Frequency and specificity of erythrocyte alloimmunization in patients at a reference hospital in Alagoas, Brazil

Frequência e especificidade da aloimunização eritrocitária em pacientes de um hospital de referência em Alagoas, Brasil

Frecuencia y especificidad de la aloinmunización eritrocitaria en pacientes de un hospital de referencia en Alagoas, Brasil

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Abstract

Objective: To determine the frequency and specificity of erythrocyte alloimmunization in patients treated by hemotherapy at the University Hospital Professor Alberto Antunes - UFAL, Maceió-Alagoas, in the period 2013-2018.

Methods: Retrospective analysis all patient records with request for red blood cell concentrate (RBC) with a positive erythrocyte antibody test.

Results: The incidence of erythrocyte alloimmunization was higher in women n=144 (80.45%) than in men n=35 (19.55%). We detected 13 antibodies, the most frequent specificities identified were anti-D (35.20%), anti-M (12.30%) and anti-E (7.82%). The combination of antibodies occurred in twenty-six cases with prevalence for combinations of antibodies of the Rh system, anti-D + anti-C (23.08%). The highest incidence of alloantibodies was observed in pregnant women.

Conclusion: The population studied had high levels of
alloimmunization through the Rh System, greater specificity of alloimmunization in women through anti-D and higher rate of alloimmunization among men over the age of 40. Further studies are necessary for a better understanding of red blood cell alloimmunization for population of Alagoas.

**Keywords:** Alloimmunization; Antibodies; Antigens; Hematological diseases; Transfusion.

**Introduction**

Erythrocyte alloimmunization occurs due to the genetic diversity of antigens present in red blood cells from blood donors and receptor. Depending on the type of antigen, it has great clinical importance due to its immunogenicity, inducing the immunological response with the production of IgG alloantibodies. Some examples include antigens from Rhesus (Rh), Kell (K system), Duffy (Fy) and Kidd (Jk) systems. In these cases, the response triggered by the immune system promotes red blood cell hemolysis in transfused patients subsequent, and as an effect, significant morbidity and mortality rates (Mitra et al., 2014; Tatari-Calderone et al., 2014; Pessoni et al., 2018).

For polytransfused patients with transfusion request, it is important to investigate the main antigens of Rh and Kell systems, in addition to the phenotyping of other antigens such as MNS, Duffy and Kidd. This is relevant in order to promote transfusion safety, reduce exposure to erythrocyte antigens and formation of alloantibodies, as well as to prevent acute and late hemolytic reactions, which can occur in the first 24h or after 24h of the transfusion, respectively (Jang et al., 2013). Additionally, it is assumed that there are other factors correlates to alloimmunization, such as the inflammatory state of the receptor and dose of immunogenicity of the antigen (Fasano et al., 2014).

Currently, blood transfusion is considered a safe and essential therapy for transfusion medicine (Peyrard et al., 2011). When indicated, red blood cell transfusion is important to compensate for acute anemia by offering oxygen support to the patient (Shah et al., 2019). For these reasons, it is crucial that these needs are met effectively, ensuring the prevention of erythrocyte alloimmunization in patients to which transfusion has been indicated.

Antibodies against the erythrocyte antigens can be produced after transfusion of an erythrocyte concentrate with
incompatible phenotype, which may cause acute or delayed hemolytic reaction in subsequent transfusions. During the gestational period, an alloantibody is formed when the fetus inherits an antigen from the father which the mother does not present. This may cause hemolytic disease of the fetus/newborn in future pregnancies, because the contact of the mother's and the baby's blood at the time of delivery causes the maternal organism to receive the child's red blood cells and begin to produce anti-Rh antibody. Thus, in a second pregnancy, if the baby is Rh+, the maternal organism has an anti-Rh antibody (Barbosa et al., 2018). In this sense, this study aimed at determining the frequency and specificity of erythrocyte alloimmunization in patients treated by hemotherapy at the University Hospital Professor Alberto Antunes - UFAL, located in the Maceió-Alagoas, in the period 2013-2018.

