

Frequency and Specificity of Red Blood Cell Alloimmunization in Chilean Transfused Patients

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Keywords

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Summary

Background: Alloimmunization is an adverse effect of blood transfusions. In Chile, alloimmunization frequency is not established, and for this reason the aim of this study was to investigate the prevalence and specificity of red blood cell (RBC) alloantibodies in Chilean transfused subjects. **Methods:** Records from 4,716 multi-transfused patients were analyzed. In these patients, antibody screening was carried out prior to cross-matching with a commercially available two-cell panel by the microcolumn gel test, and samples with a positive screen were analyzed for the specificity of the alloantibody with a 16-cell identification panel. **Results:** The incidence of RBC alloimmunization in transfused patients was 1.02% (48/4,716) with a higher prevalence in women (40/48). We detected 52 antibodies, the most frequent specificities identified were anti-E (30.8%), anti-K (26.9%), anti-D (7.7%), and anti-Fy^a (5.8%). The highest incidence of alloantibodies was observed in cancer and gastroenterology patients. **Conclusion:** The data demonstrated a low alloimmunization frequency in Chilean transfused patients, principally associated with antibodies anti-E, anti-K, anti-D, and anti-Fy^a.

Introduction

Alloimmunization is an adverse consequence of exposition to red blood cell (RBC) antigens through transfusion, pregnancy, or transplantation. Red cell antibodies can provoke hemolytic transfusion reactions (HTR) the severity of which can vary from mild, with reduced efficacy of transfusion therapy, to extremely severe causing rapid death of the transfusion recipient [1].

The development of alloantibodies can significantly complicate transfusion therapy and results in difficulties in the cross-matching of blood. The origin of alloantibodies is explained by genetic differences between blood donors and recipients, dose and route of administration, and the immunogenicity of the antigen [2]. RBC alloimmunization has been associated to female sex, diabetes mellitus, and solid malignancy. The alloimmunization risk increased with the number of RBCs transfused [3]. The reported frequency of alloimmunization is contradictory and depends on several factors [4, 5]. In Chile, alloimmunization frequency is not yet established; for this reason, the aim of this study was to determine the prevalence and specificities of RBC alloantibodies in transfused Chilean patients with diverse diseases.

Material and Methods

A case-control study was designed. The individuals were selected from a retrospective examination of 4,716 unrelated Chilean patients included in the electronic laboratory information system. The patients had been admitted to the Hernán Henríquez Aravena Hospital in the city of Temuco, Chile, between January 2007 and July 2010. Patients who developed an unexpected antibody after receiving an RBC transfusion were included in the case group, from which the following information was collected: sex, age, diagnosis, number of units of

Table 1. Characteristics of transfused alloimmunized Chilean patients and controls

	Alloimmunized (n = 48) Mean (median; range)	Controls (n = 92) Mean (median; range)	p*
Age, years	54 (55; 1-97)	57 (62; 1-98)	NS
Sex			
Male	7	47	
Female	41	45	
Units of blood transfused	7 (3.5; 1-38)	6 (3.0; 1-28)	NS
Transfusion episodes	4.2 (2; 1-24)	3.3 (2; 1-16)	NS
Patients with malignancy, %			
Hematologic	12.5	13	NS
Solid tumors	16.6	7.6	

*p values from chi-square test.
NS = No significant differences.

transfused blood, number of transfusion episodes, and alloantibody specificity. In addition, a control group for alloimmunization was selected according to criteria previously described [6]. Briefly, we randomly selected patients with RBC transfusions who fulfilled the same criteria as case patients except for the fact that they did not develop an alloantibody.

From these patients, EDTA-anticoagulated blood samples were collected by standardized venipuncture. When the patients required a transfusion, cross-matching was performed. In addition, plasma samples were screened for the presence of RBC alloantibodies using a two-cell panel of reagents group O RBCs (Immucor, Norcross, GA, USA). For the indirect antiglobulin test (IAT), we employed a LISS-enhanced gel centrifugation technique (Bio-Type AGH; A&B Commercial, Santiago, Chile) that includes a polyspecific anti-human globulin (rabbit anti-IgG and monoclonal anti-C3d). When the antibody screening was positive, an antibody identification was performed using a commercial panel of 16 reagent cells (panocell-16; Immucor) of selected phenotypes using the same method. Patients received ABO/D compatible and non-leukocyte-depleted packed RBC transfusions.

Statistical Analysis

Statistical analysis was carried out using the Sigma Stat Software, Ver. 2.0 (Jandel Sci., San Rafael, CA, USA). Differences between the means of continuous variables were evaluated by Student's t-test. Categorical variables were analyzed using the chi-square test. The level of statistical significance was $\alpha = 0.05$.

Results

4,716 transfusion recipients were analyzed for alloantibodies from January 2007 to July 2010, and 48 cases of alloimmunization (1.02%) were identified. Table 1 shows the transfusion characteristics of both groups studied. In this study no associations between variables like sex, age, number of units transfused, and transfusion episodes were found.

Alloimmunization was more prevalent in subjects with malignancies and digestive bleeding, followed by those with renal disease, those with cardiac surgery and by traumatological patients (table 2).

