

RESEARCH ARTICLE

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Evaluation of the prevalence of anti-erythrocyte alloimmunization in 100 polytransfused patients in Lomé, Togo

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ABSTRACT

Aims: Red blood cells transfusion exposes recipients to a risk of immunization. The phenotyping of red blood cells and the search for irregular erythrocyte antibodies ensure immuno-hematological safety of the transfused patients. In Togo, these tests are not systematic in transfusion. The aim of the study was to determine the rate of immunization against erythrocyte antigens in polytransfused patients in Lomé.

Methods: The search for irregular agglutinins was carried out by the indirect antiglobulin test in gel-filtration in 100 polytransfused patients selected at National Center for Research and Care of Sick Cells disease and in the care units of Campus Teaching

Hospital of Lomé in Togo, from October 1, 2021 to April 30, 2022.

Results: The prevalence of alloimmunization was 27% with a total of 13 different alloantibodies identified. The most represented were anti-K (18.52%), anti-E (14.81%), anti-C (11.11%), anti-Jka (11.11%), and anti-M (11.11%). Alloantibodies were more frequent in A+ blood group patients and those who received more than 4 bags of packed red blood cells. There was no link between age, sex, and the pathologies involved on one hand, and the occurrence of anti-erythrocyte alloimmunization on the other.

Conclusion: The rate of immunization against erythrocyte antigens is very high in polytransfused patients in Lomé. Systematic implementation of red blood cells phenotyping and irregular erythrocyte antibodies research before and after transfusion should ensure better immunological transfusion safety in Togo.

Keywords: Alloimmunization, Irregular erythrocyte antibodies, Polytransfused, Togo

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INTRODUCTION

Blood transfusion is a therapy based on the use of blood products of human origin in order to save lives. It nevertheless involves risks for the recipient, in particular the occurrence of several immunological or infectious complications for the patient. Despite the efforts made in the field of immunohematology, transfusion of packed red blood cells (RBCs) brings foreign antigens to recipients. These erythrocyte antigens unknown to the recipient could induce the appearance of anti-erythrocyte alloantibodies in the latter. The risk of occurrence of anti-erythrocyte alloimmunization is therefore greater in polytransfused subjects and increases with the number of bags of packed red blood cells transfused [1].

Indeed, Padaro et al., in 2004, in a study among major sickle cell patients followed at the University Hospital Center (CHU) Campus of Lomé in Togo had found a prevalence of alloimmunization of 50% [2]. Along the same lines, Kouacou et al. revealed an alloimmunization prevalence of 28.6% in a cohort of 42 polytransfused sickle cell patients followed in the transfusion therapy unit of the National Blood Transfusion Center of Abidjan in Côte d'Ivoire in 2017 [3].

In 2004, Noizat-Pirenne concluded that overall, the prevalence of alloimmunization in polytransfused patients in general, and in sickle cell patients in particular, fluctuated between 4% and 40% [4].

Alloimmunization constitutes a long-term obstacle to transfusion in polytransfused patients. This is why the preventive transfusion policy should avoid it because it exposes to the risk of immediate or delayed hemolytic accidents, as well as to the transfusion impasse linked to the presence of multiple antibodies with a virtual impossibility of finding compatible red blood cells. Before the first transfusion, it is desirable to determine the patient's phenotype in the most immunogenic erythrocyte blood group systems. In addition, the practice of research for irregular agglutinins (RIA) in blood donors comes up against economic difficulties. In fact, most developing countries devote a small budget to transfusion, which only allows ABO and Rhesus typing and certain serological tests that are essential to deal with medical emergencies. One way to improve the immunological safety of transfusions in developing countries could be the phenotyping of blood donors in the most immunogenic erythrocyte blood group systems. This will make it possible to give compatible blood to polytransfused patients and to patients in rare groups whenever iterative transfusions are envisaged.

In order to contribute to the strengthening of transfusion safety in Togo, it seemed appropriate to us to undertake this study to determine the prevalence of anti-erythrocyte alloimmunization in patients at risk, in particular those who are polytransfused in Lomé.

The aim of our study is to determine the frequency of anti-erythrocyte alloimmunization in polytransfused patients recruited in the care units at the CHU Campus

and the National Center for Research and Care for Sickle Cells (CNRS) in Lomé, Togo.