2. Methodology

The records of all patients who received RBC units were examined retrospectively by searching the computer database from Transfusion Agency database of Professor Alberto Antunes University Hospital (HUPAA), and administered by The Brazilian Company of Hospital Services (EBSERH). This is a federal teaching hospital, specialized in medium and high complexity, and monitoring of patients with risk pregnancy, hemoglobinopathies and oncohematological diseases in the Maceió-AL and surrounding cities.

An epidemiological study was carried out, outlined as its documentary, in which the results of RBC panels and the age, specificity and antibody combinations variables were analyzed, recorded in the database of the Immunohematology Laboratory of the HUPAA Transfusional Unit from 2013 to 2018.

This study was submitted and approved by the Ethics and Research Committee (CEP) of the Federal University of Alagoas - UFAL, under protocol No. 16666319.6.0000.5013 and under decision No. 3.516.930.

2.1 Inclusion criteria

The study considered all RBC transfusion requests with a positive agglutinin test (irregular antibodies), of patients both sexes and age, to generate quantitative data on the frequency and specificity of erythrocyte alloimmunization and of alloantibodies.

2.2 Exclusion criteria

Patients who had negative results for the search for erythrocyte antibodies were excluded.

2.3 Immunohematological tests

Whole blood samples from patients who have requested transfusion of red blood cells were selected for the detection of irregular antibodies by means of Test Red Blood Reagents for ID-System (ID-DiaCell I, II - BIO-RAD). The presence of agglutinated cells forming a red line on the gel surface or dispersed along the gel (ID-Card “LISS/Coombs - BIO-RAD) was considered a positive result. The presence of a compact cell button at the bottom of the microtube was considered a negative test.

The technique for detection for irregular antibodies consisted of dispensing 50 μL of the red blood cells I and II in the previously identified microtubes, and then adding 25 μL of the patient's plasma. The ID-Cards were incubated for 15 minutes at 37°C in an ID-Incubator. Subsequently, the ID-Cards were centrifuged for 10 minutes in ID-Centrifuge. Later, they were subjected to reading and we took notes on the results of the reactions.

The antibody specificity test was determined by using commercial red blood cell panels (BIO-RAD), performed by the Blood Center of Alagoas (HEMOAL), according to the manufacturer's instructions. The cases in which the specificity
could not be determined were released as unidentified antibodies. Cases of absence of agglutination were released as negative.

2.4 Statistical Analysis

Statistical analysis was performed using the software GraphPad Prism® Version 6.01 with the Fisher exact test, Chi-square test and Odds Ratio (OR). The level of significance was set at 5%. Alloantibody analysis (specificity, frequency and combined specificities) was only descriptive.

3. Results

3.1 Patient characteristics

During the study period, was performed 11,253 RBC transfusions. Of these, 7,618 (67.70 %) belonged female sex and 3,635 (32.30 %) male sex. A total of 179 had a positive diagnosis for erythrocyte alloantibodies prior to blood transfusion.

A total of 179 records of results of red blood cell panels were included in the study, which included patients with a request for transfusion of red blood cell concentrate on an urgent, scheduled and surgical reserve basis. Considering the investigated population, we found the presence of more alloantibodies in women n=144 (80.45%) than in men n=35 (19.55%), Chi-square test=12.56, 1; p-value=0.0004, statistically significant (α<0.05), OR=0.509 (0.351-0.739). The general data of the quantitative variables of the age groups regarding alloimmunization were subdivided into five groups. As for age, there was a higher frequency of alloimmunization among women over 20 years old up to 40 years old (G3 and G4 - 27.78 and 30.55%, respectively). For men, there was a lower incidence of alloimmunization when compared to women, with similar rates between and 11-20 to 21-30 old (G2 and G3) and 41 to >50 old (G5 and G6) (Table 1). Two cases of alloimmunization were recorded for G1, with the presence of anti- D and anti-C. These records belong to a child less than 1 year old and a newborn with 15 days of life, respectively. The case of newborn alloimmunization possibly is a passive alloimmunization, in which the circulating antibody belongs at mother, since he had not received previous transfusion. However, is the mother no evaluated in this cohort studied.

For erythrocyte alloimmunization, we found a female: male ratio of 4.1:1. The high frequency of alloantibodies in the female population was expected, since HUPAA has a maternity hospital to care for high-risk pregnant women.

