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RESEARCH ARTICLE

The ABO-RhD matched red blood cell transfusion, source of alloantigens occurrence in recipients

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Abstract

Background: The disparity of red blood cell (RBC) antigen between donor and recipient or from mother and fetus involves into red blood cell alloimmunisation. Although anti-D alloimmunization tends to disappear due to the rhesus D (RhD) prophylactic matching coupled with ABO, other antigens including Rh (C, c, E, e) and Kell (K) systems in terms of frequency of occurrence, continue to induce alloimmunization in multitransfused recipients in developing countries.
Objectives: The objective of this work is to carry out the different transfusion incompatibilities that may induce alloimmunization especially in multitransfused patients and complicate their subsequent transfusions.
Methods: Blood screening in ABO, Rh (D, C, c, E, e) and Kell (K) was performed on opaline plate and confirmed by automatic hematology analyzer on each unit of packed red blood cells and blood sample of the recipient.
Results: Different phenotypes were found in both packed red blood cells units and in recipients ($p < 0.0001$). No incompatibility antigen A, B, and D was identified; as against 89 (28.52%) incompatibilities due to antigens C, E, c, CE and K were found.
Conclusion: Our study shows that, in addition to the standard ABO-RhD, systematic phenotyping should be done especially in the mutitransfused recipients' blood as well as on packed red blood cells transfused to prevent alloantigens occurrence.

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Introduction

Blood transfusion is generally the process of receiving blood components such us red blood cells, white blood cells, plasma, clotting factors and platelets into one's circulation intravenously. Units of packed red blood cells are typically only recommended when either a patient's hemoglobin level decreases to 7-8g/dL, as a more restrictive strategy has been shown to have better patient outcomes. However, globally around 85 million units of red blood cells are transfused in a given year [1, 2]. In 2006, more than 14 million units of allogeneic red blood cells (RBCs) were transfused in the United States alone. From 1997 to 2007, the hospital discharges in the United States in which patients' record indicated RBC transfusion increased from 5% to 10.4%, with blood transfusion becoming the most common in patient hospital procedure. Red blood cell transfusion rates in other countries are variable but often

comparably high, [1, 3] particularly in developing countries where rates of sickle cell diseases (SCD), infections and parasitoses still highest [4, 5, 6, 7]. Unfortunately, RBCs transfusion remains complicated by RBC immunizations.
The disparity of red blood cell antigen between donor and recipient or from mother and fetus involves into red blood cell alloimmunisation. These phenomena are observed in RBC multitransfused patients. Although therapeutic modalities routinely undergo rigorous evaluation of their efficacy and safety before entering clinical practice, RBC transfusion has not been subjected to similar examination [3, 8] particularly in developing countries.
The most important irregular red blood cell alloantibodies in daily transfusion practice, in terms of frequency of occurrence, are directed towards the Rh (anti-D, -C, -E, -c and -e), Kell (anti-K), FY (anti-Fya and -Fyb), JK (anti-Jka and -Jkb) and the MNS (anti-M, -S and -s) blood group systems [9, 10]. The Rh system is a complex blood group system and includes > 50 different serologic specificities. The Rh locus is comprised of 2 homologous genes, RhD and RhCE, which encode the D antigen and the CE antigens in various combinations (ce, cE, Ce, or CE), respectively [11]. Of these, the D-antigen is the most immunogenic, resulting in more than 80% of immunocompetent D negative persons becoming alloimmunized after a transfusion of D-positive erytrocytes. This has resulted in prophylactic matching of red cell transfusions for the D-status. Such routine, widely observed in subtropical Africa is not common to prevent transmission of other alloantigens to recipients [10, 12].
Alloantibodies against Rh (C, E) and kell (K) also can pose serious clinical problems such as delayed haemolytic reactions and logistic problems, for example, to obtain timely and properly matched transfusion blood for patients in which new alloantibodies are detected [13]. Retrospective studies in the general population reported antibody frequencies after transfusion of less than 1 to 3 percent. However, in multitransfused patients, alloimmunizaton occurs in up to 70% of patients [13, 14]. Studies in multitransfused SCD patients, one in CHU Campus Lomé (Togo) and the other in CHU Cocody (Côte d'Ivoire) have revealed high rates of alloimmunization (50% in Lomé [6] and 62.8% in Cocody [5] against the Ag Rh and KEL. For against, in countries where phenotyping is mandatory (eg in France since 2002) in all donated blood and in recipients, post-transfusion allo-immunization have become rare [15, 16]. To highlight the Rh alloimmunization risk that recipients were expose during the non-extended matched RBC transfusion episode, this study was to determine the Rh and Kell phenotype in blood donors and patients receiving correspondent packed red blood cell (RBC).