MATERIALS AND METHODS

Study population

We carried out a prospective longitudinal study which made it possible to recruit polytransfused patients (transfused at least twice) admitted to different care services (Pediatrics, Internal Medicine, Hepato-Gastro-Enterology, Neurology, Cardiology) of the CHU Campus of Lomé and at the CNRS of Lomé from October 1, 2021 to April 30, 2022. These patients were enrolled in the laboratory department of the CHU Campus when requesting a compatibility test for a blood transfusion.

Participants were selected without distinction of age, sex, race, religion or nationality. Patients lost to follow-up and who did not undergo a post-transfusion test were excluded from the study. In the end, 100 patients who had been transfused at least twice with packed red blood cells (RBC) or whole blood, and whose last transfusion was less than three months ago, were selected for the study.

The sample size for this study was determined by the SCHWARTZ formula

$$n = t^2 \times p(1-p)/m^2$$

with the calculation factors: prevalence of the factor studied (p); risk of error accepted ($t^2 = 1.96^2$); power (m = 0.05).

According to Baby et al. in 2010 in Bamako (Mali), the frequency of erythrocyte alloimmunization with extended phenotyping was 4.23–10.3%. Therefore, the minimum sample size is 62. For greater representativeness, we opted for a sampling of 100 polytransfused patients [5].

Ethical considerations

Before the start of the study, an agreement from the Bioethics Committee for Health Research (CBRS) had been obtained. Informed consent was obtained from all study participants; and that of minors was obtained from parents, guardians, or accompanying persons.

Detection of irregular agglutinins (IAR)

The participants being known multiple transfusion recipients, irregular agglutinins were screened for at recruitment, and 21–40 days after a new transfusion or series of transfusions; using a panel of three blood group O red blood cells: ID-DiaCell I-II-II (Biorad, France) for screening, and for identification, ID-DiaPanel (Biorad, France) of eleven group red blood cells blood O on low ionic strength solution (LISS)-Coombs gel card (Biorad, France).

This technique was based on an indirect polyspecific antiglobulin test on a filtration medium. All the reactions were carried out in a low ionic strength solution (LISS) medium which facilitates the accessibility of the antigens and increases the speed of antibody binding, thus

reducing the reaction incubation time. In the event of a positive screening, the identification step consisted of determining the specificity of the antibody(ies) present in the patient's plasma by comparing the distribution of the positive and negative reactions obtained with the distribution of the antigens on the panel of test red blood cells used [6]. This analysis led to an antibody hypothesis which was confirmed by the determination of the erythrocyte phenotype of the patient in the blood group system concerned because an individual can only become immunized against an antigen that he does not possess.

Statistical analyses

Data were analyzed in SPSS software (IBM SPSS Statistics 21, Armonk, NY) and Excel 2021 spreadsheet software. Student's *t*-test and Fisher's Chi-square/exact tests were used, respectively, to compare means and frequencies, with a significance level of 0.05. A binary logistic regression was carried out by associating the different parameters (age, sex, blood groups, the different pathologies involved, and the number of transfusions received) with the alloimmunization status of the participants, with the calculation of odds ratio (OR) and adjusted odds ratio (aOR) at 95% confidence interval. OR and aOR were considered significant when $p < 0.05$ and the value 1 was not included in the confidence interval. The values of OR and aOR were interpreted as follows: OR/aOR=1, there is no association, when OR/aOR>1, the variable studied is associated with alloimmunization, and when OR/aOR<1, the variable studied is a protective factor against alloimmunization. The Office 2021 Excel spreadsheet software was mainly used to produce the prevalence graphs.

RESULTS

Socio-demographic and clinical data of the study population at recruitment

Among the 100 patients included in the study, there was a male predominance with 59 men for 41 women, either a sex ratio M/F = 1.26. The study population had an average age of 34.19 ± 19.45 years with extremes of 3 and 85 years. Blood groups O+, A+, and B+ were the most represented with 51%, 21%, and 18% respectively. Regarding the pathologies involved, sickle cell disease ranked first with 38%, followed by hepatopathy at 34% and hematological malignancies with 14%. The average number of transfusions was 4.04 ± 3.0 with extremes of 2 and 32 at recruitment. All the socio-demographic and clinical data at recruitment are presented in Table 1.

Prevalence of alloimmunization in the study population at recruitment

The search for autoantibodies in the participants at the start of the study revealed that 19% (n=19) of the participants were alloimmunized.

