Original article

Antibodies against human neutrophil antigens in non-transfused women with red blood cell alloimmunisation induced by pregnancy

Juliana Oliveira Martins, Elyse Moritz, Samira A. Abbas, Larissa B. Lopes, Melca M. O. Barros, Akemi K. Chiba, Dante M. Langhi Jr., José O. Bordin

Department of Clinical Oncology and Research, Federal University of São Paulo (UNIFESP), São Paulo, Brazil

Background - Alloantibodies against human neutrophil antigens (HNA) resulting from allogeneic exposure may be associated with transfusion-related acute lung injury and immune neutropenia. Understanding the risk factors for the formation of such antibodies could have a great impact on the adoption of measures to prevent potentially fatal transfusion reactions. The aim of the study was to determine the prevalence of anti-HNA alloantibodies in non-transfused pregnant women with and without red blood cell (RBC) alloantibodies.

Materials and methods - HNA alloantibodies were investigated in blood samples from 147 pregnant women with RBC alloimmunisation induced by pregnancy as the only allogeneic stimulus (group 1). The control group (group 2) consisted of 563 women with at least one pregnancy without RBC alloimmunisation. Both groups were investigated for the presence and identity of HNA alloantibodies using granulocyte agglutination tests, white blood cell immunofluorescence testing, and the bead-based LABScreen Multi Kit. Genotyping was performed to confirm the specificity of the HNA alloantibodies.

Results - Group 1 women had a statistically higher number of HNA alloantibodies compared to group 2 women (9/147 [6.1%] *vs* 9/563 [1.6%]; p=0.005, OR=4.01; 95% CI 1.5-10.3). Considering only multiparous women, there was a higher statistical significance for the difference in the presence of HNA alloantibodies between the two groups (7/82 [8.5%] *vs* 9/493 [1.8%]; p=0.002, OR=5.02; 95% CI 1.8-13.9).

Discussion - Our data show that RBC alloimmunisation is significantly associated with the development of anti-HNA alloantibodies, corroborating the hypothesis that some individuals are better immune responders and react strongly to allogeneic exposure. The presence of RBC alloantibodies can, therefore, facilitate the identification of individuals with a higher risk of alloimmunisation to antigens from other cells, also acting as a tool to avoid potentially fatal transfusion reactions.

Keywords: *red blood cells, white blood cells, RBC serology, blood groups.*

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Correspondence: Juliana O. Martins
e-mail: juliana.martins07@hotmail.com

INTRODUCTION

Human neutrophil antigens (HNA) can induce the formation of alloantibodies through pregnancy, blood transfusion or transplantation. HNA alloantibodies play an important

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role in the pathophysiology of various clinical conditions such as transfusion-related acute lung injury (TRALI), neonatal alloimmune neutropenia, non-haemolytic febrile transfusion reactions and ineffectiveness of transfused granulocytes[1].

Neonatal alloimmune neutropenia is caused by transplacental transfer of maternal Immunoglobulin G (IgG) antibodies against HNA expressed in the foetus inherited from the father, resulting in the destruction of the foetal neutrophils[1]. TRALI is the most serious complication of blood component transfusion and is the leading cause of transfusion-related mortality. The mechanism leading to TRALI is thought to involve two events. Briefly, the first event consists of previous inflammatory conditions and/or external factors that attract leukocytes to the pulmonary vessels and favour the appearance of the second event, in which anti-human leukocyte antigens (HLA) antibodies, anti-HNA antibodies or biologically active lipids from transfused blood bind to the pulmonary endothelium leading to pulmonary oedema[2]. Finally, non-haemolytic febrile transfusion reaction is the most common transfusion-associated adverse event, accounting for as many as 60% of all adverse reactions, and is caused by transfusion of pro-inflammatory cytokines that accumulate during component storage, and the interaction of recipient antibodies and leukocyte-specific antigens present in the transfused blood component[3].

Understanding the factors that influence alloimmunisation in humans is complicated by the large number of variables involved, as clinical, environmental, and genetic factors all appear to be directly involved in the process, although their individual roles are not yet fully understood[4].

