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ORIGINAL ARTICLE

Risk factors for red blood cell alloimmunization in polytransfused patients

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ABSTRACT

Aim Alloimmunization depends on the type of the erythrocyte antigen, as well as the number of transfused doses of blood and recipient factors reflected in genetic and immunological status, comorbidities and therapy. The aim was to determine the frequency and type of antierythrocyte antibodies, to examine the correlation between the number of transfused doses and the frequency of antibodies, as well as the influence of recipient factors on the prevalence of antierythrocyte antibodies in polytransfused patients.

Methods A retrospective study was conducted in the Department of Pretransfusion Testing and Blood Product Therapy at the Polyclinic for Transfusion Medicine of the University Clinical Centre Tuzla. The data of patients who received two or more doses of red blood cell concentrate, the frequency of antierythrocyte antibodies, as well as a dose and patient factors including age, sex, comorbidity and therapy that influenced alloimmunization were analysed.

Results The highest percentage of patients developed the anti-E (23%), anti-C (11%) and anti-K (17%) antibodies. The recipient factors that have shown to be positive predictors for the development of antierythrocyte antibodies were: surgical bleeding (OR=3.74) and autoimmune diseases (OR=2.3). The probability that patients will not develop antierythrocyte antibodies was more pronounced in oncological patients (1/OR=7.14), as well as in patients with iron supplementation (1/OR=2.22) and antihypertensive therapy (1/OR=2.32).

Conclusion The results emphasize the importance of pretransfusion testing, illuminate the positive and negative effects of frequent transfusions, and propose strategies for improving transfusion therapy with a focus on the benefits of phenotyping erythrocytes for Rh and Kell antigens.

Keywords: antierythrocyte antibodies, pretransfusion testing, transfusion

INTRODUCTION

Alloimmunization develops after the transfusion of blood products, pregnancy, or organ, cell, and tissue transplantation in circumstances where the recipient does not have the antigen expressed on the surface of the donor's red blood cells (1,2). The frequency of alloimmunized patients in the general hospital population ranges from 1 to 3%, and in groups frequently treated with red blood cell transfusions it can be as high as 59% (3-5). Researches have shown that the prevalence of immunization to red cell antigens in multitransfused adults ranges even from 1.5% to 38%, and 1-2% in children (6,7).

The speed and strength of alloimmunization depend on the type and strength of the erythrocyte antigen, as well as the number of transfused doses of erythrocyte concentrates. It was shown in their research that several factors play an important role in the development of immunization to erythrocyte antigens: antigen factors, dose factors of erythrocyte concentrate, and biological factors of the donor and recipient (8). Immunogenicity and density of antigens are defined as factors of erythrocyte antigens and are presented with the number of foreign antigens on erythrocytes (9).

Donor factors are determined by ethnic background, presence of inflammatory diseases, and the individual response of erythrocytes to actual storage conditions. The dosage factors of erythrocyte concentrates are defined by the length and method of storage of the blood products, their modification, and cell damage during storage. Recipient factors that can contribute to alloimmunization include: age and ethnic background, antigen recognition, immune system, and previous exposure to the antigen (8-10). Despite the phenotyping of erythrocyte concentrates in the Rh and Kell system, the possibility of sensitizing patients to erythrocyte antigens that are not included in

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the routine phenotyping procedure during pretransfusion blood processing still persists (11,12).

Recipient factors include various genetic causes, belonging to certain ethnic groups, the medical and immunological condition of the patient, the presence of infection, therapy, and many other causes which can lead to an increased tendency to produce antierythrocyte antibodies (13,14). In a large number of patients, associated antibodies can be detected, which can further complicate the search for an adequate blood dose for the patient (15). It has been proven that women are more prone to alloimmunization than men, and that various chronic inflammatory diseases and autoimmune diseases also favour the production of antibodies (15,16).

The number of scientific studies from the domain of alloimmunization is limited in Bosnia and Herzegovina and the region of South-East Europe. It is a result of inadequate systematic immunohematological monitoring, insufficient registry structures, and the lack of research infrastructure necessary for comprehensive, long-term investigations.

The aim of this study was to determine the frequency and specificity of antierythrocyte antibodies in polytransfused patients, as well as the influence of dose and recipient factors to the occurrence of antierythrocyte antibodies in polytransfused patients.

PATIENTS AND METHODS

Patients and study design

A retrospective study was conducted during the period between 1 January 2024 and 30 June 2024, at the Department of Pretransfusion Testing and Blood Product Therapy at the Polyclinic for Transfusion Medicine of the University Clinical Center Tuzla, Bosnia and Herzegovina.

Data were analysed from patients who were polytransfused and who received two or more doses of red blood cell concentrates. The source of data was the Renovatio RGB transfusion information system, and through the analysis of electronic medical records, data were collected of patients, as well as data of the blood products and the referred immunohematological testing: ABO blood type, Rh-factor, screening for the presence of antierythrocyte antibodies.

