each) were the third most common alloantibodies, while the remainder were anti-e (3 patients) and anti-c, anti-Kpa, and anti-Lua (2 patients each). Out of 9 patients with multiple antibodies, 4 had dual alloantibodies (anti-C and anti-Lua; anti-E and anti-K; anti-E and anti-K; anti-E and anti-C). The remaining 5 patients had triple alloantibodies (anti-C, anti-K, and anti-e; anti-E, anti-K, and anti-c; anti-c, anti-e, and anti-Lua; anti-E, anti-K, and anti-Cw; anti-C, anti-Cw, and anti-e). When the blood groups of patients with alloantibodies were examined, 8 (50%) of 16 patients had the blood group O; 3 (18.8%) had B, and 5 (3.2%) had A; no patient had the blood group AB. No significant correlation was found between the ABO blood group system and the presence of alloantibodies ($p=0.35$).
An analysis of the relationship between the age of starting transfusion and developing alloantibodies showed that 38 patients without alloantibodies had a median age of 11.5 (minimum 4-maximum 32) months. In contrast, those with alloantibodies had a median age of 15 (minimum 6-maximum 54) months. The age of starting transfusion was significantly greater in patients with alloimmunization ($p=0.03$).
An analysis of antibodies by the frequency of transfusion revealed that 5 (31.3%) of 16 patients with alloantibodies were transfused more often than every three weeks, and 11 (68.7%) every three weeks. None of the patients who were transfused every four weeks had alloantibodies. Among 38 patients with no alloantibody, 28 (73.7%) were transfused every three weeks and 10 (26.3%) every four weeks. An increased need for transfusion, thus being more frequently transfused, was significantly more common in patients with alloantibodies ($p<0.001$).
Eight patients with alloantibodies had direct Coombs positivity. Five of 38 patients without alloantibodies had direct Coombs positivity; there was a significant correlation between the presence of alloantibodies and direct Coombs positivity ($p=0.01$). Direct Coombs positivity rate was 6.6 times more common in patients with alloantibodies. The patients with direct Coombs positive + alloantibody positive and direct Coombs negative + alloantibody positive were compared in terms of lymphocyte subgroups. The two groups did not significantly

differ concerning total lymphocyte count, $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and NK cells absolute numbers (p=0.959, p=0.798, p=1, p=0.078, p=1.05, p=0.878). These findings are presented in Table 3.
When the relationship between serum ferritin level and the development of alloantibodies was examined, alloantibodies were found in 1 (6.3%) of 3 patients with a ferritin level below 500µg/L; 5 (31.3%) of 20 patients with a level of 500-1000µg/L; 6 (37.5%) of 17 patients with a level of 1000-1500µg/L; and 4 (25%) of 14 patients with a level above 1500µg/L. No significant correlation was found between the development of alloantibodies and serum ferritin level (p=0.920).
Splenectomy was needed in 5 (9%) of our patients; none of them developed alloantibodies during the study.
All of the patients used oral iron chelators due to ease of use. No comparison was made to establish a relationship between alloimmunization and iron chelator type.
Serum C3, C4, IgG, IgA, IgM levels, and lymphocyte panel were studied for an evaluation of immune functions. Table 4 shows the evaluation of differences in C3, C4, IgG, IgA, and IgM levels between groups. Serum C4 level was low in 8 (14.8%) of 54 patients; of these, 6 had alloantibodies. Serum C4 level was significantly lower in patients with alloantibodies (p=0.006).
Table 5 shows the evaluation of differences in total lymphocyte subgroups' absolute cell numbers and percentiles between groups. For patients whose lymphocyte panel was examined, the two groups did not significantly differ concerning total lymphocyte count, $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and NK cells absolute numbers (p=0.586, p=0.703, p=0.755, p=0.397, p=0.076, p=0.11). When lymphocyte subgroup percentiles were evaluated according to age, we found the presence of alloantibodies correlated with $CD19^+$ B cells percentage (p=0.023).