Buetens *et al*. suggested that individuals who form red blood cell (RBC) alloantibodies may more easily develop alloantibodies against other blood cells; therefore, some individuals can be classified as good immune responders and are more likely to form alloantibodies[5]. Past studies showed that irregular RBC alloantibodies are associated with HLA alloimmunisation in specific groups of previously transfused patients[5,6]. In addition, Schonbacher *et al*. suggested that RBC alloantibodies would also be associated with the formation of anti-HNA alloantibodies[7]. On this background, the aim of the present study was to evaluate the prevalence of HNA alloantibodies in healthy, non-transfused women with RBC alloantibodies, in whom pregnancy was the the only allogeneic stimulus.

MATERIALS AND METHODS

Study population

A total of 710 non-transfused pregnant women were included in the study. Group 1 comprised 147 women with RBC alloantibodies, while group 2 (control group) comprised 563 women without RBC alloimmunisation. Tests were performed to screen for and identify irregular RBC alloantibodies during antenatal care. For statistical purposes, the study subjects were analysed according to the number of pregnancies, being defined as primiparous (one pregnancy) or multiparous (two or more pregnancies). The number of pregnancies was based on the answers to a questionnaire given to the study participants. Only women who reported a gestational age beyond 20 weeks (term or preterm) were included and the RBC alloimmunisation was identified only during the current pregnancy or in the postpartum period. It was not assessed whether alloimmunisation came from previous pregnancies.

All individuals included in the study signed informed consent and answered a questionnaire to create a database containing information that would help in the interpretation of the results. The Research Ethics Committee of Federal University of São Paulo (UNIFESP) approved the study.

Overall, 326 pregnancies were reported in group 1, and 1,562 pregnancies in group 2. The median was two pregnancies per individual in both groups. In group 1, there was an equivalent distribution between primiparous and multiparous women (44.2% and 55.8%, respectively), whereas in group 2 there was a predominance of multiparous women (87.6%) (**Table I**).

Table I - *Characteristics of group 1 (women with RBC alloimmunisation) and group 2 (women without RBC alloimmunisation)*

Characteristics	All	Group 1	Group 2
Number of women	710	147	563
Median age (years)	29 (18-67)*	27 (18-43)	31 (18-67)
Median of pregnancies	2 (1-11)*	2 (1-9)	2 (1-11)
Total number of pregnancies	1,888	326	1,562
Primiparous, n (%)	135 (19.0)	65 (44.2)	70 (12.4)
Multiparous, n (%)	575 (81.0)	82 (55.8)	493 (87.6)

*Numbers in brackets for age and pregnancies are ranges. RBC: red blood cell.

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Laboratory studies

RBC alloantibodies were investigated by the indirect antiglobulin test using a gel matrix (Grifols, Diagnostic S.A., Barcelona, Spain). Alloantibodies were titrated to exclude individuals who had received immunoglobulins before or after delivery (anti-D immunoglobulin: titre ≤1:16). The administration of anti-RhD immunoprophylaxis was also assessed from the clinical history in all cases.

Granulocyte agglutination test

The granulocyte agglutination test (GAT) was performed, as previously described, using a panel of granulocytes from at least three individuals previously genotyped for all HNA systems[8]. Briefly, neutrophils were isolated with dextran, and 2 µL of the cell suspension (5×10^3 neutrophils/µL) were incubated on Terasaki plates with 6 µL of test serum or control serum for 2 hours at 37°C. The GAT was performed in duplicate and the results were considered positive in the presence of agglutination evaluated by an inverted microscope.