1- Methods

- Study design

This prospective study received approval agreement from the University Hospital Sylvanus Olympio of Lomé/Togo and the Ethics Committee of the University of Lomé. At first, clinicians and laboratory technicians were made aware of the importance of this study and its requirements. From February to July 2013, the purpose of this study was explained to adult patients and to parents of children patients and they advanced their agreement by signing a form that was submitted to them for this purpose. The study consisted to phenotype RBC antigens in Rh and Kell systems, both in patients receiving RBC transfusion and in correspondent packed RBC from donors matched only in ABO- RhD and to determine probable allo-antigens that occur.

- Patient population

For a patient to be transfused, following information was recorded: the clinician who takes care of the patient and patient identity as his age, sex, clinical history and service of hospitalization. About 2 ml of blood samples of patients who agreed to participate in the study were collected in Ethylene Diamine Tetra-Acetic Acid (EDTA) tubes and sent to the laboratory of the National Blood Transfusion Center of Lomé. A total of 312 patients were enrolled in this study. Allo-antigens C, c, E, e of Rh system and K of Kell system screening was carried out both on each patient blood sample and on one packet RBC intended to him.
Patients included: recipients of RBC hospitalized in CHU-Sylvanus services that had given their consent after being informed about the nature and objectives of the study.
Patients excluded from the study: recipients in situation of immediate life-threatening emergency requiring immediate transfusion of RBC, recipients aged less than one year due to the low expression of certain cell antigens in this age group, second bags of packed RBC from bags produced in duplicate to avoid duplication. In fact, for pediatric transfusions, two bags of packed RBC can be produced from one donor who gives 500 ml of total blood.

- ABO-RhD grouping

For ABO-RhD grouping, the globular test or Beth Vincent was performed [1]. To avoid cross-reactions with other plasma antibody, the recipient red blood cells and red blood cell concentrates collected from the blood unit intended to him are washed three times and suspended in 10% physiological saline. Then, in order, 50µl of antisera anti-A, -B, -AB and -D are deposited on the opaline plate. Fifty microliters of RBC suspension were added to each antiserum. After mixing, the corresponding antigen is detected by the presence of agglutination. The same test performed on the patient's blood is also carried out on blood unit intended to him.

The ABO globular test was confirmed by serum test or Simonin-Michon. To two drops of plasma deposited separately, are respectively added a drop of red cell suspensions A and B. After mixing, the interpretation is based on the fact that the blood group A contains anti-B antibodies in the plasma and vice versa.
The serum test is intended to confirm only the ABO grouping. So, RhD was confirmed simultaneously with other alloantigens phenotyping (Figure 1).

- RBC phenotyping

Alloantigens C, c, E, e of Rh system and K of Kell system screening was performed in each patient RBC and in packed RBC intended to him, using respectively anti-sera anti-C, -c, -E, -e and –K [1]. As ABO grouping on opaline plate, agglutination in an anti-serum means presence of correspondent alloantigen. The test on plate was confirmed by automatic hematology analyzer (Evolis 100 from Bio-Rad). Gel plates used contain respectively anti-sera anti-D, -C, -c, -E, -e and –K (figure 1).

2- Results

- Overall results

We collected 312 blood samples from packed RBCs recipients. Hundred eighty-one (181) recipients were female (58.01%) against 131 males (41.99%). The sex ratio was 1.38 for women. The average age of recipients was 16 years (95% CI = [1 to 33.52 years]). Most of recipients, 177 (56.73%) were recruited in pediatrics; then 71 (22.76%) in obstetrics and gynecology department and 64 (20.51%) in surgery and intensive care department.
In recipients of packed red blood cells and blood bags, group O had a majority with respective frequencies of 46.15% and 52.56%. The differences between the frequencies of ABO phenotypes in recipients and blood bags are significant ($P< 0.001$) (Figure 2).
For the standard rhesus (RhD), the majority 279 (89.42%) of RBCs recipients were RhD positive. Similarly, 271 (86.86%) RBCs pockets intended to recipients were RhD positive (Figure 2).

- Specific results to the objectives of the study

Frequency of Rh(C, E, c, e) antigens in patients and RBCs pockets

Whether in patients receiving packed RBCs or in packed RBCs bags, antigens C, E, c, e were unevenly distributed. Antigens e and c are the most common in RBCs pockets and in recipients (Table I).