Difference was observed in the rates of alloimmunization in respect to age for female sex. The frequency of alloimmunization was similar between male sex, when compared with age group. On calculating the odds ratio, higher relative risk was identified in relation to the occurrence of alloimmunization for G3 and G4. However, not difference was observed statistically significant for all group age studied (Table 1).

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Alloimmunized - n (%)</th>
<th>OR (95% CI)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>(G1) 0-10</td>
<td>0</td>
<td>2 (1.39)</td>
<td></td>
</tr>
<tr>
<td>(G2) 11-20</td>
<td>7 (20.00)</td>
<td>14 (9.72)</td>
<td>0.387 (0.016-9.137)</td>
</tr>
<tr>
<td>(G3) 21-30</td>
<td>7 (20.00)</td>
<td>40 (27.78)</td>
<td>1.080 (0.047-24.84)</td>
</tr>
<tr>
<td>(G4) 31-40</td>
<td>5 (14.28)</td>
<td>44 (30.55)</td>
<td>1.618 (0.068-38.28)</td>
</tr>
<tr>
<td>(G5) 41-50</td>
<td>8 (22.86)</td>
<td>17 (11.81)</td>
<td>0.412 (0.018-9.568)</td>
</tr>
<tr>
<td>(G6) &gt; 50</td>
<td>8 (22.86)</td>
<td>27 (18.75)</td>
<td>0.647 (0.028-14.85)</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>144</td>
<td></td>
</tr>
</tbody>
</table>

OR (Odds Ratio); *Fisher’s Exact Test; Statistically significant (alpha <0.05). Source: Authors (2022).
3.2 Erythrocyte antibodies

The specificities antibodies-prevalent found for 148 patients were anti-D > anti-M > anti-E > Anti-C > anti-K > anti-Fya > anti-c > anti Dia > anti-N > anti-Jka > anti-S > anti-Cw (Table 1). The 3 most prevalent antibodies were: anti-D (35.20%), anti-M (12.30%) and anti-E (7.82%) (Table 2).

All cases of alloimmunization by the anti-D were restricted to 63 (35.20%) female patients. From this total, 52 cases (82.54%) had a history of pregnancy. The distribution of the remaining alloimmunization cases followed the subsequent frequency: neoplasms 6 (9.52%), liver disease 2 (3.17%), ulcerative colitis 1 (1.59%), esophageal varices 1 (1.59%) and circulatory system disease 1 (1.59%). Anti-Fya and anti-M were the most frequent alloantibodies in the alloimmunization of male patients, 6 (17.14%) and 5 (14.28%), respectively.

In this study, the antibodies found in the Rh system (anti-D, C, c, E, Cw) are the most frequently in alloimmunization (51.95%). From the MNS blood group system we found anti-M, N and S in twenty-seven patients (15.09%). Despite the K System, we detected only anti-K in nine patients (5.03%). For Duffy System, nine cases with the anti-Fya (5.03%), Diego system, four cases with anti-Dia (2.23%), System Lewis, four cases with the anti-Lea (2.23%), and finally, Kidd system with two cases for the anti-Jka (1.12%).

Table 2. Specificity and frequency of antibodies identified in alloimmunized patients at the Hemotherapy Service of HUPAA-Ufal.

<table>
<thead>
<tr>
<th>Antibody specificity</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (n)</td>
</tr>
<tr>
<td>Anti-D</td>
<td>63</td>
</tr>
<tr>
<td>Anti-M</td>
<td>22</td>
</tr>
<tr>
<td>Anti-E</td>
<td>14</td>
</tr>
<tr>
<td>Anti-C</td>
<td>11</td>
</tr>
<tr>
<td>Anti-Fya</td>
<td>9</td>
</tr>
<tr>
<td>Anti-K</td>
<td>9</td>
</tr>
<tr>
<td>Anti-c</td>
<td>4</td>
</tr>
<tr>
<td>Anti-Dia</td>
<td>4</td>
</tr>
<tr>
<td>Anti-Lea</td>
<td>4</td>
</tr>
<tr>
<td>Anti-N</td>
<td>3</td>
</tr>
<tr>
<td>Anti-Jka</td>
<td>2</td>
</tr>
<tr>
<td>Anti-S</td>
<td>2</td>
</tr>
<tr>
<td>Anti-Cw</td>
<td>1</td>
</tr>
<tr>
<td>Inconclusive*</td>
<td>31</td>
</tr>
<tr>
<td>Total</td>
<td>179</td>
</tr>
</tbody>
</table>

* Unidentified antibody. Source: Authors (2022).
Interestingly, anti-Dia (2.23%) was one of the less prevalent antibody in our population (Table 2). In one patient, anti-Dia was found associated with other antibody (Table 3).