Table 1: Socio-demographic and clinical characteristics of the study population at recruitment

Variables	Values
Age (years)	
Average	34.19 ± 19.45
Median	31 [19; 46]
Minimum	3
Maximum	85
Sex, n (%)	
F	41 (41)
M	59 (59)
Blood groups, n (%)	
A–	3 (3)
A+	21 (21)
AB–	1 (1)
AB+	2 (2)
B–	2 (2)
B+	18 (18)
O–	2 (2)
O+	51 (51)
Pathologies, n (%)	
Sickle cell disease	38 (38)
Hematological malignancy	14 (14)
Hepatopathy	34 (34)
CRI*	6 (6)
HIV	5 (5)
Malaria	3 (3)
Number of transfusions	
Average	4.04 ± 3.0
Median	3 [2; 5]
Minimum	2.0
Maximum	32.0

*CRI: chronic renal insufficiency.

In the 19 alloimmunized polytransfused patients, 12 different antibodies were found with two anti-C/anti-M and anti-K/anti-NI associations (NI = Unidentified antibody). The most represented alloantibodies were anti-K antibodies (5 cases out of 19 either 26.32%), anti-M (3 cases out of 19 either 15.79%), anti-E (2 cases out of 19 either 10.53%), and anti-C (2 cases out of 19 or 10.53%). The alloantibody not identified here is the one giving a result that is difficult to interpret because it does not correspond to any case in the interpretation table, despite two different series of manipulations. Figure 1 represents the different types of alloantibodies found.

Distribution of alloantibodies by pathologies and blood groups at recruitment

When recruiting patients, the analysis of the distribution of alloantibodies according to the pathologies encountered revealed that the groups of subjects with sickle cell disease, hematological malignancies, and hepatopathies were the most alloimmunized. Only one patient with chronic renal failure had anti-C alloantibodies. No polytransfused subject with human immunodeficiency virus (HIV) or malaria was alloimmunized at this stage of the study.

Furthermore, analysis of the distribution of alloantibodies according to blood groups revealed that blood group A+ had a total of 10 alloantibodies followed by group O+ with 5 alloantibodies. Subjects in groups A– and B+ each had an anti-K antibody, and one subject in group B– had an anti-D antibody. None of the AB group subjects had developed alloantibodies. This distribution of alloantibodies is shown in Figure 2.

Factors associated with alloimmunization at recruitment

The relationship between the socio-demographic characteristics, the potential risk factors (associated pathologies and the number of transfusions), and the alloantibodies found is presented in Table 2. The Chi-square test revealed a significant difference between alloimmunized and non-immunized subjects, concerning the following variables: age, sex, blood group, pathologies, and number of transfusions. Alloimmunized subjects were significantly older than non-alloimmunized (34.0 ± 20.0 years versus 32.0 ± 13.0 , $p < 0.001$); male subjects were more alloimmunized than female subjects ($p < 0.001$); subjects with blood group A+ performed alloimmunization more than subjects with other blood groups ($p = 0.016$). Concerning the pathologies (sickle cell disease, hematological malignancies, hepatopathies, HIV, and malaria) in question, those who did not have alloantibodies were significantly more important than those who did ($p < 0.001$). Concerning the number of transfusions, the frequency of alloimmunization was higher in subjects who received more than 3 blood bags ($p = 0.029$).

After a univariate binary logistic regression analysis, polytransfused group A+ subjects showed a higher risk of developing an alloantibody (OR=6.00; 95% CI [2.04–22.00], $p = 0.002$), and polytransfused subjects who received more than 3 transfusions presented a significant risk of developing alloimmunization (OR=3.00; 95% CI [1.08–9.00], $p = 0.034$). Similarly, after adjustment in the multivariate model to eliminate all confounding factors, it emerges that the risk of anti-erythrocyte alloimmunization was linked to blood group A+ (aOR=6.00; 95% CI [1.00–21.00] (Table 2).

Socio-demographic and clinical data of the study population at the end of the study

Twenty-one to forty days after the last blood transfusion (0–3 transfusions) received by our participants who had already received multiple transfusions, the socio-demographic and clinical data showed an evolution on the average number of transfusions, which went from 4.04 ± 3.0 at enrollment to 5.0 ± 3.0 with a minimum of 2 and a maximum of 35 as shown in Table 3. In the same order, the prevalence of alloimmunization increased from 19% to 27% of the study population as shown in Table 4. It emerges from the analysis of this table that 8 new polytransfused patients were subsequently alloimmunized (Table 3).

