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Prevalence of red blood cell alloimmunization in multiply transfused patients

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ABSTRACT

Introduction: To analyze the prevalence of alloantibodies in multiply transfused patients.

Methods: This study was a retrospective, exploratory and descriptive study with a quantitative approach. The study sample comprised 185 patients transfused at a referral service in the city of Passo Fundo, Rio Grande do Sul, from January 2016 to February 2018.

Results: Overall, the antibodies identified were as follows: anti-E in 47 patients (18%), anti-D and anti-K in 28 patients each (11%), anti-C in 21 patients (8.1%), and inconclusive antibody results in 23 patients (8.9%). Females were a majority (55.7%), mean age was 48.8 years and mean quantity of blood transfused was 7.2 bags. Cardiovascular disorders were the most common comorbidities, in 39 patients (21.2%), followed by oncological disorders, in 38 patients (18.4%).

Conclusion: Alloimmunization is an important and frequent clinical condition that increases the risk of hemolytic reactions and is associated with significant patient morbidity and mortality.

Keywords: *Red blood cells; erythrocytes; alloimmunization; alloantibodies; transfusion; elderly*

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INTRODUCTION

Alloimmunization is defined as an immune response to foreign antigens after exposure to genetically different cells or tissues[1]. The increases in life expectancy and consequent population aging, has led to a greater risk of developing chronic-degenerative and oncological diseases and this, in common with the need for more complex surgeries that can require blood transfusions, has led to an increased frequency of anti-erythrocyte alloantibodies not belonging to the ABO system in the elderly population (over 60 years), demanding greater care with ensuring blood compatibility[2,3].

Chronic infectious and inflammatory diseases, neoplasms and myelodysplastic syndromes are chronic diseases that often require blood-based treatments, predisposing patients to alloimmunization. Moreover, there are also diseases such as hemoglobinopathies and autoimmune diseases that naturally predispose to alloimmunization, increasing rates of erythrocyte sensitization, to which multiparous women are also prone[4].

It is extremely important to take precautions with phenotyping and pre-transfusional compatibility testing for erythrocyte antigens, especially in the elderly population and those with critical comorbidities. Transfusion reactions in these patients can be significantly harmful, given the inherently reduced functional reserves in the elderly and the higher rate of concomitant comorbidities, which can compromise patients' general health status and aggravate possible reactions. Therefore, if blood transfusion is really necessary, it is recommended that phenotypical red blood cells should be used, identifying at least the Rh system (antigens C, c, E, and e) and Kell (K antigen), which are most strongly involved in alloimmunization and transfusion reactions[5,6].

Alloantibodies may prove clinically significant in future transfusions or pregnancy. These antibodies can cause acute or delayed hemolytic transfusion reactions or hemolytic disease of the fetus and newborn. They can also provoke lengthy and costly investigations in the blood bank and delays in locating compatible units for future transfusions. Only a fraction of the alloantibodies formed are identified. As such, the morbidity and mortality burden of RBC alloimmunization is likely underestimated[7].

This study was motivated by the facts outlined above. The objectives were to analyze the results of routine alloantibody testing performed by the Hemotherapy service at the Hospital São Vicente de Paulo (in the northern region of Rio Grande do Sul, Brazil), highlighting the prevalence rates of alloantibodies in transfused patients and correlating them with the patients' preexisting pathologies.

METHODS

This study was a retrospective, exploratory and descriptive study using quantitative methods. The study sample comprised 185 patients transfused at a referral service in the city of Passo Fundo (RS, Brazil), from January 2016 to February 2018. The results were input to an Excel spreadsheet (Microsoft, United States). Statistical analysis of the data was performed using SPSS Statistics 20.0 (IBM, United States). Qualitative variables were expressed as frequencies and percentages while quantitative variables were expressed as means ± standard deviations. The level of significance was set at 5% ($p = 0.05$). A total of 185 patients were included and no patients were excluded.