Discussion:
In this study, alloantibodies were detected in 16 (29.6%) patients. The alloimmunization rate in thalassemia patients has been reported to range between 4% and 45% in various centres [1,2,7-9]. Genetic homogeneity of blood donors in a given country, age at which regular transfusions start, and the cross-matching techniques and protocols of different centres for blood group phenotyping are known factors responsible for the wide range of alloimmunization prevalence [7]. We found a median interval of 13.7 years from the start of transfusions to alloantibody development. This was following intervals ranging from 1.5 to 14 years reported in the literature [8]. We found an alloantibody prevalence of 18.5% in males and 40.7% in females. Although alloantibodies were three times more common in females, the difference between the two genders was not significant. Some series reported so far on the presence of alloantibodies in patients with thalassemia have failed to establish any correlation between gender and alloantibody development [2,9,10]. However, several other studies have recently reported that alloantibodies are more prevalent in females [7]. Although several authors have explained this gender-based difference by the exposure of fetal erythrocytes to antigenic stimulation during pregnancy, the fact that our patients were in the pediatric age group and had no history of pregnancy suggests that some other unexplained factors may exist [7].
A total of 30 alloantibodies were detected in 16 patients—alloantibodies most commonly developed against the Rhesus system (70%). Similarly, the most common antibodies identified by previous studies were reported against the Rhesus and Kell antigens [2,7-13].
Thirteen of 16 patients with alloantibodies were older than 10 years old. This finding supported that the rate of alloimmunization increased with age. While some studies have reported a correlation between alloimmunization and age, some others have not [2,7-13]. The age at which patients started regular transfusions was significantly greater in those with alloantibodies. While some studies have reported a correlation between age of starting transfusions and alloimmunization, some others have not [2,7-10]. It has been suggested that this may be due to a resistance to alloimmunization, particularly when the immune system's antibody production is still immature [2]. In this study, none of the patients who were transfused once every four weeks had alloantibodies. The need for transfusion, thus being more frequently transfused, was significantly increased in patients with alloantibodies. This finding was in line with previous observations in the literature [1,2,8-11].
Iron overload, splenectomy, and deferoxamine use are implicated as the leading causes of immunological problems in patients with thalassemia. It is suggested that this is caused by the toxic effect of high iron levels on lymphocyte functions [14-16]. This study did not find any correlation between serum ferritin level and alloimmunization. Since no patient used deferoxamine, it could not be evaluated in this study whether this was a risk factor for the development of alloimmunization. Many studies to date have shown that splenectomy causes alloimmunization [1,2,7-9]. An increased prevalence of alloimmunization is a problem that is explained by the inability to remove antigens and damaged erythrocytes after the removal of the spleen, resulting in the development of alloantibodies and autoantibodies [1,2]. Although splenectomy was needed in 5 of our patients, none of them developed alloantibodies during the subsequent follow-up. On the contrary, our findings indicate that splenectomy does not increase the risk of alloimmunization in thalassemia. Our results are similar to the Lv et al study [17]. They reported that the total lymphocyte counts and percentages of B-lymphocytes in peripheral blood were increased after splenectomy when compared with the pre-splenectomy values in patients with cirrhotic portal hypertension. Although our patients were diagnosed with thalassemia, absolute total lymphocyte,

CD8, CD19, and NK cell numbers were significantly higher in patients who underwent splenectomy than in patients who did not undergo splenectomy.

A correlation was found between the presence of alloantibodies and direct Coombs positivity. Among patients who developed alloantibodies, no significant difference was found when patients with positive and negative direct Coombs were compared in terms of lymphocyte subgroup numbers. We detected alloantibodies in the patients. However, we do not know whether there are autoantibodies in patients with direct Coombs (+) because we did not study elution/absorption tests. Studies investigating the pathophysiology of the co-existence of autoantibodies and alloantibodies have been mostly conducted on patients with sickle cell anemia. The proposed mechanisms suggest that alloantibodies binding to transfused erythrocytes may cause conformational changes in antigenic epitopes, which in turn may stimulate autoantibody production, particularly in splenectomised patients. Another explanation, which was stated in Ofosu et al.'s study [18] on the major histocompatibility complex in patients with sickle cell anemia, is that genetic determination may be involved in autoantibody development. The presence of autoantibodies in alloimmunized patients has a concealed effect on alloantibodies in pretransfusion tests, which may fail to detect alloantibodies [7,18,19]. The mechanism of autoimmunization in chronically transfused patients is still incompletely understood, especially in patients with thalassemia.

