Prevalence of erythrocyte alloimmunization in polytransfused patients

Incidência de aloimunização eritrocitária em pacientes politransfundidos

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ABSTRACT

Objective: To determine the incidence and the rate of red blood cell alloimmunization in polytransfused patients. **Methods:** A polytransfused patient was defined as having received at least 6 units of red cell concentrates during a 3-month period. The records of all patients (n = 12,904) who had received red blood cell units were examined retrospectively by searching the computer database at Hospital Israelita Albert Einstein in São Paulo, Brazil, over a 6-year period, between 2003 and 2009. **Results:** During this time, 77,049 red cell concentrate transfusions were performed in 12,904 patients. There were 3,044 polytransfused patients, 227 of whom (7.5%) presented with irregular erythrocyte antibodies. The prevalence of alloantibody specificity was: Anti-E>anti-D>anti-K>anti-C>anti-Di^a>anti-c>anti-Jk^a>anti-S in 227 polytransfused patients. We found combinations of alloantibodies in 79 patients (34.8%), and the most common specificities were against the Rh and/or Kell systems. These antibodies show clinical significance, as they can cause delayed hemolytic transfusion reactions and perinatal hemolytic disease. About 20% of the patients showed an IgG autoantibody isolated or combined with alloantibodies. Interestingly, a high incidence of antibodies against low frequency antigens was detected in this study, mainly anti-Di^a. **Conclusion:** Polytransfused patients have a high probability of developing alloantibodies whether alone or combined with autoantibodies and antibodies against low frequency antigens. Transfusion of red blood cells with a phenotype-compatible with RH (C, E, c), K, Fy^a, and Jk^a antigens is recommended for polytransfused patients in order to prevent alloimmunization and hemolytic transfusion reactions.

Keywords: Blood transfusion/adverse effects; Erythroblastosis, fetal; Erythrocytes/immunology

RESUMO

Objetivo: Determinar a incidência e a taxa de aloimunização eritrocitária em pacientes politransfundidos. **Métodos:** Foram classificados como politransfundidos todos os pacientes que receberam no mínimo 6 unidades de concentrado de hemácias no período de 3 meses. Foram examinados retrospectivamente os prontuários de todos os pacientes (n = 12.904) que receberam transfusões de unidades de hemácias procurados nas bases de dados computadorizados do Hospital Israelita Albert Einstein, em São Paulo (SP), no período de 6 anos, entre 2003 e 2009. **Resultados:** Nesse período foram realizadas 77.049 transfusões de concentrado de hemácias em 12.904 pacientes. Os pacientes politransfundidos totalizaram 3.044, sendo que 227 (7,5%) apresentam anticorpos eritrocitários irregulares. A prevalência da especificidade dos aloanticorpos encontrados nos 227 pacientes politransfundidos foi: Anti-E>anti-D>anti-K>anti-C>anti-Di^a>anti-c>anti-Jk^a>anti-S. Em 79 pacientes (34,8%) foram encontradas associações de aloanticorpos e as combinações mais frequentes foram dos anticorpos dos sistemas Rh e/ou Kell. Esses anticorpos têm importância clínica, pois podem causar reações transfusionais hemolíticas tardias e doença hemolítica perinatal. Cerca de 20% dos pacientes apresentavam autoanticorpo IgG isolado ou em associação com aloanticorpos. Um achado interessante neste estudo foi a alta incidência de anticorpos contra antígenos de baixa frequência, com predomínio anti-Di^a. **Conclusão:** Pacientes politransfundidos têm alta probabilidade de desenvolver aloanticorpos isolados ou em associação com autoanticorpos e anticorpos contra antígenos de baixa frequência. A transfusão de concentrado de hemácias com fenótipo compatível para os antígenos RH (C, E, c), K, Fy^a, e Jk^a deve ser recomendada para o grupo de pacientes politransfundidos, com objetivo de evitar a aloimunização e a reação transfusional hemolítica.

Descritores: Transfusão de sangue/efeitos adversos; Eritroblastose fetal; Eritrócitos/imunologia

INTRODUCTION

One of the risks of blood transfusion is the formation of antibodies against one or more erythrocyte antigens resulting from genetic disparities between donor and recipient[1]. The risk depends on the recipient exposure

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to the foreign antigen and its immunogenicity[2], defined as the ability of a given antigen to stimulate antibody production in a patient lacking the antigen[3]. Whether the recipient immune system will react depends on genetic or acquired factors related to the patient, dose, number, and frequency of transfusions[4-6]. Clinically significant red blood cell (RBC) alloantibodies are developed in more than 30% of patients receiving multiple transfusions, a situation that can pose major problems in the case of long-term transfusion therapy. Several authors found that erythrocyte alloimmunization occurs mainly after the first transfusions[7,8]. Knowledge of clinical conditions that predispose to alloimmunization is important in two ways: it may influence patient management and may lead to a better understanding of the etiology of the transfusion reaction[9].