RESULTS

A total of 22,850 red blood cell transfusions were performed during the period analyzed. We identified 259 alloantibodies, found in 185 patients. Female patients accounted for 55.7% of the sample (103 patients), mean age was 48.8 years (± 21.1), with 53.9% classified as elderly (102 patients). The most common pathologies associated with detection of alloantibodies were as follows: 39 patients (21.2%) had cardiovascular disorders, 38 patients (20.5%) had oncological pathologies, and 34 patients (18.4%) had infections and traumas. Other data are shown in Table 1. The mean blood transfusion count in this group of alloimmunized patients was 7.2 bags of blood components, with a standard deviation of + 10.4.

The antibodies identified were, in descending order of frequency: anti-E in 47 patients (18%), anti-D and anti-K in 28 patients each (11%), anti-C in 21 patients (8.1%), anti-M in 14 patients (5.4%), anti-P1 in 12 patients (4.6%), anti-c and anti-Le^a in 11 patients each (4.2%), anti-Jk^a in 10 patients (3.9%), anti-S in 9 patients (3.5%), anti-Le^b in 8 patients (3.1%), anti-Lu^a and anti-Di^a in 7 patients each (2.7%), anti-Jk^b and anti-Fy^a in 6 patients each (2.3%), anti-C^w in 4 patients (1.5%), anti-s in 3 patients (1.2%), and anti-Kp^a and anti-e in 2 patients each (0.8%). The results for 23 patients (8.9%) were inconclusive, as shown in Table 2.

DISCUSSION

Alloimmunization is an adverse effect of exposure to erythrocyte antigens and is caused by genetic disparities between donor and recipient. Formation of antibodies against one or more of these antigens is one of the risks of blood transfusion and can elicit transfusional hemolytic reactions with severities ranging from mild, with reduced effectiveness of transfusion therapy, to extremely severe, with the potential to cause the transfused patient's death[8].

The risk of alloimmunization is dependent on the recipient's exposure to the foreign antigen and its immunogenicity, defined as a given antigen's ability to stimulate production of antibodies in a patient who is not an antigen carrier[6]. Some donors express antigens in a number of copies that is so low that alloantibodies are not create in the recipient. However, others may express larger numbers of copies of the antigen, forming alloantibodies after a single transfusion[9]. Nevertheless, exposure to non-self-antigens is a necessary but not a sufficient condition of formation of alloantibodies. Genetic differences between blood donors and recipients, the dose and the form of administration, and the number and frequency of transfusions all directly impact on the alloimmunization risk[10].

Alloimmunization is one cause of transfusion associated mortality, although mortality resulting directly from alloimmunization is relatively rare. More common transfusion-related complications of red blood cell (RBC) alloimmunization include 1) transfusion delays as new alloantibodies are being identified, 2) difficulties in locating compatible blood for highly alloimmunized individuals, and 3) delayed hemolytic or serologic reactions. Acute hemolytic transfusion reactions, though rare, are also possible in alloimmunized patients. However, in the absence of future transfusions, pregnancies, or transplantation, RBC alloantibodies are not inherently dangerous[7].

Clinically significant erythrocyte alloantibodies develop in more than 30% of patients given multiple transfusions, which can represent serious problems in the case of long-term transfusional therapy. Alloimmunization increases as the number of blood units transfused rises and approximately 2 to 9% of patients may develop new alloantibodies,

while anti-K formation appears to be increased in multiply transfused patients[10]. One study showed that 50% of the population studied who received more than 10 transfusions were alloimmunized, with alloimmunized patients receiving on average 6.2 transfusions of packed red blood cells, whereas in non-alloimmunized patients the mean number of blood transfusion was 3.6[2]. In our study, alloimmunized patients had received a mean of 7.2 (SD +10.4) bags of blood components.