Frequency of Rh phenotypes in recipients and in RBCs pockets

Phenotypes ce, Cce and cEe were the most frequent. The observed differences in the frequencies of the phenotypes ce, Cce and cEe in recipients and in RBCs pockets are statistically significant; in both cases, $p < 0.0001$ (Table II).

Incompatible transfusions in Rh system

Incompatible transfusions occurring was considered when one Ag found in RBCs from a pocket was absent in the RBCs of recipient to whom this pocket was given. There were a total of 86 cases (27.56%) of incompatible transfusions in the Rh system. Allo Ag found were c, C and E (Tables III, IV, V). Among the Rh incompatible transfusions, 3.49% were due to both the Ag C and E (Table VI). There was no incompatible transfusion caused by Ag e because it was present in all recipients.

Incompatible transfusions in Kell system

All RBCs recipients were Ag K negative while the Ag K was found on 3 bags of red blood cells. Therefore, 3 cases (0.96%) were registered as incompatible transfusions in Kell system.

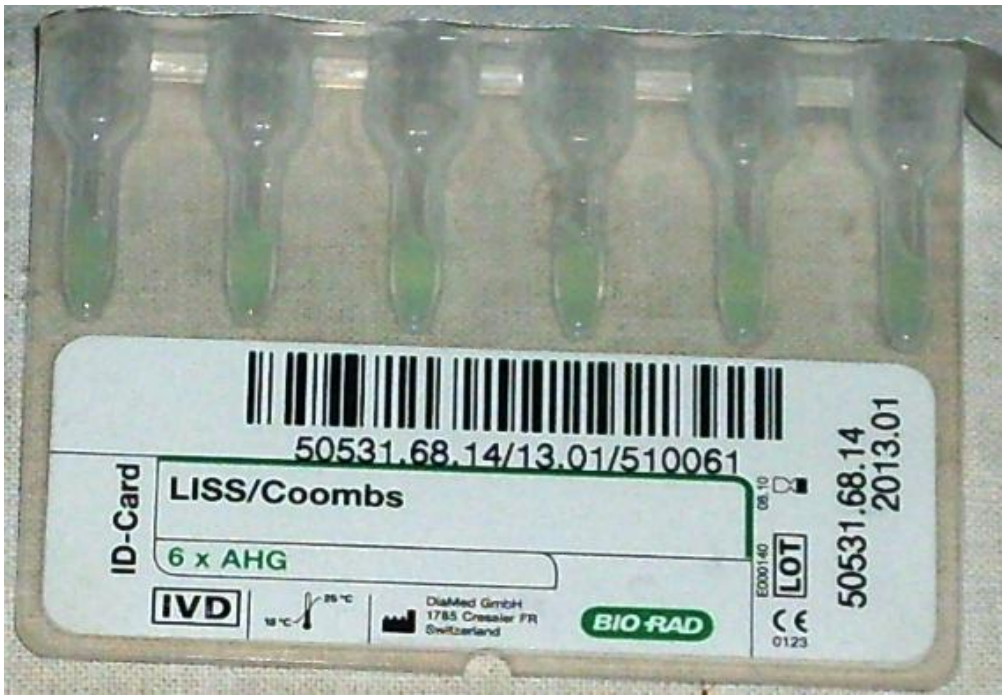

Figure 1: An Example of gel plate used containing respectively anti-sera anti-D, -C, -c, -E, -e and –K

