



## Original article

# Red blood cell alloimmunization among hospitalized patients: transfusion reactions and low alloantibody identification rate



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## ABSTRACT

**Background:** Unexpected red blood cell alloantibodies can cause hemolytic transfusion reactions. In this study, the prevalence of alloimmunization, the rate of identification of alloantibodies and the rate of blood transfusion reactions among transfused patients were identified in a clinical emergency hospital in Brazil.

**Methods:** Transfusions and clinical records of patients who had a positive indirect antiglobulin test between January and December 2013 were analyzed.

**Results:** Of 1169 patients who received blood transfusions, 28 had positive indirect antiglobulin tests, with one patient having two positive tests at different times, resulting in 29 positive tests during the period of this study. Alloantibodies were identified in 58.6% (17/29) of the cases. In 27.5% (8/29), identification was inconclusive and it was not possible to confirm alloimmunization. The rate of red blood cell alloimmunization was 1.71% (21/1169). Of 21 cases of alloimmunization, four (19%) were unidentified due to an unusual agglutination profile. All identified alloantibodies were clinically significant (10/17 anti-Rh, 5/17 anti-Kell and 2/17 anti-MNS). In two patients who had positive indirect antiglobulin tests, one had an unidentified alloantibody, and the other had an inconclusive test and developed a hemolytic transfusion reaction.

**Conclusion:** The prevalence of clinically important red blood cell alloantibodies and hemolytic transfusion reactions among patients with unidentified alloantibodies suggests that specific laboratory techniques should be performed to identify alloantibodies in cases of pan-reactivity or autoantibodies to improve transfusion safety.

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## Introduction

Alloimmunization against red blood cell (RBC), leukocyte and platelet antigens occurs due to the genetic diversity of antigens between blood donors and recipients.<sup>1</sup> Some of the clinically significant antigens that induce the production of IgG alloantibodies involve the Rhesus (Rh), Kell (K), Duffy (Fy) and Kidd (JK) systems.<sup>2</sup> These antibodies react at 37 °C and cause hemolysis in transfused patients, resulting in significant morbidity and mortality.<sup>3</sup>

The frequency and specificity of red blood cell (RBC) alloantibodies varies according to the genetic diversity of the population<sup>4</sup> and occur in 0.3–2% of the general population.<sup>5</sup> Alloimmunization rates lie between 0.009% and 0.6% in healthy donors,<sup>6</sup> 1.4% and 4.24% in previously transfused individuals,<sup>7</sup> 44% in patients with hematologic malignancies such as myelodysplastic syndrome,<sup>8</sup> 20% in patients with thalassemia<sup>9</sup> and 18.7% in people with sickle cell disease.<sup>10</sup>

Autoantibodies can also be detected concurrently with RBC alloantibodies at rates up to 28%.<sup>11</sup> These autoantibodies can exert a masking effect, leading to non-detection of antibodies, or impede the correct identification of alloantibodies by the hemagglutination gel method.<sup>12</sup> The dilution technique may be useful for discriminating autoantibodies by mimicking specificity of alloantibodies.<sup>13</sup> Furthermore, many alloantibodies are not detected because of a low titer of antibodies, the absence of the antigen in the testing panel, or multiple antibodies.<sup>14,15</sup> This often results in a higher risk of delayed hemolytic transfusion reactions because of the selection of inappropriate RBC units for transfusion.<sup>16</sup>

For multi-transfused patients, matching for major Rh and Kell antigens and further extended typing to include MNS, Duffy, Kidd and other immunogenic antigens is considered especially important to improve transfusion safety by reducing alloantibody formation and to avoid hemolytic reactions during blood transfusions.<sup>17</sup>

In this context, this study aimed to evaluate the rate of alloimmunization in transfused patients, the rate of identification of alloantibodies and the occurrence of hemolytic transfusion reactions of alloimmunization patients in a hospital in Brazil.