Bead-based assay

This test was performed in all study samples using the LABScreen Multi (LSM) Kit from One Lambda Inc. (Canoga Park, CA, USA), which is capable of identifying alloantibodies against HNA antigens through microspheres coated with the purified antigens HNA-1a, -1b, -1c, -2, -3a, -3b, -4a and -5a as well as class I and class II HLA. The bead-based assay was performed following the manufacturer's instructions. Data were acquired by the LABScan™ 100 platform (Luminex, USA) and the results were analysed by Software Fusion 4.1™ (One Lambda Inc., Canoga Park, CA, USA). We analysed the normalised background and immunofluorescence (mean fluorescence intensity), taking into account the ratio cut-off specified in the kits (lot 007): HNA-1a, HNA-1b, and HNA-1c values ≥3 (normalised background); HNA-2 values ≥30; HNA-3a, HNA-3b, HNA-4, HNA-5a, and HNA-5b values ≥10. For each test sequence, a negative control serum and a positive control serum were included and the samples with borderline or inconclusive results were tested again in a new test (batch), and the results carefully compared to those obtained with the other techniques.

Flow cytometric white blood cell immunofluorescence test

A flow cytometric white blood cell immunofluorescence test (flow-WIFT) was performed only on samples with positive results by the GAT and/or bead-based assay. The protocol used was modified from the one initially described by Nguyen *et al.*[9]. Briefly, 100 µL of whole blood were dispensed with the anticoagulant ethylenediaminetetraacetic acid (EDTA) in a 5 mL polystyrene tube and 2.0 mL of lysis solution (Pharm Lyse, BD Biosciences) were added for 10 minutes at room temperature. After RBC lysis, two washing steps were performed with phosphate-buffered saline containing 0.2% bovine serum albumin (PBS-BSA) and 0.5% Na_2EDTA. The white blood cells were incubated with 50 µL of the test plasma for 30 minutes in a water bath at 37°C. Two further washing steps were performed and then 50 µL of a Goat $F(ab')_2$ Anti-Mouse IgG (H + L) secondary antibody (Caltag, Burlingame, CA, USA) were added in a 1:200 dilution. After 20 minutes of incubation in the dark at room temperature, two washing steps were repeated, and the white blood cells were re-suspended with 500 µL of 0.2% PBS-BSA 0.5% Na_2EDTA. The presence of fluorescence emitted by immunocomplexes was observed by flow cytometry on a BD FACSCalibur cytometer. Data for 10,000 cell population events were acquired and analysed by BD CellQuest™ software (Becton Dickinson, San Jose, CA, USA). The results were considered positive when the mean fluorescence intensity reached a value two times higher than the value of the negative control serum.

The GAT and flow-WIFT are gold standard techniques for screening for HNA antibodies, while monoclonal alloantibody-specific immobilisation of granulocyte antigens (MAIGA) is used to identify HNA antibodies. However, in this study, MAIGA was replaced by the LSM test, which provides similar results[10].

Human neutrophil antigen genotyping

HNA genotyping was performed in samples with positive results for the presence of anti-HNA alloantibodies by serological techniques. A polymerase chain reaction (PCR) with sequence-specific primers was used to investigate the HNA-1 and -4 systems[11,12], while a PCR restriction fragment length polymorphism method was employed to investigate the HNA-3 and -5 systems[13,14]. The PCR products were electrophoresed on agarose gel and stained with GelRed™ (GelRed Nucleic Acid Stain, Biotium) with the addition of a molecular weight marker (DNA Ladder, Invitrogen E-Gel) on all gels. The electrophoresis result

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was read in an imager (Bio-Rad Gel Doc EZ Images, Bio-Rad).

HNA-2 phenotyping

HNA-2 expression was determined by flow cytometry on a BD FACSCalibur cytometer (Becton Dickinson, San Jose, CA, USA) using the monoclonal antibody MEM166 conjugated to phycoerythrin (Abcam, Cambridge, UK). Mouse IgG1 was used as an isotype control (Abcam, Cambridge, UK). Data were acquired and analysed by CellQuest® software (Becton Dickinson). Subjects were considered HNA-2-negative if less than 5% of their neutrophils reacted with the monoclonal antibody[15,16].

Statistical analysis

Statistical analysis was performed using the "R program" (Vienna, Austria) version 3.4.2. A chi-square test and Fisher's exact test were performed to compare proportions. A p-value less than 0.05 was considered statistically significant.

