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Determination of the Risks of Post-Transfusion Allo-Immunization in the Rhesus and Kell Systems: Case of Recipients of the Regional Hospital of Bafoussam

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Abstract: *Background*: Blood transfusion according to the World Health Organization is the transfer of blood or blood constitute from an individual donor to another transfused. Despite the efforts made in the field of immuno-haematology, the transfusion of packed red blood cells or whole blood brings foreign antigens to the recipients. The risk of occurrence of anti-erythrocyte allo-immunization is therefore greater in polytransfused subjects and increases with the number of bags of packed red blood cells transfused. The main objective was to determine the risks of posttransfusion allo-immunization of the Rhesus and Kell systems in recipients of the HRB blood bank. *Methodology*: We conducted a descriptive and cross-sectional study by successive recruitment over a period of 10 months including a one-month data collection period during which 145 participants (81 donors and 64 blood recipients) were recruited. A venous blood sample was taken on an EDTA tube and dry. ABO blood groups followed by Rhesus and Kell phenotypes were determined in the tube. *Results*: Out of the 81 donors, 82.70% (67) were men, the age group [20-30] years was more represented 56.80% (46); the familiar donor status was the most representative at 48.10% (39). Of the 64 recipients, the male sex was the most representative 53.13% (34); the age group [00-10] years was more represented, i.e. 23% (15). Blood group O was more representatives, ie 51.90% (42) /40.60% (26). In the Rhesus system, the Dce (Dccee) phenotype was the most represented, 58.0% (47) in donors and 59.4% (38) in recipients. The K1 antigen was present in 24.7% (20) of donors and 18.8% (12) of recipients. We obtained a risk of allo-immunization of 40.61% in the Rhesus system and 15.62% in the Kell system. Thus the risks of alloimmunization were more represented by the incompatibilities with the antigen E (21.87%), K (15.62%) and C (15.62%). The Chi-square test of independence and linear regression showed that the risk of alloimmunization increases with the number of non-compatible blood bags received with an OR: 0.98, ICOR [3.05-9.26]. *Conclusion*: In view of the various incompatibilities and high risk of occurrence of alloimmunization, Rhesus/Kell phenotyping is an effective means of preventing post-transfusion alloimmunization and improving transfusion safety and even the transfusion outcome of recipients.

Keywords: Risks, Post-Transfusion Allo-Immunization, Rhesus and Kell Phenotype

1. Introduction

Blood transfusion according to the World Health Organization is the transfer of blood or constituent of the blood of a donor individual to another transfused [1], which is associated with several immunological, infectious, metabolic and allergic [1]. Despite the efforts made in the

field of immuno-haematology, the transfusion of packed red blood cells (RBCs) or whole blood provides strangers antigens to recipients [2]. The risk of occurrence of anti-erythrocyte allo-immunization is therefore more important in polytransfused subjects and increase with the number of pockets of packed red blood cells transfused and therefore can lead to delayed haemolysis involving the vital prognosis of the patient as well as situations of transfusion impasses [2]. Post-transfusion allo-immunization is evoked during the observation of allo-antibodies in a transfused patient, which had not been found during previous analyzes and when the transfusion was not safe [2]. It is the consequence of the introduction into the body; erythrocyte, leukocyte or platelet allo-antigen depending on the number of transfusions, and the immunological status of the recipient [2].

The objective of blood transfusion is to provide the patient with substitution therapy compatible, effective, with maximum safety in terms of patient immunization and transmission of infection. The transfused blood is not systematically phenotyped and in this allo-immunization is a potentially real problem, hence the importance of a haemo-vigilance and implementation of the traceability of labile blood products is necessary [3]. Its main immunological risk is post-transfusion allo-immunization resulting by the production of alloantibodies against red blood cell antigens [2]. But these risks remain poorly assessed in our countries and are not always avoided, especially when transfusion is intended for a polytransfused patient [2].

In France, Duboeuf et al carried out a study in 2012 in 1575 patients, therefore one thousand and eight One hundred and fifteen alloantibodies were identified [4]. The most common alloantibodies were directed against the following antigens: RH3/E (18.7%), KEL1/K (17.3%), RH1/D (16.4%), MNS1/M (9.4%), FY1/Fya (6.9%), RH2/C (6.1%), KEL3/Kpa (4.7%), JK1/Jka (4.3%) and RH4/c (4.1%) [4]. Another study carried out in India shows that 9.8% of polytransfused patients due to chronic renal insufficiency, out of 81 respondents, had alloantibodies to erythrocytes [5].

