Red blood cell and leukocyte alloimmunization in patients awaiting kidney transplantation

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Conflict-of-interest disclosure:
The authors declare no competing financial interest

Submitted: 10/24/2012
Accepted: 12/10/2012

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DOI: 10.5581/1516-8484.20130043

***Objective**: To determine the rates of red blood cell and leukocyte alloimmunization in patients with chronic kidney disease awaiting kidney transplantation.*

***Methods**: In this cross-sectional and prospective study, the serum of 393 chronic kidney disease patients on a transplant waiting list in Ceará, Northeastern Brazil were tested for red cell and leukocyte antibodies. In addition, demographic, clinical and laboratory data were collected.*

***Results**: The average age in the sample of 393 patients was 34.1 ± 14 years. Slightly more than half (208; 52.9%) were male. The average numbers of transfusions and gestations were 3.1 ± 3.3 and 1.6 ± 6, respectively. One third (33.6%) were alloimmunized: 78% with leukocyte antibodies, 9.1% with red cell antibodies and 12.9% with both. Red cell antibodies were detected in 29 cases (7.4%), 17 of whom were women, who had received more transfusions than the males (p-value < 0.0001). The most frequently detected red cell antibodies belonged to the Rh (24.1%) and Kell (13.8%) blood group systems. Leukocyte antibodies were detected in 30.5% of cases, 83 of whom were women, who had received more transfusions than the males (p-value < 0.0001) and were more reactive to panel reactive antibodies (p-value < 0.0001). The mean alloreactivity to panel reactive antibodies was 47.7 ± 31.2%.*

***Conclusion**: Chronic kidney disease patients on the transplant waiting list in Ceará, Brazil, display high rates of red cell (7.4%) and leukocyte (30.5%) alloimmunization. In this sample, alloimmunization was significantly associated with the number of transfusions and gender.*

***Keywords**: Kidney failure, chronic; Blood transfusion; Kidney transplantation; Antibodies; Erythrocyte transfusion; Cross-sectional studies*

Introduction

Chronic kidney disease (CKD) consists of renal injury followed by progressive and irreversible loss of kidney function[1]. Complications of CKD include anemia, most often due to insufficient renal production of erythropoietin[2]. Other factors may contribute to the appearance of anemia in CKD patients, such as iron deficiency, blood loss, hyperparathyroidism and inflammation[3]. In general, CKD patients require treatment for anemia, including iron supplementation, administration of erythropoietin and red blood cell (RBC) transfusions[4].

Blood transfusions to treat anemia poses several risks, including viral and bacterial infections, and RBC and leukocyte alloimmunization[5]. Depending on the center and the population studied, the incidence of RBC and leukocyte alloimmunization is 2.5-76% and 20-65%, respectively[6-9].

CKD patients in need of RBC transfusions are preferential targets of post-transfusion alloimmunization. At each transfusion, the patient is exposed to new foreign antigens, eventually becoming alloimmunized against RBC antigens. Due to the presence of contaminating leukocytes in blood products, patients may also become sensitized to antigens of the human leukocyte antigen (HLA) system[9,10]. As a result, RBC alloimmunization may limit the availability of compatible blood for future transfusions, whereas the development of leukocyte alloimmunization often makes it necessary to postpone transplantation.

The objective of the present study was to evaluate the profile of RBC and leukocyte alloimmunization in CKD patients awaiting kidney transplantation at a referral center in Northeastern Brazil.

Methods

This study was cross-sectional and prospective and relied on data retrieved from patient records. Serum samples were collected from 393 CKD patients on the transplant waiting list of the Ceará State Transplantation Service between January and May, 2011. The study protocol was previously approved by the Research Ethics Committee of the Universidade de Fortaleza (UNIFOR) under #003/2009.

Demographic, clinical and laboratory data (including gender, age, number of transfusions and number of gestations) were obtained by an active search of the records of the histocompatibility laboratory of the Ceará Center for Research in Heart and Kidney Diseases.

The RBC antibody study was conducted at the immunohematology laboratory of the Centro de Hematologia e Hemoterapia do Ceará (HEMOCE). Serum samples were initially tested with ID-DiaCell I and II (BioRad®). Positive samples were then submitted to antibody screening with ID-DiaPanel and ID-DiaPanel-P cells (BioRad®) using a gel centrifugation system in accordance with the manufacturer's instructions.

The leukocyte antibody study was conducted at the histocompatibility laboratory of the Ceará Center for Research in Heart and Kidney Diseases. Using a complement-dependent microlymphocytotoxicity test sensitized with human antiglobulin, the sera were tested against panel-reactive antibodies (PRA) consisting of 50 individuals phenotyped with HLA class I antigens representative of the local genetic variation. Based on the observed reactivity to PRA, patients were classified into three groups: Group 1 – patients not alloreactive to PRA (0%), Group 2 – patients mildly or moderately alloreactive to PRA (1-49%) and Group 3 – patients strongly alloreactive to PRA (≥ 50%).