The analysis included age, sex, diagnoses of the underlying disease, diagnoses that required patient transfusions, presence of comorbidities and therapy, previous transfusions during life and the number of transfusions received, development of alloimmunization and the occurrence of specific antierythrocyte antibodies. The amounts, methods of storage of erythrocyte concentrates, and the type of preservative used in storage were analysed. The exclusion criteria included polytransfused patients with detected positive antierythrocyte antibody screening, where the specificity of immune antibodies in serum could not be precisely determined (false positive results due to therapy and pathological proteins) and women whose alloimmunization was exclusively the result of previous pregnancy.

All polytransfused patients during the specified period, based on whether they developed anti-erythrocyte antibodies or not, were classified into two groups. The study group consisted of polytransfused patients who developed antierythrocyte antibodies, while the control group consisted of polytransfused patients (with similar characteristics in terms of age, gender, present morbidity, and comorbidities) who did not develop antierythrocyte antibodies.

Methods

The identification of antierythrocyte antibodies was performed using a microgel method on ID DiaPanel cards. The procedure was carried out in 3 environments: NaCl medium, Liss-Coombs medium, and enzyme medium with the addition of bromelain. Each panel of cards for the identification of antierythrocyte antibodies in a specific medium consists of 12 microtubes (11 microtubes and one autocontrol).

The procedure was carried out by adding 50 μL of 0.8% red blood cell suspension from the ID-DiaPanel into an 11-cell identification panel. In microtubes for autoconfirmation, 50 μL of the patient's 0.8% red blood cell suspension was added. After that, 25 μL of the patient's serum was added to each microtube, and the microcards were incubated for 15 minutes at 37 °C, then centrifuged for 10 minutes. The NaCl medium cards were immediately centrifuged for 10 minutes in the Dia-Med Centrifuge (Bio-Rad/DiaMed, Cressier, Switzerland) after the addition was completed. For the enzymatic medium cards, 25 μL of bromelain enzyme was also added. The identification result was interpreted based on the presence of agglutination in the microtubules. LISS/Coombs ID cards contain polyspecific AHG and were used for antibody screening and identification, and the presence of agglutination was compared with the probability of the presence of a specific antibody in the identification panel.

Statistical analysis

In the statistical processing of data, standard methods of descriptive statistics were used (median, minimum, maximum). To test the statistical significance of differences among samples, both parametric and nonparametric significance tests were used. In analysing the impact of predictors on the occurrence of antierythrocyte antibodies, the principles of hierarchical logistic regression were used, which showed the contribution of each predictor to the occurrence of antierythrocyte antibodies in polytransfused patients. The contribution of each predictor was evaluated based on the z-test and the odds ratio (OR). Values of $p<0.05$ were considered statistically significant.

RESULTS

During the study period a total of 10,950 transfused patients were recorded, of which 2,160 were polytransfused. Patients were divided into 2 groups: the study group of 65 polytransfused patients who developed antierythrocyte antibodies and 65 polytransfused patients in the control group who did not develop antierythrocyte antibodies. Of the total number of transfused patients (N=10,950), 103 (1%) developed antierythrocyte antibodies. In the total number of polytransfused patients, a total of 65 (2%) patients were recorded with detected antierythrocyte antibodies. Forty-six patients (35%) included men, while 84 patients (65%) were women. In average, the patients were 63 years old (M = 62.88, SD=15.98, Min=22, Max=94). The majority of patients belonged to blood group O+, followed by blood groups A+, B+, O-, and AB+.

The largest number of patients suffered from anaemia, 48 (37%) and oncological diseases, 33 (25%). Patients used various types of medications and therapies depending on their primary illness, predominantly iron supplements, 30 (23%) and antihypertensive therapy, 30 (23%) (Table 1).

In the test group, the highest percentage of patients developed the anti-E antibody, 15 (23%), followed by patients with an-

Table 1. Frequency of patients according to sex, blood group, disease, therapy, and membership in the test (developed antibodies) or control (without antibodies) group

Variable	No (%) of patients	Cumulative %
Sex		
Man	46 (35)	35
Female	84 (65)	100
Blood type		
A+	39 (30)	30
A-	3 (2)	32
AB +	8 (6)	38
AB -	1 (1)	39
B +	21 (16)	55
B -	2 (2)	57
0 +	42 (32)	89
0 -	14 (11)	100
Disease		
Anaemia	48 (37)	37
Autoimmune diseases	13 (10)	47
Depressive disorder	1 (1)	48
Surgical bleeding	28 (22)	70
Urinary tract infection	6 (5)	75
Myeloproliferative disorder (JAK2+ mutation)	1 (1)	75
Oncological diseases	33 (25)	100
Therapy		
Anticoagulants	1 (1)	1
Antibiotics	18 (14)	1
Antidepressants	1 (1)	15
Antidiabetics	5 (4)	19
Antihypertensive therapy	30 (23)	42
Cytotherapy	21 (16)	58
Immunotherapy	1 (1)	59
Cardiotonic drugs	8 (6)	65
Corticosteroids	8 (6)	72
Iron products	30 (23)	95
Substitutive therapy with thyroid hormones	7 (5)	100
Group		
Test (appearance of antibodies)	65 (50)	50
Control (without antibodies)	65 (100)	100