Description of alloantibodies in alloimmunized subjects at the end of the study

Of the 100 polytransfused patients included in our study, 19 had presented an alloimmunization during the performance of the irregular agglutinins research (IAR) on the day of their enrolment. At the end of the follow-up period, apart from the 19 patients initially alloimmunized, 8 other patients presented an alloimmunization bringing to 27 the number of alloimmunized patients. A total of 13 different alloantibodies including 3 cases of association of antibodies (anti-E/anti-Jka, anti-K/NI, and anti-C/anti-M), and 2 unidentified antibodies were found. The most represented alloantibodies were: anti-K (5 cases out of 27 either 18.52%), anti-E (4 cases out of 27 either 14.81%), anti-C (3 cases out of 27 either 11, 11%), anti-Jka (3 cases out of 27 or 11.11%), and anti-M (3 cases out of 27 or 11.11%). The two unidentified alloantibodies are those giving results that are difficult to interpret. The first did not correspond to any case in the interpretation table and the second presented a positive autologous control, even after two different series of manipulations. Figure 3 represents the prevalence of alloantibodies (single antibodies and combinations) in alloimmunized subjects.

Distribution of alloantibodies by pathologies and blood groups in alloimmunized patients at the end of the study

Following the analysis of the distribution of alloantibodies according to the pathologies from which the patients suffered at the end of the study, 11 types of alloantibodies were identified in sickle cell patients, 7 types in subjects with malignant hemopathies and 8 types in subjects suffering from hepatopathies. Regarding blood groups, group A+ subjects had a total of 11 types of alloantibodies, followed by group O+ subjects with 6 types of alloantibodies and group B+ subjects with 3 types of alloantibodies. The distribution of these alloantibodies is shown in Figure 4.

Table 2: Descriptive analysis and logistic regression of factors related to alloimmunization at recruitment

Alloimmunization							
Descriptive analysis				Logistic regression analysis			
Alloimmunized			p-value	Univariate		Multivariate	
No	Yes			OR (95% CI)	p-value	aOR (95% CI)	p-value
Age	34.0 ± 20.0	32.0 ± 13.0	0.001*	0.00 (0.00–1.02)	0.00		
Sex			0.001*				
F	32 (39)	9 (47)		0.00 (0.00–1.00)	0.00		
M	49 (61)	10 (53)		1			
Group			0.016*				
A–	2 (2.5)	1 (5.3)		3.00 (0.00–47.00)	0.00	4.00 (0.00–66.00)	0.00
A+	11 (13.6)	10 (52.6)		6.00 (2.04–22.00)	0.002	6.00 (1.00–21.00)	0.004
AB–	1 (1.2)	0 (0)		0 (0)		0 (0)	
AB+	2 (2.5)	0 (0)		0 (0)		0 (0)	
B–	1 (1.2)	1 (5.3)		7.00 (0.00–136.00)	0.00	14.00 (0.00–296.00)	0.081
B+	17 (20.9)	1 (5.3)		0.00 (0.049–3.00)	0.00	0.00 (0.046–3.00)	0.00
O–	2 (2.5)	0 (0)		0 (0)		0 (0)	
O+	45 (55.6)	6 (31.5)		1		1	
Sickle cell disease			0.001*				
No	49 (60.5)	13 (68.4)		1.00 (0.00–4.00)	0.001		
Yes	32 (29.5)	6 (31.6)		1			
Hematological malignancy			0.001*				
No	72 (88.0)	14 (73.0)		0.00 (0.00–1.00)	0.095		
Yes	9 (12.0)	5 (27.0)		1			
Hepatopathy			0.001*				
No	54 (66.7)	12 (63.2)		0.00 (0.00–2.00)	0.00		
Yes	27 (33.3)	7 (36.8)		1			
CRI			1000				
No	76 (93.8)	18 (94.7)		1.00 (0.00–10.00)	0.00		
Yes	5 (6.2)	1 (5.3)		1			
HIV			0.001*				
No	76 (93.8)	19 (100)		1			
Yes	5 (6.2)	0 (0)		0 (0)	0.00		
Malaria			0.001*				
No	78 (96.3)	19 (100)		0 (0)	0.00		
Yes	3 (3.7)	0 (0)		1			
Number of transfusions			0.029*				
[2; 3]	48 (59.3)	6 (31.6)		1		1	
> 3	33 (40.7)	13 (68.4)		3.00 (1.08–9.00)	0.034*	3.00 (0.00–11.0)	0.051

Abbreviations: OR: odds ratio; CI: confidence interval.