The results were entered onto an Excel 2010 spreadsheet. The qualitative variables were expressed as frequencies while the quantitative variables were expressed as means ± standard deviation. The findings were submitted to Fisher's exact and ANOVA tests, followed by the Student-Newman Keuls test using the GraphPad Prism® software. The level of statistical significance was set at 5% (p-value < 0.05).

Results

Serum samples were collected from 393 patients, of whom 208 (52.9%) were male and 185 (47.1%) were female. The mean age was 34.1 ± 14 years (range: 4-77), with no significant difference between the genders (p-value = 0.1234).

Patients received 3.1 ± 3.3 transfusions on average with women receiving more than men (6.8 ± 4 vs. 1.1 ± 3; p-value = 0.0009). The mean number of gestations was 1.6 ± 6 per woman.

One hundred and thirty-two patients (33.6%) were alloimmunized, some with leukocyte alloantibodies only (103/132; 78%), some with RBC antibodies only (12/132; 9.1%), and some with both (17/132; 12.9%). Eighty-eight of the alloimmunized patients (66.7%) were female with, on average, 1.6 ± 1.3 gestations. Women received more transfusions than men (6.8 ± 4.1 vs. 0.5 ± 3; p-value < 0.0001). Table 1 shows the distribution of the 393 CKD patients with regard to mean age and the numbers of transfusions and gestations.

RBC alloantibodies were found in 29 patients (7.4%), of whom 17 (58.6%) were female and 12 (41.4%) were male. There were no significant differences between genders with regards to the mean age. In this group, the mean number of transfusions was 7 ± 3.1, with women receiving more transfusions than men (7 ± 3 vs. 3 ± 2.6; p-value = 0.0220). The most frequently detected RBC antibodies belonged to the Rh (24.1%), Kell (13.8%), Lewis (3.5%) and Diego (3.5%) blood group systems. Associations of two or more antibodies were observed in six (20.7%) patients. In ten (34.5%) patients, the antibodies could not be determined. Table 2 shows the frequency and specificity of the detected RBC antibodies.

Table 2 - Frequency and specificity of RBC antibodies detected in 29 of 393 patients with chronic kidney disease

RBC antibodies	n	%
Rh blood system	7	24.1
Anti-E	3	10.3
Anti-D	3	10.3
Anti-e	1	3.5
Other blood systems	6	20.7
Anti-Lea	1	3.5
Anti-Dia	1	3.5
Anti-K	4	13.8
Associations	6	20.7
Anti-D and Anti-C	1	3.5
Anti-K and Anti-Jk^a	1	3.5
Anti-E and Anti-Le^a	1	3.5
Anti-E, Anti-c and Anti-s	1	3.5
Anti-E, Anti-Dia and Anti-K	1	3.5
Anti-c, Anti-K and Anti-Jk^b	1	3.5
Not determined	10	34.5

Anti-Le^a: Lewis blood system; Anti-K: Kell blood system; Anti-Di^a: Diego blood system; Anti-Jk^a and Anti-Jk^b: Kidd blood system; Anti-E, anti-e, anti-D, anti-C and anti-c: Rh blood system; Anti-s: MNS blood system.

Table 1 - Distribution of 393 patients with chronic kidney disease according to age, gender and numbers of transfusions and gestations

Clinical data	Number of patients	%	Mean age (years)	Mean number of transfusions	Mean number of gestations	p-value
Alloimmunized	132	33.6	35.6 ± 13	5 ± 3.7*	1.1 ± 0.9	< 0.0001
Not alloimmunized	261	66.4	32.6 ± 13.3	1.3 ± 3.1	0 ± 1.1	
Red cell antibodies detected	12	9.1	40 ± 12.6	7 ± 3	2 ± 2	
Female	6	50	40 ± 7	7 ± 3.5*		< 0.0220
Male	6	50	36 ± 14.4	2 ± 1		
Leukocyte antibodies detected	103	78	34.8 ± 13.6	4.7 ± 3.8	1 ± 0.7	
Female	71	68.9	36.5 ± 13.4	6.8 ± 4.1*		< 0.0001
Male	32	31.1	31.5 ± 14	0.5 ± 3		
Red cell + leukocyte antibodies	17	12.9	45.9 ± 11	5.5 ± 3	0	
Female	11	64.7	44 ± 9.1	6.4 ± 2.4*		< 0.0001
Male	6	35.3	48 ± 13	4 ± 3.3		