In developing countries blood recipients are not phenotyped in several blood erythrocyte group systems [5]. Under these conditions the hello anti-erythrocyte immunization is evident [5]. In Africa, few studies have focused on assessing the risk of allo post-transfusion immunization, but rather on post-transfusion allo-immunization in blood recipients [6]. Indeed, the follow-up of a cohort of 42 polytransfused sickle cell patients in Côte d'Ivoire in 2013 had revealed a prevalence of anti-erythrocyte allo-immunization of 28% [7]. Similarly, out of 78 polytransfused in Mali, 10.3% had alloantibodies, [8] whereas this prevalence was 16.6% in a population of 84 polytransfused haemoglobinopathies in Morocco [9]. A study carried out in Bamako in 2013 on the erythrocyte phenotype in immunogenic blood group systems Baby et al; 2010 shows a difference in the frequencies of erythrocyte antigens in donors of blood and multiple transfusions in order to estimate the risk of transfusion alloimmunization [8]. It shows that in over 90 sickle cell disease patient, the Dccee phenotype is most frequently observed with a percentage of 73% followed by DCcee (9%), DccEe (7.5%) and K antigen phenotypes had no was found [8].

In Cameroon in 2022, Celianthe Guegang Guegang et al. got a frequency 55% post-transfusion allo-immunization in hospital sickle cell patients Laquintinie de Douala and Ngo Sack Françoise et al. in 2018 had obtained a frequency 33% of allo -immunization among polytransfused women in Yaoundé [10, 11]. However littlestudies have been carried out in Cameroon on the determination of the risk of allo-immunization posttransfusion by determining the Rhesus and Kell system in recipients of blood from which the interest of determining the risks of post-transfusion allo-immunization of the systems Rhesus/Kell in recipients of the blood bank of the Regional Hospital of Bafoussam.

2. Method

2.1. Study Location and Study Population

Our study was carried out at the blood bank of the Regional Hospital of Bafoussam. The This study took place over a period of 9 months from October 15, 2021 to July 15 2022 including a one month data collection period.

2.2. Collection Procedure

Sampling was done at the patient's bedside for hospitalized recipients or in the ward emergency for new patients admitted to this unit before hospitalization. Once the participant well settled and reassured. A tourniquet was applied to the arm and the participant was instructed to point the hand to make the vein turgid. The sampling area beforehand localized was disinfected with a cotton soaked in alcohol at 70°C. A venipuncture was carried out and approximately 10 mL of blood was collected, including 5 mL in the EDTA tube (Ethylene Tetraacetic Diamine) and 5 mL in the dry tube. The tourniquet was then immediately detached, the needle withdrawn and a dry cotton was placed on the puncture site. Tubes containing blood sampled were tagged with an identification code unique to each participant and transported to the laboratory for analysis.

2.3. Laboratory Analysis

For each participant (donor and recipient), the ABO/Rhesus blood grouping, the Rhesus and Kell phenotyping had been determined by the tube agglutination method.

1) Blood Grouping According to the Method of Beth Vincent:

It consists of searching the surface of red blood cells for the presence either of an antigen A (group A), or of a B antigen (group B), or of both (groups AB) and then makes it possible to define groups A, B or AB. Absence of A and B antigen defined group O.

2) Blood grouping according to the Simonin Michon method:

The 2th serum component of the test consists of looking for the presence or absence of anti-A antibodies or anti-B in serum. The presence of antigens of a certain type implied the

absence of antibodies of this specificity (under penalty of formation of an antibody-antigen complex).

These two tests, respectively of antigens (Beth-Vincent test) and of antibodies (Simonin-Michon test), are mandatory and must be concordant to establish an ABO blood group.

These two tests were validated by witnesses:

1) Allo control: this is the control of the serum test with group O red blood cells;
2) Control-auto: This control is carried out by mixing a drop of red blood cells from the participant with his serum. This mixture must be homogeneous for the reaction to be good quality. It makes it possible to verify the absence of auto- antibodies;

2.4. HR System Phenotyping

Rhesus phenotyping (Antigen D, C, E, c and e) and Kell

The Rhesus and Kell system determines, depending on the presence or absence of the D antigen, C, E, c, e and Kell1 on red blood cells, whether or not an individual has the corresponding antigen.