When clinically significant non-ABO antibodies are detected in the plasma of patients requiring red blood cell transfusions, transfusion services must find and administer erythrocytes without the corresponding antigens. Thus, in transfusion medicine, much time and effort is spent on detection and identification of blood group antibodies and on selection of a compatible product. In addition to ABO, the most clinically important antibodies are those of the Rh, Kell, Duffy, and Kidd blood group systems.[2] In a Chilean study, the most frequently identified alloantibodies were anti-E (30.8%), anti-K (26.9%), anti-D (7.7%), and anti-Fy^a (5.8%)[7]. In a Brazilian study, formation of anti-K was observed in 26.68% and antibodies against Rh system antigens were found in 20% of alloimmunized patients[2]. A Dutch study found anti-E alloantibodies in 34% of the sample, anti-K in 24.9%, anti-Fy^a in 9% and anti-c in 8.5% as the most prevalent alloantibodies[10]. In another Brazilian study, anti-E (22%), anti-D (13.18%), anti-K (11.75%), and anti-C (9.17%) were the most common[11]. In our series, the most frequently identified antibodies were: anti-E (18%), anti-D (11%), anti-K (11%), and anti-C (8.1%), while inconclusive antibody results were also prevalent (8.9%). These results confirm and are in agreement with Brazilian and international literature, showing the prevalence of antibodies with specificity for the Rh system and Kell antigen.

Additionally, some studies have shown that female patients have an increased risk of alloimmunization, mainly due to multiparity[8]. One study reports that red blood cell alloantibodies can be induced by pregnancy, being potentially harmful to fetuses and newborns. Fetal anemia, hyperbilirubinemia, and even fetal hydrops and death can result from maternal alloimmunization, with up to 1 in 300 to 1 in 600 births affected[12].

Some pathologies are also linked to a higher risk of alloimmunization, either due to the patient's immune status or due to the need for greater quantities of blood transfusions. In one study it was demonstrated that there is a higher risk of alloimmunization in patients with malignancies and digestive bleeding, followed by kidney disease, heart surgery and traumas[8]. The risk of alloimmunization is also greater in patients with autoimmune diseases (16%) and myeloid diseases (15-59%), solid tumors (up to 10%), and transplant patients, especially liver tumors (4-23%)[10]. Another study reports that alloimmunization rates are around 44% in patients with hematological neoplasms such as myelodysplastic syndrome, 20% in patients with thalassemia, and 18.7% in people with sickle cell disease[6]. Alloimmunization and autoimmunity are also linked, with an association with alloimmunization in systemic lupus erythematosus, rheumatoid arthritis, and ulcerative colitis[12]. On the other hand, immunosuppressed patients with leukemia have lower rates of alloimmunization[10,12].

It was demonstrated in another study that there is a greater probability that white rather than black or Asian individuals (regardless of Rh-D status) will respond to antibodies, as well as relatively high rates of red blood cell alloimmunization in patients with sickle cell disease (SCD)[12]. Patients with SCD have some of the highest rates of RBC alloimmunization of any population. This may be due in part to the relatively high transfusion burden of patients, and may also be due in part to the number of Rh variants (of D, E/e, or C/c) known to occur in patients of African descent.

However, some studies show no difference in the occurrence of alloimmunization when comparing by gender, age, or comorbid pathologies[2,10]. In our study, cardiovascular (21.2%), oncological (20.5%), infectious, and traumatic conditions (18.4%) were prevalent in patients with alloantibodies, while rheumatological diseases, chronic kidney disease, and anemia accounted for another 22.7% of the patients.

Knowledge of the clinical conditions that predispose to alloimmunization is important for two reasons: it can influence the treatment of a patient and can lead to a better understanding of the etiology of the transfusion reaction[13]. It is therefore necessary to evaluate previous surgery and past and current comorbidities, research the patient's family history and, in female patients, ask questions about gestational history. The clinical circumstances surrounding RBC transfusion are thought to impact the likelihood of the recipient becoming alloimmunized. Patients transfused in their baseline states of health are thought to be less likely to become alloimmunized than patients transfused in a state of inflammation[14]. In contrast, having acute chest syndrome at the time of a transfusion is a significant risk factor for becoming alloimmunized[15], as is having a viral illness or other inflammatory disorder[16,17].