It has been known that patients with thalassemia have impaired distribution and function of T and B lymphocytes, impaired production of immunoglobulin, and suppression of the complement system [20,21]. Whereas some studies on this subject have reported normal levels of immunoglobulin and complement system components, some others have reported reduced levels [22-26]. In this study, we did not find any significant difference between patients with and without alloimmunization regarding serum IgG, IgA, IgM, and C3 levels. However, serum C4 level was low in 8 of 54 patients, of whom 6 had alloantibodies. Patients with alloimmunization had a significantly lower C4 level, and the rate of having a low C4 level was increased by 10-fold by the presence of alloantibodies. Decreased levels of C3 and C4 have been reported in thalassaemia patients in some studies; however, it was not specified how many of these patients were alloimmunized [3]. We did not find such decreased levels of C3. Currently, the role of complement in the alloantibody-induced hemolysis process remains unclear. Also, the role of complement in B cell biology has been studied generally following exposure to infectious organisms. However, the role of complement in immune response to alloantigens remains unclear [27]. Our study failed to demonstrate any significant difference between the two groups in terms of total lymphocyte, $CD3^+$, $CD4^+$, $CD8^+$, and NK cell counts. However, it was shown that the $CD19^+$ B cell count was lower in the alloimmunized group. This decrease was not statistically significant according to absolute B cell count, but was substantial according to age percentiles evaluations. The role of B-lymphocytes in the production of alloantibodies against transfused erythrocytes and humoral immunity is of critical importance in patients with β-TM. It has been reported that patients with TM have an increased percentage of B-lymphocytes compared with the healthy control groups [3,28]. Bozdoğan et al. [24] found normal $CD19^+$ B cells in patients with thalassemia. While previous studies evaluated the percentage of B-lymphocytes, they did not specify how many of these patients were alloimmunized. In this study, we found a decrease in the number of $CD19^+$ cells and serum C4 levels in patients with alloimmunization. In recent studies, it is suggested that neonatal fragment crystallizable receptor (FcRn) has a role in antigen presentation and binding of immune complexes. Thus, FcRn is a central molecule in both homeostasis and immune responses [29]. Studies are being conducted on the use of FcRn inhibitors in the treatment of alloimmunization [30]. The standard explanation for the B cells and C4 low levels we found may be through FcRn functions. However, further studies are needed to say this.

Study Limitations:

The limited number of patients was a restricting factor. In the laboratory, plasma cell markers such as CD38 could not be included in the immunophenotyping panel. In addition, the lack of elution and absorption procedures for autoantibody identification in patients with direct Coombs positivity is a shortcoming of our study.

Conclusion:

We found that the age of starting regular transfusions was significantly greater in patients with alloantibodies. Also, we found the presence of alloantibodies had a strong correlation with direct Coombs positivity, low C4 levels, and $CD19^+$ B cell percentage. Alloimmunization occurs as a result of the evolution of one or more specific erythrocyte antibodies in patients undergoing repeated transfusions and is an essential complication of thalassemia treatment. From a clinical perspective, these alloantibodies cause hemolysis, difficulty cross-matching of blood, an increased frequency of transfusions, and commonly need immunosuppressive treatment. Therefore, it is essential to prevent the development of alloimmunization. For this reason, antibody screens and identification tests are carried out in every patient before each transfusion. When alloantibodies are detected, transfusions should be continued with blood products that do not contain the detected antigen. However, factors

causing alloimmunization to appear are complex and still poorly understood. Further studies are needed to explain these.

The authors have no conflict of interest.
There is no funding.
Ö.N.Ö. performed the research and wrote paper, T.H.K. designed the research, reviewed and edited manuscript, S.O.A. collected the clinical data, Y.A and Y.O. collected blood banks data, N.G and F.G. analysed immunological data.

References:

1 Singer ST, Wu V, Mignacca R, Kuypers FA, Morel P, Vichinsky EP. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly Asian descent. Blood. 2000;96:3369-73.
2 Spanos T, Karageorga M, Ladis V, Peristeri J, Hatziliami A, Kattamis C. Red cell alloantibodies in patients with thalassemia. Vox Sang. 1990;58:50-5.
3 Gluba-Brzózka A, Franczyk B, Rysz-Górzyńska M, Rokicki R, Koziarska-Rościszewska M, Rysz J. Pathomechanisms of Immunological Disturbances in β-Thalassemia. Int J Mol Sci. 2021;22:9677.
4 Bazi A, Shahramian I, Yaghoobi H, Naderi M, Azizi H. The role of the immune system in thalassemia major: a narrative review. J Pediatr Rev. 2018;6:29-36.
5 Aksu G, Genel F, Koturoğlu G, Kurugöl Z, Kütükçüler N. Serum immunoglobulin (IgG, IgM, IgA) and IgG subclass concentrations in healthy children: a study using nephelometric technique. Turk J Pediatr. 2006;48:19-24.
6 Comans-Bitter WM, de Groot R, van den Beemd R, Neijens HJ, Hop WC, Groenveld K, et al. Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations. J Pediatr. 1997;130:388-93.
7 El Kababi S, Benajiba M, El Khalfi B, Hachim J, Soukri A. Red blood cell alloimmunizations in beta-thalassemia patients in Casablanca/Morocco: Prevalence and risk factors. Transfus Clin Biol. 2019;26:240-248.
8 Jansuwan S, Tangvarasittichai O, Tangvarasittichai S. Alloimmunization to Red Cells and the Association of Alloantibodies Formation with Splenectomy Among Transfusion-Dependent β-Thalassemia Major/HbE Patients. Indian J Clin Biochem. 2015;30:198-203.
9 Ameen R, Al-Shemmari S, Al-Humood S, Chowdhury RI, Al-Eyaadi O, Al-Bashir A. RBC alloimmunization and autoimmunization among transfusion-dependent Arab thalassemia patients. Transfusion. 2003;43:1604-10.
10 Koçyiğit C, Eliaçık K, Kanık A, Atabay B, Türker M. Frequency of red cell allo- and autoimmunization in patients with transfusion-dependent beta thalassemia and affecting factors. Turk J Pediatr. 2014;56(5):487-492.
11 Azarkeivan A, Ansari S, Ahmadi MH, Hajibeigy B, Maghsudlu M, Nasizadeh S, et al. Blood transfusion and alloimmunization in patients with thalassemia: multicenter study. Pediatr Hematol Oncol. 2011;28:479-85.
12 Sadeghian MH, Keramati MR, Badiei Z, Ravarian M, Ayatollahi H, Rafatpanah H, et al. Alloimmunization among transfusion-dependent thalassemia patients. Asian J Transfus Sci. 2009;3:95-8.
13 Bhatti FA, Salamat N, Nadeem A, Shabbir N. Red cell immunization in beta thalassaemia major. J Coll Physicians Surg Pak. 2004;14:657-60.
14 Matzner Y, Hershko C, Polliack A, Konijn AM, Izak G. Suppressive effect of ferritin on in vitro lymphocyte function. Br J Haematol. 1979;42:345-53.
15 Buffe D, Rimbaut C. Immunosuppressive effect of a human hepatic glycoferroprotein, alpha2H globulin. A study on the transformation of normal human lymphocytes. Immunology. 1975;29:175-84.
16 Bryan C, Leech SH. Differential inhibition of MLR by iron and association with HLA phenotype. Immunogenetics. 1981;12:129-40.
17 Lv Y, Wu H, Lau WY, Zheng J, Wu J, Zeng M. Impact of total splenectomy on peripheral lymphocytes and their subsets in patients with hypersplenism associated with cirrhotic portal hypertension. Sci Rep. 2021;11:21246.
18 Ofosu MD, Saunders DA, Dunston GM, Castro O, Alarif L. Association of HLA and autoantibody in transfused sickle cell disease patients. Am J Hemato. 1986;22:27–33.
19 Nickel RS, Horan JT, Fasano RM, Meyer E, Josephson CD, Winkler AM, et al. Immunophenotypic parameters and RBC alloimmunization in children with sickle cell disease on chronic transfusion. Am J Hematol. 2015;90:1135-41.
20 Dwyer J, Wood C, McNamara J, Williams A, Andiman W, Rink L, et al. Abnormalities in the immune system of children with beta-thalassaemia major. Clin Exp Immunol. 1987;68:621-9.