## Methods

This retrospective study was conducted in the Hospital das Clínicas da Universidade Federal de Goiás (HC/UFG), Goiânia, Brazil. This is a general public teaching hospital linked to the Brazilian National Health Service specializing in tertiary health care and monitoring of patients with sickle cell anemia and clinical emergencies. All patients from HC/UFG between January and December 2013 with a positive indirect antiglobulin test (PIAT) regardless of age, gender and number of prior transfusions were included in this study. Transfusion and clinical records of these transfused patients were analyzed. The variables analyzed were ABO group, Rh system, PIAT, direct antiglobulin test (DAT) and autoantibodies.

According to the transfusion agency of the hospital, the indirect antiglobulin test (IAT) was processed manually by

the gel hemagglutination technique, with Serescan Diana 2 reagent (I and II) (Grifols®, Paraná, Brazil) in gel plates (Grifols®, Paraná, Brazil) according to the manufacturer's guidelines. In cases of PIAT, antibody identification was performed using the RBC 11 Identisera Diana panel (1 to 11) (Grifols®, Paraná, Brazil). The RBC 11 panel includes the following antigens: D, C, E, c, e, C<sup>w</sup>, K, k, Kp<sup>a</sup>, Kp<sup>b</sup>, Js<sup>a</sup>, Js<sup>b</sup>, Fy<sup>a</sup>, Fy<sup>b</sup>, Jk<sup>a</sup>, Jk<sup>b</sup>, Le<sup>a</sup>, Le<sup>b</sup>, P<sub>1</sub>, M, N, S, s, Lu<sup>a</sup>, Lu<sup>b</sup> and Xg<sup>a</sup>. Some samples presented unusual agglutination profiles and the antibodies were unidentified. In pan-reactive cases, the sample was considered 'inconclusive'.

All patients receiving RBCs were phenotyped for the ABO and Rh antigens and received ABO and Rh antigen-compatible RBCs. The phenotyping of RBCs for CcEe antigens and for the Kell antigen was performed on samples from patients with sickle cell anemia before the first transfusion in the hospital and they received phenotyped Rh (CcEe) and Kell-compatible RBCs whenever necessary. However, these patients may have been transfused at other hospitals where RBC phenotyping was not performed.

For the patients who had PIAT, the alloantibodies were identified, and these patients received antigen-negative RBCs for the detected alloantibody.

This study was approved by the Research Ethics Committee of the HC/UFG (CAAE N° 39854114.8.0000.5078). Data were analyzed using EpiInfo™ for Windows version 7.1 (CDC – Centers for Disease Control and Prevention).

## Results

A total of 1169 patients received 10,516 blood transfusions from January to December 2013 and 28 (2.4%) patients with a PIAT were identified. One patient had a PIAT at two different times, and two different alloantibodies were identified, resulting in 29 PIATs during the period of this study. Of 29 cases of PIATs, 17 (58.6%) RBC alloantibodies were identified, four (13.8%) were unidentified, and eight (27.5%) were inconclusive due to pan-reactivity. Thus, 21 (1.71%) cases of alloimmunization were identified in 20 patients.

The most frequent specificities of alloantibodies identified were anti-E (5/17; 29.4%), anti-Kell (5/17; 29.4%), anti-D (3/17; 17.6%), anti-S (2/17; 11.76%), anti-C (1/17; 5.8%) and anti-c (1/17; 5.8%). All alloantibodies identified were clinically significant. Autoantibodies (agglutination degree ≤2+) and DATs were positive in 4/17 (23.5%) cases of alloimmunization with identified alloantibodies (Table 1). The patient who presented PIATs at two different times was male and had leukemia. At the first PIAT, he was 37 years old, had received four transfusions and the anti-S alloantibody was identified. At the second PIAT, he was 38 years old and had received 28 transfusions; the anti-D alloantibody was detected (Table 1).