When clinically significant non-ABO antibodies are detected in the plasma of patients requiring RBC transfusions, transfusion services must find and administer RBCs lacking the corresponding antigens. Thus, in transfusion medicine, much time and effort are spent in detecting and identifying blood group antibodies. Besides ABO, the most clinically significant antibodies are those in Rh, Kell, Duffy, and Kidd blood group systems[10].

When non-ABO blood group antibodies disappear, patients are at risk of unknowingly receiving incompatible RBC transfusions and developing delayed hemolytic transfusion reactions (DHTRs). DHTRs are probably the least recognized and most underreported type of transfusion reactions, partly due to their temporal dissociation from the causative transfusion[11].

The prevalence of blood group alloantibodies was reported in several study populations, including hospital-based patients, patients with hematological disorders requiring chronic transfusion therapy, and blood donors[9,12].

Giblett calculated the relative immunogenicity of a number of RBC antigens compared to the K antigen. The author compared the frequency that certain particular antibodies are encountered with the calculated probability of exposure. Based on her calculations, the relative likelihoods of non-D blood group formation are K(0.05) > c(0.0205) > E(0.0169) > Fy^a(0.0023) > Jk^a(0.0007)[3].

This retrospective study reports the prevalence and rate of alloimmunization in polytransfused patients at Hospital Israelita Albert Einstein (HIAE), São Paulo, Brazil.

OBJECTIVE

To determine the incidence and the rate of red blood cell alloimmunization in polytransfused patients.

METHODS

The records of all patients (n = 12,904) who received RBC units were examined retrospectively by searching the computer database from HIAE, a 530-bed general hospital in São Paulo (SP), over a 6-year period, between 2003 and 2009.

A polytransfused patient was defined as that receiving at least 6 RBCs units in 3 months. We selected all patients with detected RBC auto- and/or alloantibodies. The data collected included patient age, sex, history of RBC transfusion and pregnancy, RBC extended typing, and RBC antibody detection results.

Immunohematology testing

Blood samples from patients submitted to blood transfusions were screened for RBC alloantibodies using a selected three-cell set of reagent RBCs for antibody detection. The technique for antibody detection involved the use of 25 μL of serum and 50 μL of 0.8-percent RBCs in low ionic-strength solution gel tests (DiaMed AG, Cressier Switzerland, and Grifols, Barcelona, Spain.) Antibody identification was accomplished with commercial panels of cells tested by similar methods or additional techniques (e.g., polyethylene glycol and enzyme) whenever needed. If specificity could not be clearly determined, the blood sample was sent to our reference immunohematology laboratory for further analysis. The results of both antibody screening and antibody identification were valid for 72 hours (a transfusion episode). Complete crossmatching, including an indirect antiglobulin phase, was performed.

Statistical analysis

Data were analyzed using descriptive statistical analysis by means of absolute frequencies and percentages, and the results were organized in tables.

RESULTS

Patient characteristics

During the study period, we examined 12,904 patients who received 77,049 RBC units. Of these, 3,044 belonged to the selected polytransfused patient category, 227 (7.5%) of whom developed alloantibodies and/or autoantibodies. We found more alloantibodies in men than in women in that population, including patients who already had a RBC antibody before their first transfusion in this hospital. The risk of alloimmunization, defined as the total number of alloantibodies divided by

the total number of units transfused, was 0.3%. Patient age did not influence the alloimmunization rate.

RBC antibodies

The incidence rates of antibody formation and antibody specificities are presented in table 1. The prevalent antibody specificities found in those 227 polytransfused patients were anti-E > anti-D > anti-K > anti-C > anti-Di^a > anti-c > anti-Jk^a > anti-S. The most prevalent single antibodies found were: anti-E (20%), anti-D (12%), anti-K (11%), and anti-C (8%) (Figure 1). Combinations of antibodies were present in 79 (34.8%) patients. Forty-four patients (19.4%) had 2 antibodies; 20 (8.8%) had 3; 6 (2.6%) had 4; 5 (2.2%) had 5; and 4 (1.7%) had more than 5 antibodies (Figure 2). The most frequent combinations were found within the Rh system and/or anti-K specificities and accounted for 20% of antibody combinations (Table 2). Forty-nine (21.6%) patients presented with an IgG autoantibody alone or in combination with alloantibodies.

In this retrospective study, we observed 57 (3.1%) antibodies to low-incidence antigens (LIA-Ab) alone or in combination, and the anti-Di^a was the most prevalent antibody found (Table 3). The single LIA-Abs found was developed after the patient's first transfusion.