Table 3: Number of transfusions and prevalence of alloimmunization at the end of the study

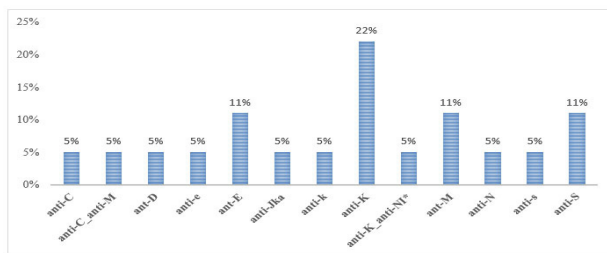
Number of transfusions	
Average	5.0 ± 3.0
Median	4 [3, 6]
Minimum	2.0
Maximum	35.0
Alloimmunized, n (%)	
No	73 (73)
Yes	27 (27)

Table 4: Descriptive analysis and logistic regression of factors related to alloimmunization at the end of the study

	Alloimmunization						
	Descriptive analysis			Logistic regression analysis			
	Chi-square			Univariate		Multivariate	
	No	Yes	p-value	OR (95% CI)	p-value	aOR (95% CI)	p-value
Age	34.0 ± 20.0	32.0 ± 13.0	0.001*	0.000 (0.000–1.023)	0.001		
Sex			0.001*				
F	28 (38.5)	13 (48.15)		1.000 (0.000–3.000)	0.001		
M	45 (61.65)	14 (51.85)		1			
Group			0.017*				
A–	2 (2.74)	1 (3.70)		2.000 (0.000–28.000)	0.001	1.000 (0.000–25.000)	0.001
A+	10 (13.70)	11 (40.76)		5.000 (1.000–15.000)	0.004	4.000 (1.000–15.000)	0.008
AB–	0 (0)	0 (3.70)		NA	0.001	NA	0.001
AB+	1 (1.37)	1 (3.70)		4.000 (0.000–81.000)	0.001	2.000 (0.000–48.000)	0.001
B–	1 (1.37)	1 (3.70)		4.000 (0.000–81.000)	0.001	9.000 (0.000–192.000)	0.001
B+	15 (20.55)	3 (11.11)		0.000 (0.000–3.000)	0.001	0.000 (0.000–3.000)	0.001
O–	2 (2.74)	0 (0)		0 (0)		0 (0)	0.001
O+	42 (57.53)	9 (3.33)		1		1	
Sickle cell disease			0.001*				
No	45 (61.65)	17 (62.96)		1.058 (0.000–2.000)	0.0001		
Yes	28 (38.35)	10 (37.04)		1			
Hematological malignancy			0.001*				
No	65 (89.04)	21 (77.78)		1			
Yes	8 (10.96)	6 (22.22)		2.000 (0.000–7.000)	0.001		
Hepatopathy			0.001*				
No	48 (65.75)	18 (66.67)		1.042 (0.000–2.000)	0.001		
Yes	25 (34.25)	9 (33.33)		1			
CIR			0.001*				
No	68 (93.15)	26 (96.30)		1			
Yes	5 (6.85)	1 (3.70)		0.000 (0.058–4.000)	0.001		
HIV			0.001*				
No	68 (93.15)	27 (100)		1			
Yes	5 (6.85)	0 (0)		0 (0)	0.001		
Malaria			1.000				
No	71 (97.26)	26 (96.30)		0 (0.064–8.000)	0.001		
Yes	2 (2.74)	1 (3.70)		1			
Number of transfusions			0.013				
[2, 4]	42 (57.53)	8 (29.63)		1		1	
>4	31 (42.47)	19 (7.37)		3.000 (1.000–8.000)	0.016	3.000 (1.000–11.058)	0.019*

Distribution of alloimmunized patients according to the number of transfusions at the end of the study

Figure 5 represents the distribution of polytransfused patients who had a positive IAR according to the number of transfusions received, subdivided into three sections. Polytransfused patients who received between 6 and 10 transfusions are the most represented with 48%. Conversely, polytransfused patients who received more than 11 transfusions are the least represented with 11%; however, only three patients are in this situation in our study (Figure 5).