Of 17 alloimmunized women, 11 (64.7%) had a history of pregnancy. The anti-Kell was the most common alloantibody (4/11; 36.4%), followed by anti-E (3/11; 27.3%). Anti-C was identified in one patient of four females without any history of pregnancy or history of transfusion; this was an 11-year-old girl with sickle cell anemia and without any history of surgery. The other three women without a history of pregnancy had a

**Table 1 – Transfusion and clinical characteristics according to identified (n = 17) and unidentified alloantibodies (n = 4).**

Alloantibody	Gender	Pregnancy history (n)	Age (years)	Clinical indication	Transfusion history (n)	Surgery history	DAT	Autoantibody	Blood type	Phenotype
Anti-E	F	7	38	SCA	5	Yes	–	–	O+	NR
Anti-E	M	#	10	SCA	20	NR	–	–	O+	ce
Anti-E	F	NR	63	Other	3	Yes	–	–	O+	ce
Anti-E	F	0	8	SCA	47	NR	+	+	O+	Cce
Anti-E	F	2	54	Other	10	Yes	+	+	A+	Ce
Anti-Kell	F	2	44	PA	2	Yes	–	–	AB+	NR
Anti-Kell	F	3	47	PA	2	Yes	–	–	A+	NR
Anti-Kell	F	5	46	Other	2	Yes	–	–	A+	NR
Anti-Kell	F	1	49	SC	2	Yes	–	–	B+	ce
Anti-Kell	M	#	54	Other	12	Yes	2+	2+	B+	NR
Anti-D <sup>a</sup>	M	#	38	Leukemia	28	NR	–	–	A–	NR
Anti-D	F	3	48	PA	1	Yes	+	–	O–	NR
Anti-D	F	1	30	Other	0	Yes	+	+	O–	ce
Anti-S <sup>a</sup>	M	#	37	Leukemia	4	NR	3+	–	A–	NR
Anti-S	F	5	57	PA	0	Yes	–	–	A+	ce
Anti-C	F	0	11	SCA	0	No	–	–	O+	cEe
Anti-c <sup>b</sup>	F	0	13	SCA	1	NR	–	–	O+	Cec
Unidentified										
1	F	4	30	SC	0	Yes	–	–	O+	ce
2	F	NR	55	PA	0	NR	–	–	A–	ce
3	F	2	58	Other	2	Yes	–	–	O–	ce
4 <sup>c</sup>	F	0	27	SCA	3	No	–	–	B+	CC <sup>w</sup> eKell

DAT: direct antiglobulin test; M: male; F: female; NR: not reported. SCA: sickle cell anemia; Other: other clinical indications; PA: postoperative anemia; SC: hemoglobinopathy SC; #: not applicable.

<sup>a</sup> Patient with positive IAT at two different times.

<sup>b</sup> Likely anti-c (CW not tested).

<sup>c</sup> Case of blood transfusion reaction.

**Table 2 – Transfusion and clinical characteristics of patients with inconclusive test results (n = 8).**

Gender	Pregnancy history (n)	Age	Clinical Indication	Transfusion history (n)	Surgery history	Autoantibody	DAT	Blood type
M	#	69	Lymphoma	15	Yes	+	+	O+
F	3	53	SLE	2	NR	4+	4+	O+
F	2	63	Chagas disease	0	NR	3+	3+	A+
F	5	66	Lymphoma	0	Yes	4+	4+	AB+
M	#	71	Leukemia	0	No	3+	+	A+
F	8	76	Leukemia	28	No	2+	3+	A+
M	#	63	Lymphoma	2	NR	2+	4+	O+
F	0	13	SCA	43	NR	+	4+	O+

DAT: direct antiglobulin test; M: male; F: female; SLE: systemic lupus erythematosus; SCA: sickle cell anemia; NR: not reported. #: not applicable.

history of transfusions. For two of the women (11.7%), there was no information about prior pregnancy.