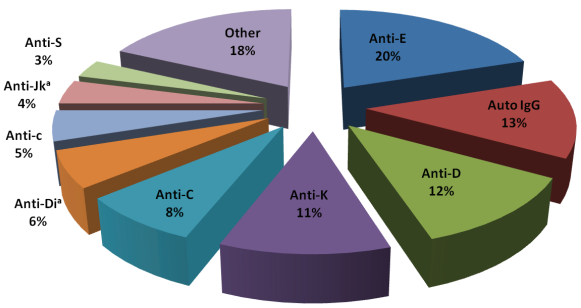

Figure 1. Incidence of red cell antibodies in patients with multiple transfusuons in HIAE the period 2003 to 2009

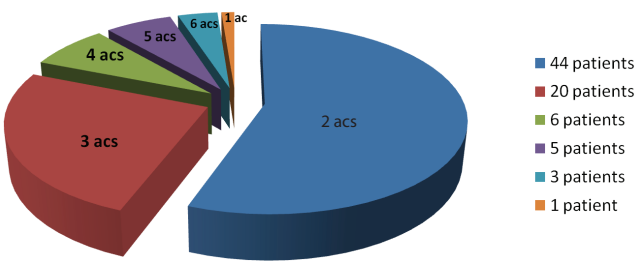

Figure 2. Associated antibodies in polytransfused patients

Table 1. Antibody specificity and frequency in 227 alloimmunized patients

Antibody specificity	Frequency	
	Number	Percent
E	77	22,06
D	46	13,18
K	41	11,75
C	32	9,17
Di^a	21	6,02
c	19	5,44
Jk^a	15	4,3
S	11	3,15
Kp^a	10	2,86
C^w	10	2,86
Lu^a	7	2
Js^a	6	1,72
M	6	1,72
HI	6	1,72
I	5	1,43
Fy^a	5	1,43
G	5	1,43
P_1	5	1,43
Le^a	4	1,17
e	3	0,86
Yt^b	3	0,86
V	3	0,86
Jk^b	2	0,57
N	2	0,57
Co^b	2	0,57
Go^a	1	0,29
H	1	0,29
f	1	0,29
Total	349	100

Table 2. Antibody specificities combinations found in 28 patients

Antibody specificity	Patients
Anti-E, -C^w	1
Anti-E, -K	1
Anti-E, -Jk^a	1
Anti-E, -Le^a	1
Anti-D, -C	1
Anti-C, -e	1
Anti-C, -Le^a	1
Anti-c, -Kp^a	1
Anti-D, -C, -E	2
Anti- E, -K, -Lu^a	1
Anti- E, - C^w, -Di^a	1
Anti- e, -K, -Fy^a	1
Anti-D, -C, -E, -S	2
Anti-E, -c, -Jk^a, -Fy^a	1
Anti-D, -C, -E, -Jk^a	1
Anti-E, -c, -C^w, -Di^a	1
Anti-E, -Jk^b, -Di^a, -Yt^b	1
Anti- E, -c, -S, -P1, -Co^b	1
Anti- D, -E, -C^w, -Kp^a, -Js^a	1
Anti- E, -c, -K, -Kp^a, -H	1
Anti- E, -K, -Jk^a, -S, -Di^a	1
Anti-D, -C, -E, -Le^a, -Co^b	1
Anti-E, -C^w, -K, -Kp^a, -Lu^a, -I	1
Anti-C, -E, -K, -Jk^a, -S, -Di^a	1
Anti-D, -C, -E, -K, -Jk^a, -V	1
Anti-C, -Kp^a, -Js^a , -Di^a , -Lu^a, -Go^a, -V	1

Table 3. Low incidence antibody (LIA-Ab) specificities found alone or in combination in 44 patients

LIA-Ab specificities		
LIA-Ab	**Associated antibodies**	**Patients**
C^w		1
C^w	E	2
C^w	E + K	1
C^w	E + c	1
C^w	E + c + Di^a	1
C^w	E + Di^a + auto IgG	1
C^w	D + E + Kp^a + Js^a	1
C^w	E + K + Kp^a + Lu^a + I	1
C^w	c + Jk^b + auto IgG	1
Di^a		4
Di^a	E	2
Di^a	C	1
Di^a	G	1
Di^a	S	1
Di^a	Lu^a	1
Di^a	Fya	1
Di^a	E + Kp^a	1
Di^a	C + S	1
Di^a	C + E	1
Di^a	E + K + Jka + S	1
Di^a	E + Jk^b + Yt^b + auto IgG	1
Di^a	C + E + K + Jka + S	1
Di^a	C + Kp^a + Js^a + Lu^a + Go^a + V	1
Kp^a	c	1
Kp^a	K	1
Kp^a	E + K	1
Kp^a	E + V	1
Kp^a	E + K + auto IgG	1
Kp^a	E + c + K + H	1
Lu^a		3
Lu^a	E + K + auto IgG	1
Js^a		1
Js^a	E	1
Js^a	E + K	1
Js^a	E + auto IgG	1
Yt^b		2

LIA-Ab: low incidence antibody.