The alloimmunized subjects produced a total of 52 RBC antibodies. The most frequent specificities identified were anti-E (30.8%), anti-K (26.9%), anti-D (7.7%), and anti-Fy^a (5.8%) (table 3). In 3 cases, a combination of antibodies was detected (anti-c + anti-E; anti-Fy^a + anti-Le^b; and anti-K+ anti-Js^b). Finally,

Table 2. Distribution of pathologies in alloimmunized Chilean patients

Disease group	Patients	
	n	%
Malignancies		29.2
Hematologic	6	
Solid tumors	8	
Gastrointestinal bleeding	8	16.7
Renal disease	5	10.4
Heart disease	5	10.4
Trauma	3	6.25
Infectious (sepsis)	2	4.2
Other diagnostic	11	22.9
Total	48	100

Table 3. Alloantibody specificity detected in transfused Chilean patient

Alloantibody	Frequency	
	n	%
Anti-E	16	30.8
Anti-K	14	26.9
Anti-D	4	7.7
Anti-Fya	3	5.8
Anti-c	2	3.8
Anti-Jka	2	3.8
Anti-M	2	3.8
Anti-Leb	2	3.8
Anti-Lub	1	1.9
Anti-Jsb	1	1.9
Anti-s	1	1.9
NI	4	7.7
Total	52	100

NI = Not identified.

4 patients (7.7%) showed pan-reactive antibodies. Table 4 shows the sex distribution of alloantibodies detected in transfused patients, and differences between both sexes were not significantly different.

Table 4. Sex distribution of alloantibodies detected in transfused Chilean patients

Alloantibody	Male, n (%)	Female, n (%)	p*
Anti-E	3 (42.9)	13 (31.7)	NS
Anti-K	4 (57.1)	10 (24.4)	NS
Anti-D	0	4 (9.8)	NS
Anti-Fy ^a	0	3 (7.3)	NS
Anti-c	0	2 (4.9)	NS
Anti-Jk ^a	0	2 (4.9)	NS
Anti-M	0	2 (4.9)	NS
Anti-Lu ^b	0	2 (4.9)	NS
Anti-Le ^b	0	2 (4.9)	NS
Anti-Jsb	0	1 (2.4)	NS
Anti-s	0	1 (2.4)	NS
NI	0	4 (9.8)	NS
Total subjects	7	41	

*p values from chi-square test.
NS = No significant differences; NI = not identified.

Discussion

Alloimmunization is an unfavorable effect of blood transfusions than can make transfusion therapy difficult. The alloimmunization frequency detected in transfused patients was 1.02%. A similar frequency was reported in Malaysia in a group of 5,719 patients, with an overall prevalence of 1.13% [7]. In the Costa Rican population, the frequency was 1.93% [8]. Contrarily, in China the prevalence of alloantibodies in a group of hospitalized patients was 0.21%, and a high rate of alloimmunization (10.49%) was observed in Brazilian transfused patients [5, 6]. These apparently contradictory frequencies may be explained, at least in part, by the ethnicity, based on the knowledge that different ethnicities have varying frequencies of erythrocyte antigens. According to several studies, the Chilean population presents a genetic background with Amerindian predominance [9–11].

Elevated frequencies of alloimmunization have been associated with hematology pathologies as thalassemia and sickle cell anemia [12, 13]. In this study, we did not detect this kind of patients because these pathologies are not frequent in our country. However, 12.5% of alloimmunized individuals were patients with hematologic malignancy, e.g. acute lymphocytic leukemia, non-Hodgkin's lymphoma, myelodysplastic syndromes or multiple myeloma, and 16.6% of patients had solid tumors that required continued RBC transfusions. In addition, we found a high rate of antibody production in patients with gastrointestinal bleeding (16.7%). In India the major incidence of alloimmunization was observed in gastroenterology patients [14].

The mean of units transfused and transfusion episodes in alloimmunized subjects was 7 and 4.2, respectively. No significant differences to the control group were established. This result is different to previous reports, where alloimmunization was associated with the number of units of blood received and the number of transfusion episodes [5, 15]. In a recent study, it was established

that the risk to developing alloantibodies increased with the cumulative number of RBC units transfused, rising to 6.5% at 40 units transfused [4]. In the present study, the antibody formation occurs relatively early, with a mean of 4.2 transfusion episodes, which agrees with other observations [16]. Only one subject received 38 units of RBCs.

Some studies suggest that the female sex could be a risk factor for the production of alloantibodies [3], but these results are controversial. Recently, a revision concludes that women should not be considered an at-risk group [17]. In this study, in the alloimmunized group, females were more prevalent (85.5%), but no differences exist when compared with the control group. In addition, we did not detect any significant differences in alloantibody production between both sexes with regard to the specificity of antibodies (table 4).

Regarding the specificity of antibodies, the most prevalent were anti-E (30.8%), anti-K (26.9%), anti-D (7.7%), and anti-Fy^a (5.8%). All antibodies detected were potentially hemolytic, which is in concordance with previous reports [4, 14, 15, 18]. It is important to note that the transfusions in the study hospital were not antigen-matched. All RBC transfusions done were compatible as shown by IAT at 37 °C. The implementation of extended blood typing for receptors that required periodical transfusions might be a useful alternative to improve the safety of blood transfusion in our institution.

Blumberg et al. [19] suggested that humoral immune responses to RBC antigens may be reduced by leukodepletion. However, despite leukoreduction, alloimmunization continues in RBC transfusion recipients. RBC-free hemoglobin and lipid mediators accumulate in microparticle and supernatant fractions of leukoreduced stored RBCs; moreover, apoptosis and loss of viability of residual white cells in leukodepleted units release immunostimulatory antigens, and mediators can sensitize the recipients [20, 21]. In our study, the units transfused are non-leukoreduced, only neonatal and hematocology patients received some leukoreduced hemocomponents. Recently, has been signed that leukoreduction and saline washing of the RBC concentrates may be an effective way to minimize immune effects in transfused patients [22]. Currently, universal leukoreduction has been adopted as quality policy in our hospital.

In summary, the data from the present study demonstrated a low alloimmunization frequency in Chilean transfused patients, principally associated with antibodies anti-E, anti-K, anti-D, and anti-Fy^a. However, this observation needs to be validated in a much larger Chilean population.

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Disclosure Statement

The authors have no conflict of interest to disclose.

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