* p-value < 0.05

Leukocyte antibodies were detected in 120 patients (30.5%), 83 of whom (69.2%) were female. There were no significant differences between genders with regards to the mean age. In this group, the mean number of transfusions was 4.7 ± 3.8, with women receiving more transfusions than men (5.6 ± 4.1 vs. 0 ± 2.9; p-value < 0.0001). The mean number of gestations per women was 1.2 ± 1.4. The mean level of alloreactivity to PRAs was 47.7 ± 31.2%. Sixty-six patients (55%) were classified as Group 2 (1-49% alloreactivity) while 54 (45%) were classified as Group 3 (> 50% alloreactivity). Females displayed greater alloreactivity than males (52.6 ± 28.2% vs. 23 ± 24%; p-value < 0.0001).

Discussion

The detection of RBC and leukocyte alloantibodies in, respectively, 7.4% and 30.5% of our sample of 393 CKD patients confirms the risk of alloimmunization to which this patient population is exposed through RBC transfusions. The 29 patients with RBC alloantibodies had been submitted to, on average, 7 ± 3.1 transfusions. The corresponding figure for the 120 patients with leukocyte alloantibodies was 4.7 ± 3.8 transfusions. These findings are supported by the literature showing that the risk of alloimmunization is dependent on the number and frequency of transfusions[11].

The RBC alloantibodies most frequently observed in our sample belonged to the Rh (24.1%) and the Kell (13.8%) blood group systems. Similar findings were obtained for multiply-transfused patients from Uberaba (Minas Gerais) and Catanduva (São Paulo)[10,12,13]. Thus, to help prevent RBC alloimmunization, phenotyping for the Rh and Kell blood group systems should be considered in patients requiring multiple transfusions[10]. RBC phenotyping has been performed at the Uberaba blood center since 1996 for patients with sickle cell disease, thalassemia, aplastic anemia, lymph and myeloproliferative diseases, refractory anemia and CKD[12]. Following the adoption of this transfusion policy, the incidence of RBC alloimmunization at the Uberaba blood center has been reduced to 0.75%, as opposed to the incidence observed in our study (7.4%) and in other Brazilian studies (3.2-20.8%)[12,14,15].

Associations of two or three RBC alloantibodies were observed in 27.2% of cases, and all included antibodies against the Rh blood group system. According to Baptista et al., the presence of associated antibodies makes it difficult to perform compatibility tests and identify compatible donors causing further delays due to the additional tests[16]. Furthermore, these antibodies can cause delayed hemolytic transfusion reactions and perinatal hemolytic disease[10].

Leukocyte alloimmunization can have complications with considerable clinical impact on transfusion medicine and organ transplantation, including platelet transfusion refractoriness and humoral rejection, respectively[9,17]. In the present study, 30.5% of the patients presented leukocyte alloantibodies with greater alloreactivity to PRA among women (52.6 ± 28.2%) than men (23.0 ± 24.0%), possibly because of the greater number of transfusions received (5.6 ± 4.1 vs. 0 ± 2.9) and additional sensitization through gestations (1.2 ± 1.4 per woman). These findings are supported by the literature which shows that anti-HLA antibody production is directly influenced by the number of previous transfusions, gestations and transplantations[18]. In fact, women sensitized to anti-HLA antibodies are commonly seen on the transplant waiting list[8]. Currently, 42% of the patients on the transplant waiting list in Ceará are > 50% reactive to PRA. Over two thirds (69.2%) are women with an average reactivity of 79 ± 24.3%[19].

Although 52.9% of our patients were male, RBC and leukocyte alloimmunization was more prevalent among women (58.6% and 69.2%, respectively). In the absence of significant age differences between the genders, gestations and a greater number of transfusions seem to be determining factors for the observed differences in alloimmunization. In a study on patients with sickle-cell disease, gender was not considered a risk factor but the number of transfusions received significantly influenced the incidence of alloimmunization in the sample[20]. On the other hand, in a study involving multiply-transfused patients, gender and alloimmunization were found to be strongly associated, as RBC alloantibodies were detected in 72.8% of the women in the sample[12]. Thus, transfusion to female CKD patients on the transplant waiting list should be carefully considered in view of the additional risk of alloimmunization from maternal-fetal incompatibility.

Conclusion

CKD patients on the transplant waiting list in Ceará, Brazil, display high rates of RBC (7.4%) and leukocyte (30.5%) alloimmunization. In our sample, alloimmunization was significantly associated with the number of transfusions and gender. The most frequently observed RBC alloantibodies belonged to the Rh and the Kell blood group systems. The average alloreactivity to PRA was 47.7 ± 31.2%, with higher rates of leukocyte alloantibodies among women (52.6 ± 28.2%).

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