NI* = Not identified

Figure 1: Prevalence of alloantibodies in alloimmunized at recruitment.

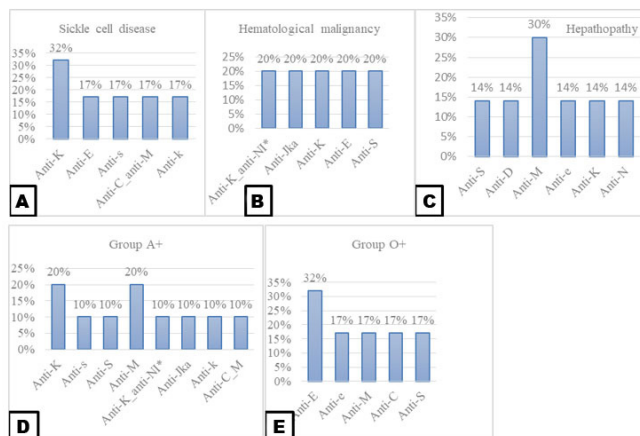


Figure 2: Prevalence of alloantibodies at recruitment: (A) in sickle cell patients (n = 38), (B) in polytransfused patients with hematological malignancy (n = 14), (C) in polytransfused patients with hepatopathy (n = 34), (D) in polytransfused blood group A+ (n = 21), (E) in polytransfused group O+ (n = 50).

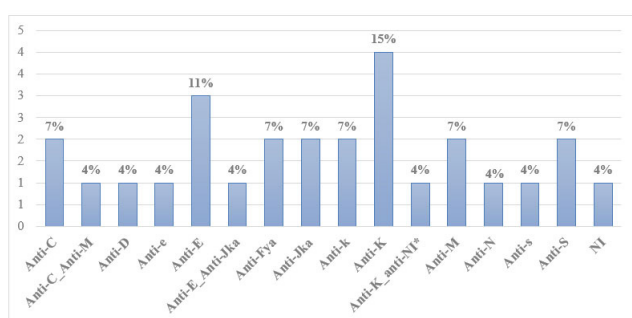


Figure 3: Prevalence of alloantibodies in alloimmunized at the end of the study.

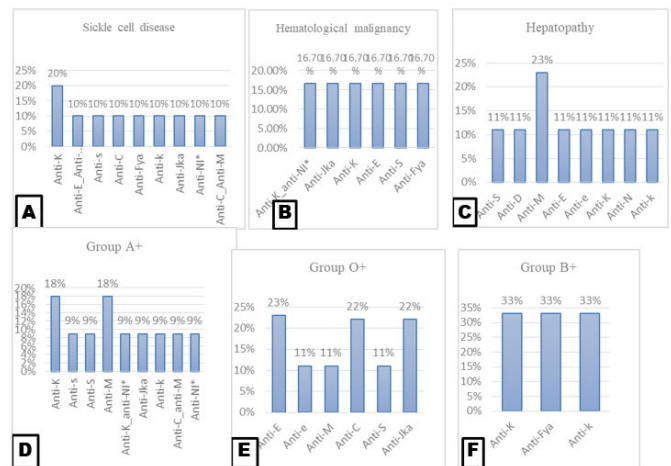


Figure 4: Prevalence of alloantibodies: (A) in sickle cell patients (n = 38), (B) in polytransfused patients with hematological malignancy (n = 14), (C) in polytransfused patients with hepatopathy (n = 34), (D) in polytransfused blood group A+ (n = 21), (E) in polytransfused group O+ (n = 50), and (F) in polytransfused group B+ (n = 17) at the end of the study.

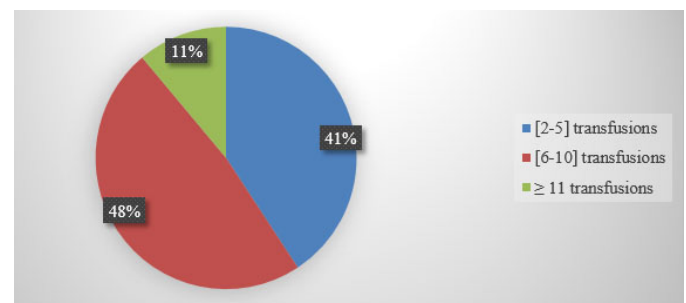


Figure 5: Distribution of polytransfused patients with a positive IAR according to the number of transfusions.