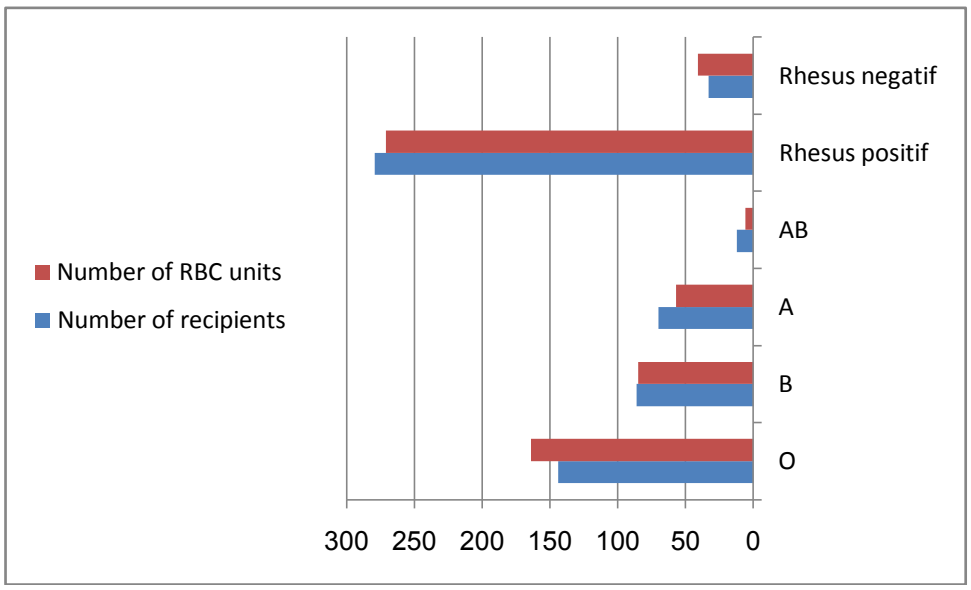

Figure 2: Distribution of the 312 recipients and 312 RBC units according to ABO grouping and rhesus status

Table I: Frequency of antigens C, E, c, and e

	Number of RBC units (%)	Number of recipients (%)
e	310 (99,36 %)	312 (100 %)
c	306 (98,08 %)	307 (98,40 %)

C	55 (17,63 %)	57 (18,27 %)
E	47 (15,06 %)	53 (16,99 %)

Table II: Frequency of Rh phenotypes

	Effectifs des poches (%)	Effectifs des receveurs (%)
ce	216 (69,23 %)	209 (66,99 %)
Cce	47 (15,06 %)	49 (15,71 %)
cEe	39 (12,50 %)	46 (14,74 %)
CEe	4 (1,28 %)	4 (1,28 %)
CcEe	2 (0,64 %)	3 (0,96 %)
Ce	2 (0,64 %)	1 (0,32 %)
cE	2 (0,64 %)	0
Total	**312 (100 %)**	**312 (100 %)**

Table III: Incompatible transfusions caused by Ag c

Phenotype of RBC unit	Phenotype of recepient	incompatible Ag	Number of cases
ce	Ce		1
ce	CEe	Ag c	1
Cce	CEe		2
cEe	CEe		1
Total	**NA***		**5**

NA = Non Applicable.

Table IV: Incompatible transfusions caused by Ag C

Phenotype of RBC unit	Phenotype of recepient	incompatible Ag	Number of cases
Cce	ce		30
Cce	cEe	Ag C	10
CEe	cEe		2

Ce	ce	1
Total	**NA**	**43**

Table V: Incompatible transfusions caused by Ag E

Phenotype of RBC unit	Phenotype of recepient	incompatible Ag	Number of cases
cEe	ce	Ag E	20
cEe	Cce		13
cE	ce		1
CcEe	Cce		1
Total	NA		**35**

Table VI: Incompatible transfusions caused by both the Ag C and E

Phenotype of RBC unit	Phenotype of recepient	incompatible Ag	Number of cases
Cee	ce	Ag C and Ag E	2
CcEe	ce		1
Total	-		**3**

3- Discussion

In countries where blood grouping is extended to major alloantigens of Rh, Kell, Duffy, MNS systems, allogeneic differences persist and polytransfused patients are not spared of alloimmunisations [17, 18]. We implement this study to assess pathways patients develop the Rh and Kell allo-immunization with only ABO-RhD matched RBCs transfusions in developing countries.

The overall results show that the majority of recipients were children and young people with an average age of 16 years. Also, samples were collected more in women (58.01%) than males (41.99%). These two observations show that blood products are primarily for children and women. Indeed, according to the World Health Organization (WHO) up to 65% of blood transfusions are administered to children under five in developing countries [19]. In addition, 56.73% of recipients of pediatrics could also be explained by the period of sample collection. In Lomé, months from May to August coinciding with the rainy season and the resurgence of malaria infection rates. Children anemic forms due to infection with Plasmodium falciparum are common and therefore the need for transfusions increases [20].

In this study , both in recipients and RBCs pockets, the O blood group had a majority with respective frequencies of 46.15% and 52.56 % with no significant difference ($p = 0.2472$) between the pockets and recipients. After O, come successively groups B, A and AB. This order of frequency in the ABO system was one found by Padaro E. et al. [15]. The prevalence of group O and the low representation of subjects group AB were found in most studies in the black African populations [21, 22, 23]. By cons, in French [2], the group A majority (45%) followed by the group O (43%). A study of 9280 voluntary blood donors in India, found a predominance of group B (37.39%) followed by

the group O (31.85%) [9]; this unequal distribution of ABO blood groups reflects the antigenic diversity of people in different geographical areas.