21 Dua D, Choudhury M, Prakash K. Altered T and B lymphocytes in multitransfused patients of thalassemia major. Indian Pediatr. 1993;30:893-6.
22 Walter PB, Fung EB, Killilea DW, Jiang Q, Hudes M, Madden J, et al. Oxidative stress and inflammation in iron-overloaded patients with β-thalassemia or sickle cell disease. Br J Haematol. 2006;135:254-63.
23 Walter PB, Macklin EA, Porter J, Evans P, Kwiatkowski JL, Neufeld EJ, et al. Inflammation and oxidant-stress in beta-thalassemia patients treated with iron chelators deferasirox (ICL670) or deferoxamine: an ancillary study of the Novartis CICL670A0107 trial. Haematologica. 2008;93:817-25.
24 Bozdogan G, Erdem E, Demirel GY, Yildirmak Y. The role of Treg cells and FoxP3 expression in the immunity of β-thalassemia major and β-thalassemia trait patients. Pediatr Hematol Oncol. 2010;27:534-45.
25 Musumeci S, Schiliro G, Romeo MA, Sciotto A, Rosalba A, Pizzarelli G. Lymphocyte changes in beta-thalassaemia major. Arch Dis Child. 1979;54:954-7.
26 Bao W, Zhong H, Li X, Lee MT, Schwartz J, Sheth S, et al. Immune regulation in chronically transfused allo-antibody responder and nonresponder patients with sickle cell disease and β-thalassemia major. Am J Hematol. 2011;86:1001-6.
27 Chonat S, Mener A, Verkerke H, Stowell SR. Role of complement in alloimmunization and hyperhemolysis. Curr Opin Hematol. 2020;27:406-14.
28 Al-Awadhi A, Alfadhli S, Al-Khaldi D, Borhama M, Borusly M. Investigation of the distribution of lymphocyte subsets and zinc levels in multitransfused β-thalassemia major patients. International journal of laboratory hematology. 2010;32:191-6.
29 Gjølberg TT, Mester S, Calamera G, Telstad JS, Sandlie I, Andersen JT. Targeting the Neonatal Fc Receptor in Autoimmune Diseases: Pipeline and Progress. BioDrugs. 2025;39:373-409.
30 Jacobs JW, Booth GS, Raza S, Clark LM, Fasano RM, Gavriilaki E, et al. Current state and potential applications of neonatal Fc receptor (FcRn) inhibitors in hematologic conditions. Am J Hematol. 2024;99:2351-66.

Table 1- Evaluation of differences in lymphocyte subgroups between patients with splenectomy and non-splenectomy

Cells counts (/mm^3)	Total patients (n=54)	Patients with splenectomy (n=5)	Patients with non-splenectomy (n=49)	p value
Lymphocyte counts mean±SD median (min-max)	 2671,53±1691,59 2165 (780-9500)	 4168±1542,76 4760 (1540-5240)	 2518±1562,34 2130 (780-9500)	**0.047**
CD3$^+$ counts mean±SD median (min-max)	 1888,37±1086 1611,5 (478-5948)	 2774,8±1115,92 2878 (1044-3812)	 1797,91±1053,16 1595 (478-5948)	0.063
CD4$^+$ counts mean±SD median (min-max)	 1037,75±567,6 908 (196-2948)	 1465,8±625,8 1806 (463-1995)	 994,08±549,6 901 (196-2948)	0.089
CD8$^+$ counts mean±SD median (min-max)	 757,96±531,82 617(213-3307)	 1150,2±463,75 959 (613-1660)	 717,93±526,03 612 (213-3307)	**0.02**
CD19$^+$ counts mean±SD median (min-max)	 490,87±455,38 339,5 (90-2965)	 863,4±341,49 986 (296-1122)	 452,85±450,92 333 (90-2965)	**0.013**
NK counts mean±SD median (min-max)	 235,12±161,56 176,5 (55-753)	 423±248,58 506 (165-753)	 215,95±140,07 170 (55-716)	**0.034**

Table 2- Distribution of the presence of alloantibodies by age groups

	Alloantibody	**status**	
	Present	**Absent**	**Total**
Age Range	**n* (%)**	**n (%)**	**n (%)**
1-5 years old	3 (5.4%)	2 (3.6%)	5 (9%)
5-10 years old	0 (0%)	11 (20%)	11 (20%)
10-15 years old	4 (7.5%)	5 (9.5%)	9 (17%)
15-18 years old	3 (5.4%)	8 (16.6%)	11 (20%)
18-24 years old	6 (11.3%)	12 (22.7%)	18 (34%)
Total	16 (29.6%)	38 (70.4%)	54 (100%)

*n: Number of patients

Table 3- Evaluation of differences in lymphocyte subgroups between direct Coombs positive and negative patients with alloantibodies