Factors associated with alloimmunization at the end of the study

The Chi-square test revealed a significant difference between alloimmunized and non-immunized subjects, concerning the following variables: age, sex, blood group, pathologies, and number of transfusions. Alloimmunized subjects were significantly older than non-alloimmunized (34.0 ± 20.0 years versus 32.0 ± 13.0 , $p < 0.001$). For gender, male subjects were more alloimmunized than female subjects ($p < 0.001$); subjects with blood group A+ had alloimmunization more often than subjects with other blood groups ($p = 0.017$). Concerning the pathologies in question (sickle cell disease, hematological malignancies, hepatopathies, chronic renal failure, and HIV) in question, those who did not have alloantibodies were significantly more important than those who did ($p < 0.001$). Regarding the number of transfusions, the frequency of alloimmunization was higher in subjects who received more than 4 blood bags ($p = 0.013$).

After a univariate binary logistic regression analysis, polytransfused group A+ subjects always had a higher risk of developing alloantibodies (OR=5.000; 95% CI [1.000–

15.000], $p=0.008$). Similarly, polytransfused subjects who received more than 4 transfusions presented a greater risk of developing alloimmunization (OR=3.000; 95% CI [1.000–8.00], $p=0.016$). However, these risks were still not statistically significant. After multivariate adjustment, the risk of anti-erythrocyte alloimmunization was always higher in subjects in group A+ (aOR=4.000; 95% CI [1.000–15.000], $p=0.008$) and in subjects who received more of 4 transfusions (aOR=3.000; 95% CI [1.000–11.058], $p=0.019$). All these results are recorded in Table 4.

It emerges from the analysis of Table 4 that the polytransfused subjects of blood group A+ and those who received more than 4 transfusions presented a higher risk of developing alloimmunization. On the other hand, age, sex, and the various pathologies from which polytransfused patients suffered were not risk factors for the development of alloimmunization.

DISCUSSION

Blood transfusion, whatever the precautions, presents a risk for the recipient. Therefore, even in countries where blood transfusion is performed with extensive erythrocyte phenotyping and IAR before and after each transfusion of red blood cells, alloantigenic differences persist and polytransfused patients are not spared from alloimmunizations [7]. We conducted this study to assess the prevalence of alloimmunization to major alloantigens in polytransfused patients who received compatible red blood cells only for the ABO and Rhesus standard D systems in Lomé, Togo.

The search for anti-erythrocyte alloantibodies was carried out by the indirect antiglobulin test in a medium of low ionic strength solution (LISS) on a filtration medium in accordance with the recommendations of the manufacturer of the reagent relating to the proper execution of the analyzes of medical biology [8]. This technique is very sensitive, well standardized and allows the detection of all clinically significant antibodies. It has often been used by other authors in the literature [9].

The relationship between the socio-demographic characteristics, the potential risk factors (associated pathologies and the number of transfusions), and the alloantibodies found did not change much compared to those obtained at recruitment. The socio-demographic results show a male predominance with 59 men for 41 women and an average age of 34.19 ± 19.45 years. These results differ from those observed by Kouacou et al. in Côte d'Ivoire in a study of major sickle cell patients who reported gender parity and an average age of 24.45 years [3]. It should be noted that Kouacou's study only concerned patients with sickle cell disease, a disease that affects both sexes equally, because it is of autosomal recessive transmission; therefore, not linked to sex [10].

We found an alloimmunization frequency of 27% overall at the end of the study. These results

can be superimposed on those found in 2014 in Togo by Magnang et al. in a study on the occurrence of alloimmunization with the Rhesus and Kell systems which reported a prevalence of 28.52% [11]. This prevalence is also similar to that found by Kouacou et al. who reported a prevalence of 28.6% of alloimmunization in 2017 [3]. On the other hand, this prevalence in our patients is higher than that found by Baglo et al. in a study of multiple transfusion recipients in Cotonou, Benin, where they reported a prevalence of 13.73% [12]. In addition, Diarra in Mali in 2011 and Kolou in Côte d'Ivoire in 2005 reported much lower prevalences of 4.4% [13] and 4% [14] respectively. Similarly, Pahuja et al. in 2010 in India reported in a study in thalassemia patients a prevalence of 3.79% [15] and Yétéma et al. in 2022 in Bobo Dioulasso in Burkina Faso reported in a study on multiple transfusion recipients a prevalence of 5.67% [16]. It should be noted that these studies are difficult to compare due to the variability of the study periods, the different antibodies sought, the methods of data collection, the underlying pathologies of the recipients as well as the regulations concerning mandatory pre-tests—transfusionists from the countries where each of the studies took place.