All the results of inconclusive samples n=31 (17.32%) showed irregular antibodies testing positive. Most of these patients were suffering from cancer n=17 (54.84%), including onco-hematologic diseases such as myeloma (n=6) and leukemia (n=2). Of the remaining patients, eight had a gestational history (25.81%), three had anemia (9.69%), one with sickle cell anemia (3.22%), one with adrenocortical insufficiency (3.22%) and one case of megaesophagus (3.22%).

The combination of antibodies occurred in twenty-six cases with prevalence for combinations of antibodies of the Rh system, anti-D + anti-C (23.08%) (Table 3). However, the differentiation of combined anti-D, -C and -G is not realized for routine transfusion in our service of hemotherapy. There was only one case (3.85%) of combination with three antibodies (anti-E, anti-K and unidentified antibody (possibly a low-frequency antibody). The remaining cases, n = 25 (96.15%) reported the presence of two antibodies.

Table 3. Combined specificities of antibodies identified in HUPAA patients.

<table>
<thead>
<tr>
<th>Antibody specificity</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-D, - C</td>
<td>6</td>
</tr>
<tr>
<td>Anti-C, - inconclusive *</td>
<td>3</td>
</tr>
<tr>
<td>Anti-D, - E</td>
<td>2</td>
</tr>
<tr>
<td>Anti-D, - inconclusive*</td>
<td>2</td>
</tr>
<tr>
<td>Anti-M, - inconclusive*</td>
<td>2</td>
</tr>
<tr>
<td>Anti-E, - M</td>
<td>1</td>
</tr>
<tr>
<td>Anti-E, - Fya</td>
<td>1</td>
</tr>
<tr>
<td>Anti-E, - Diα</td>
<td>1</td>
</tr>
<tr>
<td>Anti-K, - Fya</td>
<td>1</td>
</tr>
<tr>
<td>Anti-D, K</td>
<td>1</td>
</tr>
<tr>
<td>Anti-Jka, - inconclusive*</td>
<td>1</td>
</tr>
<tr>
<td>Anti- D, - M</td>
<td>1</td>
</tr>
<tr>
<td>Anti-E, - S</td>
<td>1</td>
</tr>
<tr>
<td>Anti-Fya, - inconclusive*</td>
<td>1</td>
</tr>
<tr>
<td>Anti-K, - inconclusive*</td>
<td>1</td>
</tr>
<tr>
<td>Anti- E, - K, inconclusive*</td>
<td>1</td>
</tr>
</tbody>
</table>

Total combinations 26

* Unidentified antibody. Source: Authors (2022).
4. Discussion

This retrospective study was conducted in order to verify the frequency and specificity of erythrocyte alloantibodies in the population of patients treated by the Hemotherapy Service of HUPAA-UFAL-EBERH. We demonstrated that 13 different antibodies were responsible for alloimmunization in 148 patients. In addition, 31 results were expressed as an unidentified antibody, which may be correlates to low-frequency antigens not found in the red cell pool testing, which suggests a limitation of the method adopted in the laboratory routine. Some studies (Hill et al., 2017) defend another reason for the incidence of unidentified alloantibodies. These authors agree that some alloantibodies are detected only with the use of special techniques such as prolonged incubation, use of red blood cells treated with enzyme and through low ionic concentration. In all cases in which blood transfusion was necessary in patients with unidentified alloantibodies, we considered the donor/receptor compatibility test compatible.