Cells counts (/mm^3)	Total patients with alloantibodies (n=16)	Patients with d. coombs positive (n=8)	Patients with d. coombs negative (n=8)	p value
Lymphocyte counts mean±SD median (min-max)	 2583,12±2089,33 2125 (780-9500)	 3017,5±2817,15 2210 (780-9500)	 2148,75±993,28 2125 (970-4230)	 0.959
$CD3^+$ counts mean±SD	 1832,12±1315,59	 2005±1768,83	 1659,25±715,36	 0.798

median (min-max)	1607,5 (478-5948)	1569,5 (478-5948)	1720 (637-3014)	
$CD4^+$ counts mean±SD median (min-max)	 978,87±548,13 936 (196-2271)	 1007,5±694,67 908 (196-2271)	 950,25±399,21 998,5 (303-1642)	 1
$CD8^+$ counts mean±SD median (min-max)	 754,25±733,71 617 (213-3307)	 871,37±1014,47 617 (213-3307)	 637,12±305,08 612 (266-1185)	 0.878
$CD19^+$ counts mean±SD median (min-max)	 510,12±711,07 267 (90-2965)	 742,62±954,7 368 (90-2965)	 277,62±220,14 212 (93-787)	 1.05
NK counts mean±SD median (min-max)	 179,62±106,23 147 (80-456)	 193±131,72 129 (96-456)	 166,25±80,15 159 (80-343)	 0.878

Tablo 4- Evaluation of Differences in C3, C4, IgG, IgA, IgM Levels Between Groups

	Presence of Alloantibodies				**P Value**
C3		**Low**	**Normal**	**High**	**Total**
		n* (%)	**n (%)**	**n (%)**	**n (%)**
	No	7 (18.4%)	31 (81.6%)	0 (0%)	38 (100%)
	Yes	3 (18.8%)	13 (29.5%)	0 (0%)	16 (100%)
C4					
	No	2 (5.3%)	36 (94.7%)	0 (0%)	38 (100%)
	Yes	6 (37.5%)	10 (62.5%)	0 (0%)	16 (100%)
IgG					
	No	2 (5.3%)	34 (89.5%)	2 (5.3%)	38 (%100)
	Yes	0 (0%)	16 (100%)	0 (0%)	16 (100%)
IgA					
	No	0 (0%)	28 (73.7%)	10 (26.3%)	38 (100%)
	Yes	0 (0%)	11 (68.8%)	5 (31.2%)	16 (100%)
IgM					
	No	0 (0%)	34 (89.5%)	4 (10.5%)	38 (100%)
	Yes	1 (6.3%)	14 (87.5%)	1 (6.3%)	16 (100%)

1.00	
0.006	
0.403	
0.747	
0.272	

*n: Number of patients

Table 5-Evaluation of differences in lymphocyte subgroups between Groups

Cells counts (/mm^3) and percentage according to age (%)	Total patients (n=54)	Alloantibody positive patients (n=16)	Alloantibody negative patients (n=38)	p value
Lymphocyte counts mean±SD median (min-max)	 2671,53±1691,59 2165 (780-9500)	 2583,12±2089,33 2125 (780-9500)	 2708,76±1408,15 2235 (980-7070)	0.58
Lymphocyte percentage Low Normal High	 2 47 5	 1 15 0	 1 32 5	0.27
$CD3^+$ counts mean±SD median (min-max)	 1888,37±1086 1611,5 (478-5948)	 1832,12±1315,59 1607,5 (478-5948)	 1912,05±993,45 1611,5 (614-5020)	0.70
$CD3^+$ percentage Low Normal High	 3 45 6	 2 13 1	 1 32 5	0.29
$CD4^+$ counts mean±SD median (min-max)	 1037,75±567,6 908 (196-2948)	 978,87±548,13 936 (196-2271)	 1062,55±580,99 887,5 (365-2948)	0.75
$CD4^+$ percentage Low Normal High	 4 45 0	 3 13 0	 1 32 5	0.05
$CD8^+$ counts mean±SD median (min-max)	 757,96±531,82 617(213-3307)	 754,25±733,71 617 (213-3307)	 759,52±432,30 624 (249-2185)	0.39
$CD8^+$ percentage Low Normal High	 0 46 8	 0 14 2	 0 32 6	1.00
$CD19^+$ counts mean±SD median (min-max)	 490,87±455,38 339,5 (90-2965)	 510,12±711,07 267 (90-2965)	 482,76±303,06 399,5 (114-1160)	0,076
$CD19^+$ percentage Low Normal	 5 43	 4 11	 1 32	**0.023**

High	6	1	5	
NK counts mean±SD median (min-max)	 235,12±161,56 176,5 (55-753)	 179,62±106,23 147 (80-456)	 258±175,85 188,5 (55-753)	 0.11
NK percentage Low Normal High	 4 49 1	 2 14 0	 2 35 1	 0.537