DISCUSSION

This retrospective study was undertaken to determine the incidence of antibody formation after RBC transfusion. We demonstrated 349 unexpected antibodies in 3,044 patients characterized as polytransfused.

No influence of age was demonstrated; however, a greater prevalence of alloimmunization among males was found, with a male:female ratio of 1.5:1. The high prevalence of antibodies within the male population was unexpected given that pregnancy as an alloimmunization stimulus was not a factor[1,3,6]. As noted above, the majority of antibodies detected among males were preexisting antibodies, and most patients had a prior history of surgery. Of all patients 7.5% formed antibodies after consecutive transfusions and 35% of those showed a combination of two or more antibodies. Antibodies within the Rh system (anti-E, -D, -C) combined with anti-K were the most common specificities found. Anti-E was implicated in 16/20 patients with multiple antibodies.

Kidd antibodies represented 5% of the antibodies (17 patients) found and were more frequent than anti-Fy^a (5 patients). Fifteen patients developed anti-Jk^a shortly after transfusion, suggesting a rapid primary immune response. In two patients, anti-Jk^a was demonstrated in the eluate, indicating that these patients probably experienced hemolysis. Heddle et al. prospectively studied alloimmunization after transfusion in 2,082 patients and found 32 new RH, K, FY, JK, or MNS antibody specificities, 9 (28%) of which were anti-Jk^a, detected after a median period of 4 days (range, 1 to 94 days) after transfusion. A positive antiglobulin test was found in 31% of the newly alloimmunized patients. Only three patients showed hemolysis, one of which was an anti-Jk^a case[13]. Schonewille et al. found 2.8% of anti-Kidd in 1,795 patients with 2,257 antibodies[14].

These findings differ from those found by Schonewille et al. regarding alloimmunization to the Fy^a antigen. A prevalence of 1% of anti-Fy^a was found while these authors reported 7.3% of anti- Duffy in their study[14].

Twenty patients (8.8%) developed multiple antibodies. Several studies reported on the presence of multiple RBC antibodies in chronically transfused patients, with up to a fourfold increased risk of multiple antibodies as compared to the general risk of antibody formation[15-20].

The high incidence of RBC alloimmunization found by different authors has posed the question as to whether patients who are transfusion-dependent over a long period, such as those with sickle cell disease or thalassemia, should receive blood matched for antigens other than ABO and D in an attempt to prevent the formation of RBC alloantibodies[21-24]. The incidence of alloimmunization in patients receiving extended antigen-matched RBC transfusions was quoted as 0 to 6.4%. Extended antigen matching (e.g., c, E, and K) to prevent the formation of the majority of RBC antibodies in chronically transfused patients has been advocated for selected patient populations. Some reports applying this policy showed a significant drop in

the alloimmunization rate and delayed hemolytic transfusion reactions[25,26].

In this study, we had a high incidence of IgG autoantibodies (22% patients) alone or in combination with alloantibodies. One female patient developed a RBC autoantibody after an allogeneic blood transfusion and alloimmunization leading to temporary bystander immune hemolysis. She had a history of two pregnancies a long time prior and her antibody screening was negative. Anti-c was identified in her serum and eluate. At that time, she received another two units of compatible blood according to her phenotype. The direct antiglobulin test was positive and an IgG autoantibody was detected in the eluate[27]. Vichinsky et al. recognized a bystander hemolysis effect in patients with hemoglobinopathies, sickle cell disease, and thalassemia[25].

Fifty-seven (3.1%) cases of LIA-Ab alone or in combination were observed. Interestingly, anti-Di^a (6%) was the most prevalent antibody in our population. The Di^a antigen is a low-incidence antigen found predominantly in Indians and Asians. Anti-Di^a has been implicated in severe immediate or delayed transfusion reactions; however, finding compatible blood in cases where this antibody is present is not a problem[28]. In 16 patients, anti-Di^a was found associated with other antibodies. According to Schonewille et al., the prevalence of LIA–Ab varied between 0.3% and 10% and they found no anti-Di^a. Anti-Wr^a was the most frequent antibody found in this study[14].

CONCLUSION

Polytransfused patients have a high probability of forming RBC alloantibodies alone or in combination, autoantibodies, and antibodies against low-incidence antigens, and our findings are consistent with many studies published previously. Extended matching for RH (C, E, c), K, Fy^a, and Jk^a antigens should be considered for this group of patients in order to avoid alloimmunization and hemolytic transfusion reactions.

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