RESULTS

Red blood cell alloimmunisation

We detected 178 RBC alloantibodies in women in group 1, with 125/147 (85.0%) women having a single RBC alloantibody and 22/147 (15.0%) having two or more RBC alloantibodies. Anti-D and anti-Le^a were the most commonly found alloantibodies (44/178; 24.7% each), followed in frequency by anti-E (n=15), anti-D + C (n=11), anti-M (n=9), anti-Le^b (n=7), anti-K (n=7), anti-Jk^a (n=6), anti-S (n=2), and other alloantibodies (n=33) (**Table II**).

Human neutrophil antigen alloimmunisation

Overall, it was found that 18/710 (2.5%) women had anti-HNA alloantibodies, including 9/147 (6.1%) belonging to group 1 and 9/563 (1.6%) belonging to group 2 (p=0.005, odds ratio [OR]= 4.01; 95% confidence interval [95% CI]: 1.5-10.3) (**Table III**).

Women with multiple RBC alloantibodies had a higher risk of HNA alloantibodies compared to women with a single alloantibody (OR=5.3; 95% CI: 1.3-21.7), while women with one RBC alloantibody did not appear to have a significantly increased risk of anti-HNA compared to women without RBC alloantibodies (OR=2.3; 95% CI: 0.8-6.9).

Considering only multiparous women, as they were more homogeneously distributed between groups 1 and 2 (55.8% and 87.6%, respectively) and since all anti-HNA antibodies in group 2 were found in multiparous women, a more significant statistical difference was observed between the groups regarding the presence of HNA alloantibodies.

Table II - *Red blood cell and human neutrophil antigen alloantibodies found in non-transfused women with a history of pregnancy (group 1; n= 147)*

RBC alloantibody Specificity	Number of RBC alloantibodies (%)
Le^a	38 (25.8)*,‡
D	26 (17.7)
E	14 (9.5)*
D + C	11 (7.5)‡
M	9 (6.1)
Le^b	7 (4.7)‡
K	7 (4.7)
Unspecified antibodies (IgM)	7 (4.7)*,‡
Jk^a	6 (4.1)*
Unspecified antibodies (IgG)	5 (3.4)*
Le^a + Le^b	2 (1.3)‡
S	2 (1.3)
D + C + K	1 (0.7)*
D + M + Le^a	1 (0.7)*
C	1 (0.7)
C	1 (0.7)
N	1 (0.7)‡
D + Jk^a	1 (0.7)
D + C + Jk^a	1 (0.7)
D + E + Le^a	1 (0.7)
D + C + E + K + Le^a + Le^b	1 (0.7)
P1	1 (0.7)‡
C + e	1 (0.7)
D + Le^a	1 (0.7)
S + Jk^b + K	1 (0.7)
Total	147 (100)

*One individual with anti-HNA alloantibody; ‡Two individuals with anti-HNA alloantibodies; ‡RBC alloantibody(ies) without clinical significance. RBC: red blood cells; HNA: human neutrophil antigens.

In this analysis, 7/82 (8.5%) *vs* 9/493 (1.8%) individuals were found to have anti-HNA alloantibodies (p=0.002, OR=5.02; 95% CI: 1.8-13.9).

Statistical analysis was also performed including only individuals who had clinically significant RBC alloantibodies; for this analysis, the following individuals with RBC alloantibodies were excluded: anti-Le^a (n=38), anti-Le^b (n=7), anti-Le^a + anti-Le^b (n=2), anti-N (n=1), anti-P1 (n=1) and unspecified antibodies (IgM) (n=7). Clinically significant RBC alloantibodies were observed in 91/147 (61.9%) women; seven of these individuals also had

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Table III - *Frequency and specificity of alloantibodies to anti-human neutrophil antigens detected in groups 1 and 2*