In this study, most cases of alloimmunization were female patients with a gestational history and Rh negative. The most frequent cause of erythrocyte alloimmunization is RhD negativity, approximately 80% (Lindenburg et al., 2012). According to another study (Xu et al., 2012), women are at higher risk for developing red blood cell alloimmunization. From the same perspective (Verduin et al., 2012) correlates the rate of alloimmunization in women by the number of previous pregnancies, in view of the greater allogeneic exposure. We must remember that women of childbearing age and multiparous women have a higher rate of fetal-maternal alloimmunization. In contrast, women with chronic diseases have a lower parity and alloimmunization index, as highlighted (Neto et al., 2018) which may be correlates to our results.

The diagnosis of RhD negative patients in prenatal screening and the prophylactic use of RhD immunoglobulin reduced the incidence of severe hemolytic disease of the fetus/newborn. On the other hand, non-RhD antigens still contribute to perinatal morbidity and mortality (Li et al., 2017). For this reason, it is recommended to prevent the synthesis of alloantibodies in Rh negative women of childbearing age, conducting irregular antibody testing (PAI) and ABO and RhD phenotyping, as well as other unexpected antibodies (AABB, 2017; Webb & Delaney, 2018).

Despite our findings, we did not correlates gender as a predisposition for erythrocyte alloimmunization in line with other inspiring studies (Ameem et al., 2002; El-Danasoury et al., 2012; Thedsawad et al., 2019). Data found in literature review point to divergences, with a higher risk of erythrocyte alloimmunization in women (Reisner et al., 1987) and other more recent studies in male patients (Saied et al., 2011). Our study was unable to demonstrate associations between the number of blood transfusions and the development of erythrocyte alloimmunization in polytransfused patients. The previous transfusion data was not available for these patients. Therefore, it was not a parameter analyzed.

Investigations on the relationship between hyperbilirubinemia and alloimmunization in 107 newborns and detected irregular antibodies in seven cases (6.5%) (Orgun et al., 2010). According to the authors, ABO/Rh incompatibilities are the most common causes of discrepancies in blood groups of newborns, which may progress to hyperbilirubinemia and perinatal hemolytic disease. In our study, we verified the existence of only two cases of alloimmunization in minors during the study period, revealing a lower rate of alloimmunization than that described (Orgun et al., 2010).

The presence of anti-M was diagnosed in seven patients with a history of previous pregnancy. Despite Anti-M is a relatively common antibody and IgM occurs naturally and is considered to be clinically insignificant, we not evaluate if these antibodies they were IgG or IgM. According to the literature the anti-M alloimmunization can cause of fetal hemolytic disease and few cases are reported (Li et al., 2017). The authors reported a diagnosis of severe fetal anemia attributed to anti-M in a pregnant woman with a history of three intrauterine deaths. After regular monitoring during pregnancy and five intrauterine transfusions, they managed to correct anemia until the baby was born. Therefore, the formation of this antibody in the gestational period is a high risk factor for the fetus, since there may be a compromise to its development in the intrauterine phase.
Despite the low frequency, some antibodies against natural erythrocyte antigens can be developed without any prior exposure to foreign red blood cells (Hudson et al., 2010) and can be associated with a hemolytic transfusion reaction (Jens et al., 2005). The development of natural antibodies has not yet been fully elucidated, and it is suggested that the stimulus for the production of one or more of these antibodies starts from antigenic similarities between environmental or microbial substances with blood group antigens (Zimring et al., 2016).

The transfusion agency adopts the recommendations of the current Brazilian legislation (BRASIL, 2016) for phenotyping of erythrocyte antigens in the blood of the recipient of the Rh (E, e, C, c), Kell (K) and Kidd (Jka) systems for alloimmunized patients. However, excluding cases of gestational alloimmunization, patients may possibly have received blood transfusions with incompatible phenotype in other health services. These cases reinforce the importance of determining the profile of erythrocyte antigens in blood donors and in patients who are candidates for blood transfusion, in order to prevent the occurrence of alloimmunization. These measures require greater efforts by government agencies and hemotherapy services, in view of the exhaustive searches for compatible red blood cells, causing difficulties and delays in carrying out transfusions, in addition to the economic expenses with the consumption of more inputs needed for the performance of immunohematological tests needed.