The clinical consequence of transfusing RBCs expressing an antigen against which a recipient has alloantibodies varies by situation. Some seemingly incompatible RBCs may continue to circulate in the transfusion recipient for the remainder of their lives. In contrast, other RBCs may be completely cleared within days after transfusion in the form of a delayed hemolytic transfusion reaction. In delayed hemolytic transfusion reaction, the clinical team may observe

fever, dark urine, or hemoglobin that drops back to the pre-transfusion level. Blood bank work-up may reveal a seemingly new antibody, which was likely present pre-transfusion but below the level of detection[7].

Increases in life expectancy and technological developments have led to an increase in the number of people with chronic-degenerative diseases and to more complex surgeries that require greater amounts of blood transfusions. Thus, multiply transfused patients are highly likely to form erythrocyte alloantibodies alone or in combination, autoantibodies, and antibodies to low-incidence antigens[7,11,12].

Judicious transfusion or transfusion avoidance is one strategy for preventing RBC alloimmunization. However, this is often not feasible. Matching for some blood group antigens is recommended for patients with SCD, to reduce formation of alloantibodies to matched immunogenic antigens such as D, C/c, E/e, and K91[18].

CONCLUSION

Alloimmunization is an important and frequent clinical condition that increases the risk of hemolytic reactions and is associated with significant patient morbidity and mortality. It is an important possibility in multiply transfused patients, mainly among those with onco-hematological diseases, hemoglobinopathies, and chronic renal failure, among other pathologies and should always be remembered as a differential diagnosis.

Erythrocyte phenotyping for the antigens of the Rh (D, C, E, e), Kell (K), Duffy (Fy^a, Fy^b), and Kidd (Jk^a, Jk^b) systems is an important procedure and aims to offer the patient the blood group with the lowest possible antigenic rate. Signs and symptoms of transfusion reactions should be observed and the possibility of emergency procedures should be considered in cases of hemolytic reaction, excluding other types of transfusion reactions such as Transfusion-Related Acute Lung Injury (TRALI), transfusion-related anaphylaxis, and sepsis.

Significant attention must be drawn to the elderly population, since they are more likely to have alloantibodies on account of previous blood transfusions, medical pathologies, and multiple pregnancies. Thus, clinical and transfusional history should be carefully investigated in order to minimize the risks of transfusion reactions, and when there is a risk that the patient is alloimmunized, transfusion of phenotyped blood components should be indicated.

Table 1: Distribution of pathologies in alloimmunized patients seen at the Hospital São Vicente de Paulo Hemotherapy Service

Disease	N	%
Cardiovascular Diseases	39	21.2
Malignant Diseases	38	20.5
Infections and Traumas	34	18.4
Gastrointestinal Diseases, Respiratory Diseases, Myelofibrosis or Malformations	32	17.3
Rheumatological and Renal Diseases	30	16.
Benign Hematological Diseases	12	6.4
	185	100%

Table 2: Frequency of alloantibodies identified in multiply transfused patients.

Alloantibodies	N	%
Anti-E	47	18
Anti-D	28	11
Anti-K	28	11
Inconclusive	23	8.9
Anti-C	21	8.1
Anti-M	14	5.4
Anti-P1	12	4.6
Anti-c	11	4.2
Anti-Le^a	11	4.2
Anti-Jk^a	10	3.9
Anti-S	9	3.5
Anti-Le^b	8	3.1
Anti-Lu^a	7	2.7
Anti-Di^a	7	2.7
Anti-Jk^b	6	2.3
Anti-Fy^a	6	2.3
Anti-C^w	4	1.5
Anti-s	3	1.2
Anti-Kp^a	2	0.8
Anti-e	2	0.8
	259	100%

Conflict of Interests

Autores declaram não ter conflito de interesse.

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