All samples with inconclusive results (n=8) had autoantibody-positive DATs and PIATs. The majority (6/8; 75%) of these patients had hematological diseases, including three (50%) with lymphoma, two (33.3%) with leukemia and one (16.6%) with sickle cell anemia. Of the remaining patients (2/8, 25%), one had systemic lupus erythematosus and the other had Chagas heart disease (Table 2).

The overall rate of transfusion reactions in the period of the study was 0.34% (4/1169). Two of the patients had non-immune transfusion reactions and negative IATs. One patient, a 69-year-old male with lymphoma, had an inconclusive alloantibody test due to a positive DAT and a positive autoantibody test sixteen days after a blood transfusion reaction. The IAT was negative on the day of the reaction. He

suffered anaphylactic shock, which was not related to RBC alloantibodies, after the third RBC infusion. A tracheostomy was performed, and the patient was hospitalized in the intensive care unit for seven days after which he recovered and he was discharged from the hospital. Another patient, a 27-year-old female with sickle cell anemia had an unidentified alloantibody and presented an acute hemolytic transfusion reaction related to RBC alloantibodies; her hemoglobin level dropped while her lactic dehydrogenase level increased. No cases of transfusion reactions were observed for the 17 patients who had identified alloantibodies.

## Discussion

The frequency of alloimmunization detected in transfused patients at a clinical emergency hospital in Brazil was 1.71%.

Elevated frequencies of alloimmunization are associated with hematologic pathologies such as myelodysplastic syndrome, thalassemia and sickle cell anemia and are correlated to the large number of transfusions and the proinflammatory state of patients.<sup>18,19</sup> Considering that 55% of the patients in this study were hospitalized due to clinical emergencies and the other 45% were hospitalized due to hematologic diseases, the frequency of alloimmunization was lower than the frequency described in these populations in Brazil.<sup>20</sup> However, it is known that the characteristics of the population of the study, the sensitivity of the laboratory tests used, and the follow-up of patients are some factors associated with this large variation in alloimmunization frequencies.<sup>21</sup>

In this study, the majority of alloimmunized patients were female and three of the five women without previous transfusions had gestational history. Females have a higher risk for RBC alloimmunization<sup>22</sup> with some studies showing a positive correlation between the number of previous pregnancies and the rate of alloimmunization due to greater allogeneic exposure.<sup>23</sup> An 11-year-old female patient with sickle cell anemia but without any history of transfusion and without a history of pregnancy, developed anti-C alloantibodies. In some situations, some 'naturally occurring' RBC antibodies can be produced without prior exposure to foreign RBCs.<sup>24</sup> The stimulus for most of these antibodies is not clear, and it is often suggested that antigenic similarities between environmental or microbial substances with blood group antigens may result in their production.<sup>25</sup>

Alloimmunization is associated with the number of units of blood received and the number of transfusion episodes.<sup>18</sup> In the present study, most alloimmunized patients (16/21:76.2%) had a history of blood transfusions prior to alloimmunization. However, we cannot state that the number of transfusions was the major factor associated with alloimmunization in this sample because alloimmunization depends on dose, immunogenicity and clinical aspects, such as proinflammatory conditions. In addition, some patients are non-responders, even when exposed to high levels of RBC antigens.<sup>26,27</sup>

Regarding the specificity of antibodies, anti-Rh antigens and anti-Kell antigens were the most prevalent. These data are in concordance with previous Brazilian reports.<sup>20</sup> The specificity of RBC alloantibodies varies according to the study population. In Japan, anti-Rh antigens (E, c) and anti-Lewis (Lea) are the most prevalent<sup>4</sup>; in India and China, anti-Rh (E, D) and anti-MNS (M)<sup>6,22</sup>; in Korea, anti-Lewis (Lea)<sup>28</sup>; in Chile, anti-Rh (D, E) and anti-Kell (K)<sup>29</sup>; and in Iran, anti-Kell (K).<sup>30</sup>