The techniques and process are the same as that of ABO blood grouping.

2.5. Statistical Data Analysis

The different variables and the results obtained after compliance verification were recorded in Excel software, then analyzed with SPSS software. The risk factors have were sought and the association was demonstrated with univariate logistic regression tests. The Level of significance was set at P-value < 0.05 and the confidence interval at 95%.

2.6. Ethical Consideration

In order to carry out our study in a well-defined ethical context, we have obtained:

1) An Ethics Clearance from the Institutional Ethics Committee of the University of Douala (AUTHORIZATION N^{oh}3042IEC-Udo/04/2022/M);
2) An authorization to collect data issued by the Regional Hospital of Bafoussam;
3) A free and informed consent of each participant;
4) We ensured in our study that the confidentiality of the participants was respected by using codes and not their names to identify them.

3. Results

3.1. Description of Recipients

Most of the blood recipients were in the age group of [00-10] years (23%), the average age was 31.15 ± 25 years with a minimum of one month and a maximum of 90 years. The male sex was the most represented (53.13%) with a sex ratio of 0.53.

The most represented matrimonial group was singles (48.40%). Finally, the Emergency service was the more represented (23.40%).

Table 1. *Description of recipients.*

Variables	Numbers (%)
Age (year)	
Mean ± Standard Deviation 31.15 ±25	31,15 ±25
Minimum; Maximum 18 years old; 91 years old	18 ans; 91ans
[00 – 10]	15 (23,0)
[10 – 20]	5 (7,90)
[20 – 30]	6 (9,40)
[30 – 40]	10 (15,70)
[40 – 50]	9 (14,10)
[50 – 60]	2 (3,20)
[60 – 70]	9 (14,10)
[70 – 80]	5 (7,90)
[80 – 90]	2 (3,10)
[90 – 100]	2 (3,10)
Sex	1 (1,60)
Male	34 (53,13)
Female	30 (46,87)
Marital status (n, %)	
Married	31 (48,40)
Singles	26 (40,70)
Widowed	7 (10,90)
Service of recipients (n, %)	
Emergency service	15 (23,40)
Pediatrics	13 (20,30)
Surgery	11 (18,80)
Gyneco-obstetrics	10 (15,50)
Neonatology	8 (12,60)
Internal Medicine	4 (6,30)
Gastroenterology	2 (3,10)

3.2. Description of Donors

Most of the blood recipients were in the age group of [00-10] years 23% (15/64), the average age was 31.15 ± 25 years with a minimum of one month and a up to 90 years old. The male sex was the most represented 53.13% (34/64) with a sexratio of 0.53. The most represented marital status was single 48.40% (31/64).

Finally the Emergency service was the most represented 23.40% (15/64).

Table 2. *Description of donors.*

Variables	Numbers (%)
Age (year)	
Minimum; Maximum	18 ans; 60ans
[10 – 20]	4 (4,90)
[20 – 30]	46 (56,80)
[30 – 40]	9 (11,10)
[40 – 50]	16 (19,80)
[50 – 60]	6 (7,40)
Sex (n, %)	
Male	67 (82,70)
Female	14 (13,7)
Sex-ratio	4,78
Marital status (n, %)	
Singles	44 (54,30)
Married	37 (45,70)
Donor status (n, %)	
Male	67 (82,70)
Female	14 (13,7)
Sex-ratio	4,78

3.2.1. Distribution of Donors According to ABO Blood Group

The majority blood group in blood donors was blood

group O, i.e. 51.90% (42/64).

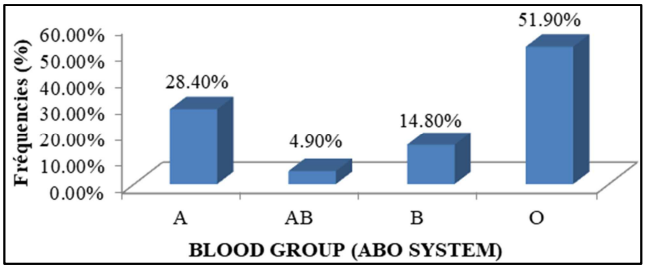

Figure 1. *Distribution of Donors according to ABO blood group.*

3.2.2. Distribution of Recipients According to ABO Blood Group

The majority blood group in blood recipients was blood group O, i.e. 40.60% (26/64).