For alloantibody specificities, we found a total of 13 types of alloantibodies including 3 cases of antibody association and 2 unidentified antibodies. These results can be superimposed on those reported by Kouacou et al. in Ivory Coast who had found 14 alloantibodies including 2 cases of association of 2 alloantibodies [3].

The most represented alloantibodies were anti-K antibodies (5 cases out of 27 either 18.52%), anti-E (4 cases out of 27 either 14.81%), anti-C (3 cases out of 27 either 11.11%), anti-Jka (3 cases out of 27 or 11.11%), and anti-M (3 cases out of 27 or 11.11%). According to the literature, the most immunogenic blood group antigens are, in decreasing order of immunogenicity, the D, K, E, c, Fya, and Jka antigens [17]. Along the same lines, Castro et al. have in a study on transfusion in sickle cell patients concluded that if all transfusions had been selected by limited phenotypic matching (C, c, E, e, and K, as well as for ABO and D), all alloantibodies would have been avoided for more than half (53.3%) of the 137 alloimmunized patients. And if all transfusions had been matched for C, c, E, e, K, S, Fya, and Jkb, all antibodies would have been avoided in 70.8% of the 137 alloimmunized patients. About 13.6% of random white blood donors would be expected to match a limited phenotype matching protocol, while only 0.6% would match an extended phenotype matching protocol [18].

The two cases of unidentified alloantibodies are those giving results that are difficult to interpret. The first did not correspond to any case in the interpretation table and the second presented a positive autologous control, even after repeating the manipulation twice. A positive autologous control could reflect the presence of an anti-erythrocyte auto-antibody, proof of which could be provided by tests of auto-adsorption of the antibody

associated with an RAI carried out on the adsorbed serum [6]. This could not be achieved during our study. However, a conflict by transfusion incompatibility is not formally ruled out because all the patients recruited had received at least two bags of packed red blood cells or whole blood during the last 3 months.

Regarding the number of transfusions received in relation to the onset of alloimmunization, our study shows that patients who received between 6 and 10 transfusions of packed red blood cells are the most represented with 48%, and that only 11% of patients received more than eleven bags of packed red blood cells. In addition, in statistical studies, we noted that polytransfused subjects who received more than 4 transfusions presented a higher risk of having alloimmunization. Our results are close to those found by Khachaa et al. who reported that alloimmunization was greater in polytransfused patients who received more than two bags of packed red blood cells [19]. They are different from those found on the one hand by Baglo et al. [12] and secondly by Dembélé et al. [20] who respectively reported 15 transfusions and more than 15 transfusions.

With regard to ABO and rhesus blood groups, polytransfused subjects of group A+ presented a higher risk of alloimmunization ($p=0.004$). These results are similar to those found by Akpan et al. in 2022 in Nigeria who reported that blood group was significantly associated with alloimmunization ($p=0.013$) in a study of polytransfused sickle cell patients and blood donors [21].

On the other hand, age, sex, and the various pathologies from which polytransfused patients suffered were not risk factors for developing alloimmunization. Like most similar studies, age and sex were not associated with the appearance of anti-erythrocyte alloantibodies but rather with the number of transfusions received as in our case [1, 20]. However, the size of our study population could be related to the non-correlation found.

CONCLUSION

At the end of our study, we can say that the prevalence of alloimmunization of 27% that we found reflects the risk of immunization incurred by polytransfused patients. This demonstrates that the practice of simply respecting ABO/Rhesus compatibility according to the protocol of the Ottenberg and Schultz scheme is insufficient. It is desirable on the one hand to carry out an extended erythrocyte phenotyping in voluntary blood donors and in recipients, and on the other hand to carry out an IAR before and after transfusion in patients who have received multiple transfusions or are likely to be. These measures could make it possible to optimize immunological transfusion safety in Togo.

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Authors declare no conflict of interest.

Data Availability

All relevant data are within the paper and its Supporting Information files.

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