Among the recipients, 89.42 % were RhD positive. This result is consistent with that of other researchers because all studies have shown a predominance of RhD positive people; the rates varied across the different geographical areas [9, 24]. All transfusions were compatible in the ABO -RhD standard grouping. This result, even if it allows solving major cases of post-transfusion immunizations, the prophilactic ABO-RhD grouping alone does not allow to conclude a complete resolution of alloimmunisations including Rh and Kell systems [25].

Four antigens C, c, E and e are present in all human populations, but their frequencies differ considerably across the world. Almost all of our study population has Ag e and c respectively with higher frequencies (99% and 98%). The antigens C (17.63% in pockets and 18.27% in RBCs recipients) as the Ag E (15.06% in pockets and 16.99% in RBCs recipients) are not rare but less frequent compared to Ag c and e; however, these antigens C and E appear high to cause serious problems of alloimmunisation [25]. These frequencies are comparable to those obtained by other researches which did similar work among black Africans. Indeed, Traoré et al. found in Mali that Ag e and c were significantly more frequent with the following distribution: Ag e (99.5%), Ag c (100%), Ag C (9.6%) and Ag E (17.8%) [15]. In general, according to reported data, respective frequencies 98%, 96%, 22% and 27% of Ag e, c, E and C are found in Black people. In against, in Caucasian Ag C is the most common (70%) as in Asians (93%) [26].

In total, eight rhesus phenotypes were identified in our study population (Table IV). In RBCs pockets and in recipients, rhesus phenotype most common was “ce” with respective frequencies of 69.23% and 66.99%. Then followed the phenotypes Cce and cEe with statistically significant differences ($p < 0.0001$) between the frequencies of these phenotypes. These results are similar to those obtained in Black Africa, by other researchers. In Togo, Padaro et al. had found in sickle cell multitransfused a clear predominance of the phenotype ce (71.56%), followed by Cce (14.67%) and cEe (11.93%) [15]. Baby et al. in Mali found in multitransfused patients that the most common Rh phenotypes were: ce (67.90%), cEe (15.4%) and Cce (10.30%) [27]. In general, the phenotype "ce" is more common in Black people but it is found at least in 10% of White people [22] and less than 7.5% in Asian [23].

Although the phenotype "ce" is the most common in RBCs pockets (69.23%) and in recipients (66.99%), there were some phenotypic differences responsible for the cases of incompatible transfusions. Eighty nine (28.53%) cases of incompatible transfusions were identified in which the majority (86) comes from Rh system and 3 cases from Kell system. The risk of alloimmunization against the Rh Ag was therefore higher than that of Ag K. This could be explained by the low frequency (0.96%) Ag K in our study population. In the Rh system, incompatible transfusions were dominated by Ag C (50%) followed by E Ag (40.70%). These results are worrying because alloimmunization against Ag C or Ag E may be responsible for hemolytic disease in newborn (HDN). So it can cause blood inefficiency especially in multitransfused patients such as delayed haemolytic reactions and time required for properly matched transfusion blood for patients in which new alloantibodies are detected [13]. The risk of immunization against Ag C (50%) and against Ag E (40.70%) is superimposed with the results obtained by Akre et al. in Côte d'Ivoire [16] and Baby et al. in Mali [27] which showed that the majority of patients were immunized against Ag C and Ag E. There was no incompatible transfusion caused by Ag e because it was almost present in all recipients. The eighty nine (28.52%) cases of incompatible transfusions confirm compliance with the ABO-RhD compatibility according to the rules of Ottenberg and Schultz [2] only is insufficient. It is desirable to establish systematic phenotyping of RBCs in pockets and in recipients (especially multitransfused patients) to avoid these incompatible transfusions.

Conclusion

The major findings of this study show that transfusion of patients with packet RBCs using donor units’ antigen matched only for ABO-RhD is not the best strategy to reduce alloimmunizations; rather, the alloantigens appear in an alarming proportion particularly in Rh and Kell systems. To reduce any alloimmunisation, phenotyping and pre-transfusion compatibilization for C, c, E, e (Rh system) and K (Kell system) antigens should be extended to all patients with programmed surgeries or acute clinical events that do not need emergency transfusions and especially in multitransfused patients.

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