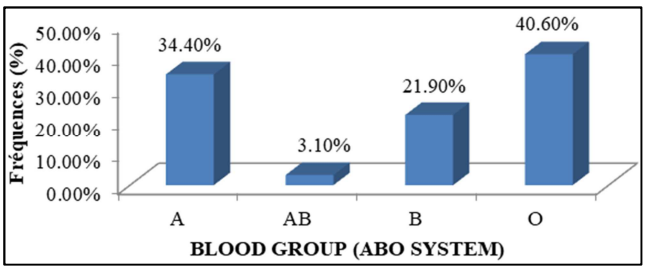

Figure 2. *Distribution of recipients According to ABO Blood Group.*

3.2.3. Frequencies of Donor Phenotypic Profiles in Rhesus and Rhesus Systems Kel

From this table 6, it appears that the majority of the donors carry the Dce phenotype (Dccee) with a frequency of 58.0% (47/81), followed by the DEce phenotype i.e. 24.7% (20/81).

Table 3. *Frequencies of phenotypic profiles of donors in the Rhesus system.*

RH SYSTEM	
Phénotypes	**Fréquencies**
DCce	4,9% (4/81)
Dce	2,5% (2/81)
DcedC	58,0% (47/81)
	1,2% (1/81)
EceDC	6,2% (5/81)
EceDE	24,7% (20/81)
Cede	2,5% (2/81)
Total	100,0% (81/81)

Table 4 below shows the frequency of Kell 1 antigen in donors of blood.

From this table, it emerges that 24.7% (20/81) of the donors carry the K1 antigen.

Table 4. *Frequency of Kell 1 antigen in blood donors.*

KELL SYSTEM	
Phénotypes	**Fréquencies**
K1 positif	24,7% (20/81)
K1 negatif	75,3% (61/81)
Total	100,0% (81/81)

3.2.4. Frequencies of Phenotypic Profiles of Recipients in the Rhesus and Kell Systems

From this table 5, it appears that the majority of the recipients carry the Dce phenotype

(Dccee) with a frequency of 59.4% (38/64), followed by the DEce phenotype, i.e. 17.2% (11/64).

Table 5. *Frequencies of phenotypic profiles of recipients in the Rhesus system.*

RH SYSTEM	
Phénotypes	**Fréquencies**
Dcce	3,1% (2/64)
DCce	6,3% (4/64)
Dce	3,1% (2/64)
Dce	59,4% (38/64)
DCe	1,6% (1/64)
DCEc	1,6% (1/64)
DCEce	6,3% (4/64)
DEce	17,2% (11/64)
Dee	1,6% (1/64)
Dcce	3,1% (2/64)
Total	100,0% (64/64)

Table 6 below shows the frequency of the Kell 1 antigen in recipients of blood.

From this table, it appears that 18.8% (12/64) of the recipients carry the K1 antigen.

Table 6. *Frequency of Kell 1 antigen in blood recipients.*

SYSTEME KELL	
Phénotypes	**Fréquencies**
K1 positif	18,8% (12/64)
K1 negatif	81,3% (52/64)
Total	100,0% (64/64)

3.2.5. Frequencies of the Different Antigens of the Rhesus System in the Study Population

From this table 7, it appears that the antigens of the Rhesus system in a decreasing way were: RH(e): 99.3% (144/145), RH(c): 98.6% (143/145), RH(D): 97.2% (141/145), RH(E): 31% (45/145) and finally HR (C): 15.2% (22/145).

Table 7. *Frequencies of the different antigens of the Rhesus system in of study he population.*

Antigènes	**Fréquences**
RH1 (D)	97,2% (141/145)
RH2 (C)	15,2% (22/145)
RH3 (E)	31% (45/145)
RH4 (c)	98,6% (143/145)
RH5 (e)	99,3% (144/145)

3.2.6. Frequency of Incompatible Antigens in the Rhesus and Kell System

Table 8, presents the frequency of incompatible antigens in the RH/Kell system in receivers.

36 out of 64 patients are incompatible in the Rhesus/Kell system with transfused blood either 56.25%.