Anti-HNA	Group 1 (n=147)		Group 2 (n=563)		Total (n=710)	p-value	OR (95% CI)
	Primiparous (n= 65)	Multiparous (n= 82)	Primiparous (n= 70)	Multiparous (n= 493)			
HNA-1a	1 (1.5%)	4 (4.9%)	0	4 (0.8%)	9		
FcγRIIIb	1 (1.5%)	1 (1.2%)			2		
HNA-2				1 (0.2%)	1		
HNA-3a				1 (0.2%)	1		
HNA-3b		2 (2.4%)		1 (0.2%)	3		
HNA-5a				2 (0.4%)	2		
Total of anti-HNA in primiparous	2 (3.0%)		0			NS	
Total of anti-HNA in multiparous		7 (8.5%)		9 (1.8%)		0.002	5.02 (1.8-13.9)
Total anti-HNA	9 (6.1%)		9 (1.6%)			0.005	4.01 (1.5-10.3)

HNA: human neutrophil antigen; OR: odds ratio; 95% CI: 95% confidence interval; NS: not significant.

anti-HNA alloantibodies (group 1=7/91 [7.7%] *vs* group 2= 9/563 [1.6%], p= 0.003, OR=5.13; 95% CI: 1.8-14.1]).

Considerations about the techniques performed

Table IV summarises the results of anti-HNA antibody detection in both groups, according to the techniques used. The LSM test and GAT were performed on all subjects included in the study. All samples with a positive result were genotyped and investigated by flow-WIFT.

The LSM test detected 54/710 (7.6%) positive reactions to HNA alloantibodies. Only 18/54 (33.4%) were confirmed as true-positive by additional tests, and 36/54 (66.6%) samples were classified as false-positive. Of the 36 samples classified as false-positive, 17 (47.2%) were for the HNA-1 system, 1 (2.8%) for the HNA-2 system, 10 (27.8%) for the HNA-3 system and 8 (22.2%) for the HNA-5 system. We did not find non-specific reactions with beads for the HNA-4 system.

The GAT is considered the gold standard test for confirming anti-HNA-3 antibodies. Therefore, the panel that contemplated the GAT was expanded to six different cells, including three homozygous for HNA-3aa and three homozygous for HNA-3bb. In total, 10/710 (1.4%) samples were positive for the beads of the HNA-3 system by the LSM test, and negative by the GAT. As the GAT is considered the gold standard, and only the LSM test showed a positive reaction in the beads for the HNA-3 system, these samples were considered negative for anti-HNA alloantibodies.

The LSM test was performed in duplicate in all cases that showed non-specific or background reactions against HNA beads.

DISCUSSION

The present study aimed to investigate the frequency of anti-HNA alloantibodies in women in whom pregnancy was the only stimulus to alloimmunisation. Women with a history of pregnancy account for a considerable fraction of the blood donor population, which makes alloimmunisation studies clinically relevant in transfusion medicine. We observed evidence of an association between RBC alloimmunisation and the development of HNA alloantibodies. The high frequency of anti-HNA alloantibodies found in this study may be due to the wide miscegenation found in some countries, including Brazil[17]. Previous studies have found that irregular RBC alloantibodies may be associated with HLA alloimmunisation in some specific groups of transfused patients[5,6]. However, few studies have addressed the potential association between RBC and HNA alloimmunisation. The data show that the presence of RBC alloantibodies may increase the risk of HNA alloimmunisation, as suggested by Schonbacher *et al.*[7]. Interestingly, we observed that multiparous women with RBC alloimmunisation formed more anti-HNA alloantibodies than did multiparous women who were not RBC alloimmunised. The biological basis to explain the association between the presence of anti-RBC antibodies and anti-HNA antibodies in the case of multiparous women has not yet been fully elucidated, but in addition to the increased allogeneic exposure in multiparous women, RBC alloimmunisation can denote

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Table IV - *Genotyping and phenotyping results of the cases with anti-HNA antibodies in both groups, according to the different techniques performed*