Considering the cases of erythrocyte alloimmunization (n=179), we found a lower alloimmunization rate for patients with oncohematological diseases (4.73%). In our service, these results may be correlates to the blood transfusion protocol adopted by the HUPAA immunohematology sector, where it is recommended to perform erythrocyte phenotyping for all patients with oncohematological disease, as well as donors, for compatible phenotypic transfusion, in order to avoid cases of alloimmunization according to the current guidelines of Brazilian legislation. Another factor that could be correlates, would be the low sensitization in immunocompromised individuals, both for the basic oncological pathology, as well as the chemotherapy and radiotherapy treatments carried out to control the pathology (Schonewille, 2009).

An inspiring study determined the rate of erythrocyte alloimmunization in patients with hematological neoplasms and found rates of variable alloimmunization according to the type of pathology (Asare et al., 2016). For the authors, the highest rates were for myelodysplastic syndromes (8/71; 11.2%), acute leukemia (1/34; 2.9%) and myeloproliferative disorders (2/75; 2.7%). Other hematological neoplasms were found with rates below 2% in the studied patient population. Thus, the data in this study, as well as ours, demonstrated that in patients with hematological neoplasms, the rates of erythrocyte alloimmunization are lower than in other diseases.

In this study, the most common antibodies in the studied population corresponded to the clinically significant Rh (51.95%) and MSN (15.09%) systems (Pessoni et al., 2018). A similar result for the Rh System was found in the another survey (Neto et al., 2018) who assessed the clinical and epidemiological profile of alloimmunized and autoimmunized multi-transfused patients reporting 53.11% of Rh system antibodies. However, in that same study, the result for the MSN System (6.25%) was lower than that found by us. Also was investigated erythrocyte alloimmunization in hospitalized patients and identified 58.6% of cases of alloantibodies (Pessoni et al., 2018). In addition, the authors revealed 27.5% of cases of inconclusive identification, a result superior to ours (17.32%). In both cases, it was not possible to confirm alloimmunization.

Determined the incidence and rate of erythrocyte alloimmunization in polytransfused patients and reported that the most frequent combinations were through the Rh System and anti-K specificities, corresponding to 20% of the antibody combinations (Cruz et al., 2011). We have shown results below these, with a rate of 14.52% of combined antibody cases.

Although our results has been revealed frequency of 23.08% for the Anti-D, -C combination, it is not part our routine perform anti-G detection even in pregnant women. For this reason, is not possible to distinguishing presence of anti-G antibody together with anti-D and anti-C in this study. The differentiation of anti-D and anti-C from anti-G is not necessary for routine transfusion - on the other hand, during pregnancy, it is necessary because anti-G can masquerade as anti-D and anti-C
with initial antibody testing. Additionally, pregnant with positive anti-D or anti-G are at risk of developing hemolytic disease of the fetus and newborn and need close obstetrician monitoring (Yousuf et al., 2017).

Dia antigen frequency is variable among populations. In this study we reveal outcomes different of those found in São Paulo. We showed a low incidence of anti-D-Dia when compared to the literature, 2.26 vs 6.02%, respectively. This is important because these antibodies are great clinical importance, as they can cause delayed hemolytic transfusion reactions (Cruz et al., 2011).

We also found that there is a heterogeneity of alloantibodies present in the population studied and they can cause sensitization or destruction of the recipient's red blood cells during or after blood transfusion (Younesi et al., 2016). On the other hand, these risks decrease when there is homogeneity between the blood donor and recipient blood erythrocyte antigens (Pai Schun-Chung, et al., 2010). For these reasons, we were motivated to know the profile and specificity of alloantibodies of patients treated by the hemotherapy service of HUPAA-UFAL, so far not studied.

5. Conclusion

Our results demonstrated high levels of Rh System alloimmunization, greater specificity of alloimmunization in women through anti-D and higher rate of alloimmunization among men over the age of 40. Several factors may have contributed to these findings, such as disproportionality between sex and age variables, blood transfusion without phenotypic compatibility and the presence of natural alloantibodies. Further studies are necessary for a better understanding of the etiopathogenesis and pathophysiology of red blood cell alloimmunization of HUPAA-UFAL users, mainly due to the lack of reports in the literature for alloimmunization in the population of Alagoas.

Acknowledgments

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References