Table 8. *Summary of incompatible antigens in the Rhesus and Kell system.*

RH SYSTEM		
ANTIGENS	**EFFECTIVE**	**FREQUENCY OF INCOMPATIBILITY**
Antigène C	10	15,62%
Antigène E	14	21,87%
Antigène c	1	1,56%
Antigène e	1	1,56%
Total	26	40,61%
KELL SYSTEM		
Kell1	10	15,62%
TOTALS	36	56,2%

Relationship between the number of blood bags received by the recipients (Compatible or not) and the probability of occurrence of allo-immunization in the Rhesus/Kell system.

Table 9, presents the relationship between the number of blood bags received by recipients (Compatible or not) and the probability of occurrence of allo-immunization.

Table 9. *Relationship between the number of blood bags received by recipients (Compatible or not) and the probability of occurrence of alloimmunization.*

Blood bag	**Risk of alloimmunization**		**Total**
	No risk of allo-immunization	**Risk of allo-immunization**	
1	22 (34,4%)	17 (26,6%)	39 (60,9%)
2	6 (9,4%)	18 (28,1%)	24 (37,5%)
3	0 (0,0%)	1 (1,6%)	1 (1,6%)
Total	28 (43,8%)	36 (56,2%)	64 (100,0%)

Khi-2: 6,746; ddl: 2; p: 0,034

Table 10. *Univariate analysis of the risk of occurrence of allo-immunization according to the compatibility and the number of blood bags received in univariate analysis; the risk of occurrence of alloimmunization in the Rhesus and Kell system is significantly associated with non-compatibility and the number of non-compatible blood bags received.*

Variables	**Risk of alloimmunization**		**IC bully**	**GOLD 95%**	**P-value**
	YES	**No**			
	N (%)	**n (%)**			
Compatibility					
Non	28 (43,8%)	36 (56,2%)	Ref	2,06-6,96	0,034
Oui	0 (0,00%)	0 (0,00%)	0,96		
Number of blood bags					
1	22 (34,4%)	17 (26,6%)	Ref		
2	6 (9,4%)	18 (28,1%)	0,98	3,05-9,26	0,0209
3	0 (0,0%)	1 (1,6%)			

4. Discussion

Our study focused on determining the risks of allo-immunization posttransfusion by determining Rhesus/Kell phenotypes in recipients of the blood bank of the Regional Hospital of Bafoussam. In our study 145 participants including 81 donors and 64 blood recipients were recruited on free and informed consent. The laboratory analysis was carried out taking into account the rules of good practice immuno-haematological and all patients were recruited on the basis of consent free and enlightened. We used tube agglutination method for Grouping blood ABO and Rhesus and Kell phenotyping.

The blood donors were relatively young, the most represented age group was that between [20-30] years with a frequency of 56.80%. Mahajanga et al. we find still this young age of blood donors with a proportion of 55.69% in the class 20 to 35 year olds [12]. This predominance could be explained by the rejuvenation of the Cameroonian population and blood transfusion criteria which favor young people more.

The most represented age group among recipients was between [00-10] years or 23.4%. Young children were in the majority in the study by Azonhouec et al. in 2008 and that carried out by Traore at the CHU Point-G Bamako, Mali in 2015 [13]. our average age was 31.15 years with extremes of one month and 90 years. This result is comparable to those obtained by DE Broche in France [14] and Eddakhchehicham in Morocco respectively 28.26 years and 31.07 years [15].

Among blood donors, men outnumber women with respectively 82.70% (67/81) versus 17.30% (14/81). Sanogok at the CNTS [16] had found 84% for men and 16% for women. The M/F sex ratio was 4.78 in favor men. This male predominance is constantly found in most studies carried out in recent years, in particular in Antananarivo at the CHU JRA, at CENHOSOA Soavinandriana and Mahajanga with respectively a proportion of 80.46% [17], 92.90% [18] and 81.75% [19]. This male predominance could be explained on the one hand by the selection criteria for blood donation limiting female participation.

The male sex was the most representative among the recipients in our sample with a frequency of 53.13% (34/64). Our result corroborates with those of Chemalak in Algeria and Soumaya in Morocco who had reported that the male gender was in the majority with respectively 62.10% [20] and 64.7% [21]. Our result is also opposite to that reported by Drame [20] who had found that the female sex was in the majority with 68%. That this difference could be explained by the socio-demographic context of the study populations.