	RBC	LSM		GAT*	Flow-WIFT*		Genotype
	Alloantibodies	**HNA Alloantibodies**	**HLA Alloantibodies**	**HNA Specifitity**	**Lymphocytes**	**Granulocytes**	
Group 1	Unspecified antibody (IgG)	1a	Negative	1aa (2x)‡	Negative‡	1aa (2x)	FCGR3B*02/FCGR3B*02
	Unspecified antibody (IgM)	1a	Negative	1aa (2x)‡	Negative‡	1aa (2x)	FCGR3B*02/FCGR3B*02
	D + M + Le^a	1a	Class I	1aa (2x)	1aa	1aa (2x)	FCGR3B*02/FCGR3B*02
	D + C + K	1a	Class I	1aa (2x)	1aa	1aa (2x)	FCGR3B*02/FCGR3B*02
	D + C	1a	Class I and II	1aa (2x), 1ab	1aa (2x), 1ab, 1abc	1aa (2x)	FCGR3B*02/FCGR3B*02
	Le^a	FCγRIIIb	Class I	1aa (2x), 1ab	1aa, 1ab	1a (2x), 1ab	FCGR3B**null*
	D + C	FCγRIIIb	Class I and II	1aa (2x), 1ab, 1abc	1aa (2x), 1ab, 1abc	1aa (2x), 1ab, 1abc	FCGR3B**null*
	E	3b	Class I and II	3bb (2x)	3aa, 3bb (2x)	3bb (2x)	SLC44A2*01/SLC44A2*01
	Jk^a	3b	Class I and II	3bb (2x)	3aa, 3bb (2x)	3bb (2x)	SLC44A2*01/SLC44A2*01
Group 2	Negative	1a	Class I and II	1aa, 1ab	1aa (2x), 1ab	1aa (2x)	FCGR3B*02/FCGR3B*02
	Negative	1a	Negative	1aa, 1ab	Negative	1aa, 1ab	FCGR3B*02/FCGR3B*02
	Negative	1a	Negative	1aa	Negative	1aa	FCGR3B*02/FCGR3B*02
	Negative	1a	Negative	1aa	Negative	1aa	FCGR3B*02/FCGR3B*02
	Negative	2	Class I	Negative	HNA-2+ (87%)§	HNA-2+ (87%)	Not tested
	Negative	3a	Class I	3aa (2x), 3ab	3aa, 3ab	3aa (2x), 3ab	SLC44A2*02/SLC44A2*02
	Negative	3b	Class I	3bb	3ab, 3bb	3bb	SLC44A2*01/SLC44A2*01
	Negative	5a	Class I	5ab	5aa	5ab	ITGAL*02/ITGAL*02
	Negative	5a	Negative	5ab	Negative	5ab	ITGAL*02/ITGAL*02

*GAT and flow-WIFT were performed with cells from at least three different donors; ‡(2x): Positive results with cells from two different donors; ‡Negative: negative results with all tested cells; §HNA-2+ (87%): positive results with only neutrophils from one donor with an expression level of 87% HNA-2 (determined by flow cytometry).

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a state of increased immune response which would make the individual more likely to develop alloantibodies to other blood cells[18]. Besides being associated with haemolytic disease of the foetus and newborn, anti-RBC alloantibodies in multiparous women may indicate a higher risk of alloimmunisation against other cells, and the identification of such individuals could prevent the development of neonatal alloimmune neutropenia, TRALI and non-haemolytic febrile transfusion reactions.

No significant difference was found between the groups when comparing the presence of anti-HNA alloantibodies in primiparous women, although this result could have been influenced by the small number of individuals with a single pregnancy included in the control group (results not reported). Alloimmunisation against multiple blood cells in this population may be related to the profile of the individuals included in the study, *i.e.*, only pregnant women or those with a gestational history. During pregnancy, the amount of foetal-maternal bleeding is usually small, with less than 0.1 mL of foetal blood coming into contact with the mother's blood in 90% of pregnancies[19]. A small volume of foetal blood was able to alloimmunise 6.1% of women against RBC and HNA, indicating that these women were good immune responders. Several elements may have contributed to this result, such as population heterogeneity, antigenic differences between mother and baby, and immunomodulatory effects of pregnancy[20].