ti-K, 11 (17%) and anti-C, 5 (11%). Among patients who developed two or more antibodies, the most common combinations were anti-E and anti-C, 8 (12%) (Table 2).

Patients were divided into two groups: patients who developed one antibody and patients who developed two or more antibodies. A $\chi 2$ test was conducted to determine whether the frequency of patients with one versus patients with two or more antierythrocyte antibodies statistically differed. The test was statistically significant, $\chi 2\ (1)=16.75$; $p < 0.001$, indicating that patients with one antibody were significantly more represented than patients with two or more antibodies (Table 2).

A total of 46 patients (70.77%) developed single antierythrocyte antibody and 19 patients (29.33%) patients were detected with multiple antibodies. It is important to emphasize that one of the detected antibodies in combination always belonged to the Rh-system.

In the test and control group, the blood product factors (number of transfused doses and type of preservative used during

Table 2. Frequency of patients with antierythrocyte antibodies by antibody specificity

Antibody type	No (%) of patients	Cumulative (%)
Single antibodies		
Anti E	15 (23)	43
Anti K	11 (17)	91
Anti C	7 (11)	11
Anti Fy a	5 (8)	65
Anti Jk a	4 (6)	72
Anti M	2 (3)	98
Anti Jk b	1 (2)	66
Anti Lu a	1 (2)	95
Anti Le b	1 (2)	100
Multiple antibodies		
Anti E, Anti c	8 (12)	55
Anti C, Anti E	2 (3)	14
Anti D, Anti C	2 (3)	20
Anti E, Anti Fy a	1 (2)	57
Anti E, Anti C, Anti K, Anti Jk a	1 (2)	74
Anti E, Anti K	1 (2)	92
Anti K, Anti Fy a	1 (2)	94

product preparation and storage) and recipient factors (primary diagnoses and type of medication applied) were analysed. Patients were divided into 4 subgroups depending on the number of doses of erythrocyte concentrate they received before the ap-

Table 3. Results of hierarchical regression analysis considering dose and recipient factors

Predictors	Model 1				Model 2			
	b	OR	Z	p	b	OR	z	p
Segment	0.15	1.16	0.52	0.600	1.38	3.98	1.58	0.115
Doses with CPDA-1	0.02	1.02	0.18	0.857	0.11	1.12	0.81	0.417
Doses with CPDA-SAGM	0.09	0.91	1.18	0.237	0.10	0.90	1.19	0.234
Age of patients					0.02	0.98	1.43	0.154
Sex (male vs. female)					0.09	1.10	0.21	0.831
Surgical diseases vs. all diseases					1.32	3.74	2.55	0.011
Autoimmune diseases vs. all diseases					0.83	2.30	1.20	0.232
Anaemia vs. all diseases					0.36	1.44	0.74	0.461
Iron therapy vs. all medicaments					0.80	0.45	1.54	0.123
Cytotherapy vs. all medicaments					1.92	6.85	2.07	0.039
Antihypertensive therapy vs. all medicaments					0.85	0.43	1.76	0.079
Antibiotics vs. all medicaments					0.09	1.09	0.13	0.898
	AIC = 184.73 AUC = 0.55				AIC = 182.72 AUC =0.55			

Model 1 includes: number of doses conserved with CPDA-1 and CPDA-SAGM; Model 2 includes: number of doses conserved with CPDA-1 and CPDA-SAGM, age of patients, sex, diseases and therapy,
b, non-standardized regression coefficient; OR, odds ratio.z, value of z-test which determines the significance of individual contribution of the predictor. AIC, Aikake's information criterion; AUC, The area under the Roc curve.

pearance of antierythrocyte antibodies. A total of 33 (50.77%) patients received 2 doses, 24 (36.92%) patients received 3-5 doses; four (6.15%) and 10 (6.15%) and more than 10 doses.

The applied preservative in the blood bag as a dose factor has not proven to be a reliable predictor in the development of alloimmunization in polytransfused patients, regardless of whether it involved blood preservation with CPDA-1 or CPDA-SAGM preservative (OR=1.1; OR=0.9).