The familiar donor status was the most representative with a number of 48.10% (39/81). Regular voluntary donors represent only 12.3%. Similar results are already reported to the Soavinandriana Antananarivo Hospital Center in July and August 2011 on the prevalence of malaria in blood donors [18]. Likewise, the study on the distribution of erythrocyte antigens among blood donors in Antananarivo led by Randriamanantany et al. [17] published in 2012 also noted this low proportion of volunteer donors ranging from 13.14% to 24.14%. Furthermore, the study led by Enosolease in the Niger Delta regions reports that 44.57% of their study population were volunteer donors [22]. This could be explained by the loyalty of these donors, by mobile collections.

The Emergency department was the department that had more blood recipients, i.e. 23.40% (15) followed by pediatrics, i.e. 20.30% (13/64). Our result is in agreement with the study conducted by Azonhouec et al. In 2008 in Cotonou [23] and Traore at the CHU Point-G Bamako, Mali in 2015 [13]. Moreover, it is different from the study conducted by Ouadghiri et al. In 2017 in a retrospective study from 1999-2013 found 53.3%in the medical department followed by surgery general 25.5% [24]. The difference between our study and that carried out by Ouadghiri might be explained by the fact of a great frequency of cases of traffic accident in the course of our study.

The majority blood group in blood donors was blood group O Rhesus (D) positive with a frequency of 51.90% (42/81). This result is similar to the blood group distribution in some studies carried out in Mali which all reported a predominance of the O Rhesus positive group, i.e. 41.7% [25] and 45.7% [26]. This is explained by the fact that in the black race this group is the most represented [27]. The major part of donors carries the Dce phenotype still represented Dccee (RH1(D)+, RH2(C)-, RH3(E)-, RH4(c)+, RH5(e)+) with a frequency of 58.0% (47/81). Mornandji PC [28] in Mali in 2001 had found a predominance of this same phenotype in patients with renal failure.

The majority blood group in blood recipients was blood group O with a frequency of 40.60% (26/81) [29, 30]. This result is similar to that of Drame at Mali and Sawadogo in Burkina Faso who had 42.2% and 40.9% respectively [31]. The major part recipients carry the Dce phenotype also called Dccee (RH1(D)+, RH2(C)-RH3(E)-, RH4(c)+, RH5(e)+) with a frequency of 59.4% (38/81). The Dccee phenotype is the most usual among blacks [31, 32]. Our 59.4% frequency is similar to those provided by O. Traore, Guindo and Tolo who respectively reported frequencies of: 70.2%; 60.5%; 78.6% and 67.9% [33-34].

Our study shows that 36 patients out of the 64 recruited are incompatible in the Rhesus and Kell systems to transfused blood, i.e. a frequency of 56.2% recruited are at risk of develop post-transfusion alloimmunization for one or more antigens of Rhesus and Kell systems. The rate of alloimmunization reported in the literature varies according to studies between 4 and 47% with a median of 25% [35-36]. Regarding the relationship between the number of blood bags received by recipients (Compatible or not) and the risk of occurrence of allo-immunization, we obtained statistically by the Chi-test two independence and by univariate analysis that the risk of allo-immunization in the Rhesus and Kell systems is likely to increase depending on the number of bags received incompatible. This result corroborates those reported by I. Ben et al. in Tunisia [37] and Zidouh et al. In 2014 in Morocco [38] that allo-immunization increases according to the number of blood bag received incompatible.

5. Conclusion

At the end of our study, we recruited a hundred participants who gave their informed consent. Among these hundred participants, about eighty were donor and about sixty was recipient. We performed blood grouping, Rhesus and Kell phenotyping on these participants. Blood type O was the majority follow-up of blood group A. The frequency of antigens of the Rhesus system in a decreasing way was as follows: RH (e) > RH (c) > RH (D) > RH (E) > RH (C). Moreover, the most represented in the study population, in donors and in recipients was Dce (Dccee).

We obtained a risk of allo-immunization of about forty percent in the Rhesus system and fifteen percent in the Kell system. Thus the risks of alloimmunization were more represented by incompatibilities with the E antigen (twenty in percentage), K (about fifteen as a percentage) and C (about fifteen as a percentage). Regarding the relationship between the number of blood bags received by recipients (Compatible or not) and the risk of occurrence of allo-immunization, we obtained in a way statistically by the Khi-square test of independence and by univariate analysis that the risk of allo immunization in the Rhesus and Kell systems is likely to increase depending on the number of pouches received incompatible.

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