Alloimmunisation against the HNA-1 system has been reported as the main cause of neonatal alloimmune neutropenia in Caucasian populations[21]. Most HNA alloantibodies found in the present study were against HNA-1a antigens, with the genotyping showing only the FCGR3B*02 allele, ruling out the possibility of HNA autoantibodies. In addition, two (0.3%) individuals, with HNA-1 *null* confirmed by genotyping, were found to have anti-FcγRIIIb isoantibodies in their plasma. Although rare, anti-FcγRIIIb alloantibodies have already been associated with severe cases of neonatal alloimmune neutropenia[22,23].

Anti-D and anti-Le^a were the most commonly found alloantibodies in this study. Despite the prophylactic use of Rhesus immunoglobulin, anti-D is still an alloantibody identified in pregnant women who come to hospitals. The lack of adequate antenatal tests or even prophylactic failure of anti-Rh immunoglobulin (D) may explain this fact. Koelewijn *et al*. found that in 43% of cases of failed anti-D immunoglobulin prophylaxis, risk factors were not present[24]; however, foetal-maternal haemorrhage, which can cause sensitisation to RhD, is an important obstetric complication that occurred in 21.2% of pregnant women in an African study[25].

A high frequency of anti-Lewis alloantibodies was observed in our study. Such alloantibodies are generally considered not clinically significant because Lewis antigens are not intrinsic to RBC, being type 1 glycosphingolipids passively adsorbed by the RBC membrane[26]. However, even with the exclusion of all individuals having anti-Lewis alloantibodies, we still observed evidence for the association between RBC alloimmunisation and the development of anti-HNA alloantibodies.

Regarding the techniques used, the GAT was positive in 89.5% of the samples, detecting anti-HNA alloantibodies confirmed by additional tests. However, it is known that reactivity in the GAT is variable and can be negative, depending on the specificity and the alloantibody titre. The LSM test detected 14 samples that were positive for the HNA-3 system, but only four were confirmed by other techniques. A non-specific reaction against the HNA-3 system may be due to the great challenge of the LSM test in imitating the natural conformation of the recombinant CTL-2 protein[10]. In addition, the presence of anti-HLA class I alloantibodies in more than half of these samples may have contributed to a non-specific reaction.

Despite the high efficiency of the LSM test in large-scale, routine practice, the GAT is still useful for the detection and identification of alloantibodies against the HNA-3 system. According to the literature[9,27], the flow-WIFT proved to be a valuable and viable technique for screening for HNA alloantibodies, since it is less laborious than the standard granulocyte immunofluorescence test, its results are more objective than microscopic evaluation; the test has the potential to be automated and it can also detect lymphocytic alloantibodies. In addition, the LSM and MAIGA are recommended to confirm the specificity of the HNA alloantibody.

CONCLUSIONS

In conclusion, our data show that RBC alloimmunisation is significantly associated with the development of anti-HNA alloantibodies, corroborating the hypothesis

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that individuals who have RBC alloimmunisation have a stronger immune response to allogeneic antigens. As the presence of RBC alloantibodies may be a risk factor for alloimmunisation against antigens from other cells, the identification of such individuals could prevent potentially fatal transfusion reactions and neonatal cytopenias.

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AUTORSHIP CONTRIBUTIONS

JOM designed the research, collected the samples, performed the genotyping, GAT, flow-WIFT and LSM tests, analysed the data, and wrote the manuscript; EM performed the GAT, flow-WIFT and LSM tests and edited the paper; SAA collected the samples, performed the research and identified the tests for RBC alloantibodies; LBL edited the article and contributed to the resolution of the cases with anti-HNA antibodies; MMOB collected the samples and contributed to the resolution of the cases with anti-HNA antibodies; AKC performed the genotyping, the research, and identified the tests for RBC alloantibodies; DMLJr designed the research and contributed to the resolution of the cases with anti-HNA antibodies; JOB designed the research and edited the paper.

The Authors declare no conflicts of interest.

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