The analysed recipient factors that were found to be positive predictors for the development of antierythrocyte antibodies were: surgical bleeding (OR=3.74), autoimmune diseases (OR=2.3), anaemia (OR=1.44), and cytostatic therapy (OR=6.85). The probability that patients will not develop antierythrocyte antibodies was more pronounced in patients with oncological diseases (1/OR=7.14), as well as in patients who used iron supplementation (1/OR=2.22) and antihypertensive therapy (1/OR=2.32) (Table 3).

DISCUSSION

The risk of the erythrocyte alloimmunization in transfused patients depends on a number of factors including the number and frequency of transfusion procedures, previous pregnancies, immunogenicity of erythrocyte antigens in the blood dose, and the immune response of the recipient and donor. The frequency of antierythrocyte antibodies in the studied population of polytransfused patients was 2.0%, which was higher than the results of other studies, where the frequency of antibody occurrence ranged from 0.9-1.1% (1,3).

The research results showed a higher proportion of the female population in the category of alloimmunized individuals, which is consistent with the findings of all conducted studies published on this topic (1, 8-13). This result is explained by greater exposure to erythrocyte antigens during gestational periods in life, which also leads to higher vulnerability of women regarding alloimmunization (17,18). Other studies, on the other hand, did not show a statistically significant difference in the incidence of antierythrocyte antibodies among polytransfused patients based on sex (19,22).

The most common antibodies detected as a result of alloimmunization to erythrocyte antigens are anti-E, anti-C, anti-K, and anti-Fya, which are also the clinically most significant antibodies due to their potency in causing posttransfusion haemolytic reactions and haemolytic disease of the newborn (23,24).

The great significance of our results lies in the fact that at the Polyclinic for Transfusion Medicine of the University Clinical Centre in Tuzla, all blood doses are regularly phenotyped only if they belong to RhD-negative blood groups, while RhD-positive doses and all other doses are phenotyped in the Rh and Kell system only occasionally and depending on the need for blood and blood products within the hospital. Therefore, it is not surprising that the largest number of antibodies comes from these two very extensive blood group systems.

Our research has also shown that the frequency of transfusions affects the rate of occurrence of antierythrocyte antibodies, as repeated blood transfusions lead to greater exposure to antigens, thus increasing the risk of alloimmunization (25-27).

The type of substance used during blood conservation has not been shown to be a reliable factor in predicting the occurrence of antierythrocyte antibodies, and such scientific analysis has not been found in the available literature.

The results of the research showed that the patient's factors have a much more significant impact on the frequency of the occurrence of antierythrocyte antibodies in polytransfused patients, primarily the influence of existing diseases, pathological conditions, and the therapy being applied. The analysis was quite complicated, as all patients, in addition to their primary disease, have many additional comorbidities that complicate the statistical analysis of data. Therefore, the results were presented as the probability of antibody occurrence in patients with a certain disease compared to all other patients in the observed group. Thus, patients suffering from oncological diseases had a 7.14 times greater chance of not developing antibodies after transfusion due to the immunosuppressive effect of therapy.

Patients who were transfused during various surgical operations had a higher risk of developing antibodies, with the explanation that massive surgical operations, especially solid organ surgeries with complications, involve the transfusion of more blood doses in a shorter time span (26). From the perspective of therapy usage, patients who underwent cytostatic therapy had a higher risk of developing antibodies, which can be explained by the fact that cytostatic therapy lowers blood count values, thereby increasing the need for frequent transfusions of red blood cell concentrates (27).

An interesting fact was that patients with autoimmune diseases predominately Hashimoto's thyroiditis, rheumatoid arthritis, and ulcerative colitis had 2.3 times greater chances of developing alloimmunization, which is a result consistent with all available scientific achievements that have examined the inflammatory risk for diseases in transfused patients (27, 28).

None of the patient factors (age, sex, comorbidity and therapy) can be taken as a single predictive factor for the development of alloimmunization, but in combination with other factors and laboratory findings that are regularly monitored alongside the primary disease, one can assume the risk and dynamics of the development of antierythrocyte antibodies.

During the study, donor factors were not taken into account, although from a scientific point of view, various enzymatic and immunological mechanisms in the blood may likely influence the probability of the occurrence of antierythrocyte antibodies to some extent.

The limitation of this research is the inability to completely analyse certain patient factors such as genetic predisposition and the immunocompromised state of the patient. This should be included as a potential topic for some future analyses. The very occurrence of autoimmunity and the immune reactions that arise at that time certainly contribute significantly to the emergence of antierythrocyte antibodies, but due to the lack of laboratory equipment, we were unable to investigate this in more detail.

In conclusion, the results of this study emphasize the importance of pretransfusion immunohematological testing of blood donors and recipients of blood and blood products, highlighting the positive and negative effects of frequent blood transfusions, and proposing potential strategies for improving transfusion therapy with an emphasis on the benefits of phenotyping erythrocyte antigens of the Rh and Kell systems in order to minimize immunohematological complications in polytransfused patients.

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