The transfusion agency performed RBC phenotyping for patients with sickle cell anemia before initiating chronic transfusions to avoid alloimmunization as recommended by current Brazilian legislation.<sup>31</sup> However, in this study, patients with sickle cell disease were alloimmunized. These patients may have received blood transfusions in other medical services prior to RBC phenotyping. In addition, other studies have demonstrated high rates of alloimmunization despite prophylactic phenotypic matching in patients with sickle cell anemia.<sup>32</sup>

The screening for autoantibodies was positive in twelve samples in the present study. Alloantibodies were identified in four samples with a degree of agglutination of  $\leq 2$ . The

rate of alloimmunization in patients with a positive DAT is significantly higher (12.5%) when compared to the alloimmunization rate in DAT-negative patients (1.6%).<sup>33</sup> Ahrens et al.<sup>11</sup> demonstrated that alloimmunization is the most important cause of autoimmunity and may occur simultaneously to blood transfusions in up to 75% of recipients. In addition, autoantibodies can mimic the specificity of alloantibodies in up to 27% of cases of PIAT.<sup>13,34</sup> Therefore, the use of techniques such as ZAP adsorption (combination of papain and dithiothreitol) and polyethylene glycol (PEG),<sup>35</sup> and the dilution technique<sup>13</sup> to remove autoantibodies, allows the identification of alloantibodies. This differentiation of autoantibodies from the mimicking of real alloimmunization is very important, due to the possibility of blood transfusion reactions. However, some studies demonstrated that mimicking autoantibodies cannot cause transfusion reactions.<sup>13,34,36</sup> In the current study, techniques to remove autoantibodies were not used and it was not possible to know if there was a case of mimicry.

Eight samples with high degrees of agglutination due to autoantibodies were inconclusive. These high concentrations of autoantibodies have a masking effect, leading to non-detection of alloantibodies.<sup>12</sup> If the reactivity of alloantibodies is stronger than that of autoantibodies, such interference can be gradually reduced until it disappears by the continuous dilution method, so that the specificity of alloantibodies can be shown.<sup>37,38</sup> However, the dilution procedure was not performed, so it was impossible to prove that these cases were RBC alloimmunization.

Thus, a frequency of almost 20% (4/21) of unidentified alloantibodies was demonstrated in this study. This frequency was higher than that found in the literature.<sup>30,39</sup> This percentage is even higher if we consider the total PIATs. Of 29 PIATs, it was not possible to identify alloantibodies in 12 (41.4%). The methods used to identify alloantibodies, the characteristics of the alloantibody and very low titers are some factors associated with this.<sup>14,15</sup> Some alloantibodies are detected only with the use of special techniques, such as prolonged incubation, the use of enzyme-treated RBCs using ficin for example and low ionic concentration medium.<sup>37</sup> No special technique was used by the service to detect and identify alloantibodies in inconclusive cases using the gel hemagglutination technique.

Hemolytic transfusion reactions due to RBC alloimmunization are the main cause of morbidity and mortality related to transfusions<sup>40</sup> and have a great impact on patients dependent on regular transfusions.<sup>19</sup> In this study, transfusion reactions did not occur in patients with identified alloantibodies but occurred in one patient with unidentified alloantibodies or autoantibodies. This shows the great importance of implementing techniques to eliminate possible interference to allow correct identification.

## Conclusion

This study showed a low prevalence of alloimmunization in patients with clinical emergencies, but all of the antibodies identified were clinically significant. One patient had a hemolytic immune transfusion reaction and did not have their alloantibodies identified by the pre-transfusion tests. The low



rate of identification of alloantibodies (58.6%) among the PIATs found in the study demonstrates that there are shortcomings in the laboratory techniques used and the need to implement laboratory techniques capable of identifying alloantibodies in cases of pan-reactivity with the gel hemagglutination technique and in the concomitant presence of autoantibodies.

## Conflicts of interest

The authors declare no conflicts of interest.

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