





Prevention of alloimmunization in patients with sickle cell disease in Chad

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ABSTRACT: The objective of this study was to contribute to the prevention of anti-erythrocyte alloimmunization in patients with sickle cell disease (DS) in Chad. This was a descriptive and analytical cross-sectional study from December 2021 to June 2022 in sickle cell patients regularly followed in two University Hospital Centers (CHU) in N'Djamena. The search for irregular antibodies was carried out by a combination of three techniques: indirect anti-globulin test (Coombs), enzymatic test (Papain) and the test in a low ionic strength medium, in a partner laboratory of the National Blood Transfusion Center in France. The study included 57 sickle cell patients, 45 of whom (78.9%) were actually transfused. Sickle cell patients accounted for 84.2% of cases. The sex ratio Male/Female (M/F) was 1.03. The average age of the transfused was 9.3 ± 5.4 years. Four of the transfused patients (8.8%) had produced 7 antibodies including 85.7% anti-rhesus and 14.3% anti-Kell. Age, sex and number of blood units were associated with antibody production with probabilities of 0.046, 0.041 and 0.035, respectively. In view of the results obtained from this study, we recommend the application of the blood transfusion rules and procedures in force in Chad.

KEYWORDS: Allo-immunization, sickle cell disease, prevention, Chad

INTRODUCTION

Anti-erythrocyte alloimmunization corresponds to an individual's immune response to foreign erythrocyte antigens (Ag), that is to say not present on the surface of their red blood cells [1].

The main problem is linked to the genetic polymorphism of erythrocyte blood groups resulting in the identification by the International Society of Blood Transfusion (ISBT) of more than 300 erythrocyte antigens, most of them listed in 30 blood group systems [1]. Clinically, anti-erythrocyte allo-immunization remains a major problem for patients whose pathology justifies chronic transfusions [2]. In the event of Ag/Ab conflict, antibodies (Ab) have the ability to induce destruction of target red blood cells, the clinical consequences of which range from transfusion inefficiency to renal failure, disseminated intravascular coagulation (DIC), hypovolemic shock, even the death of the patient [1].

In the literature, the prevalence of anti-erythrocyte allo-immunization varies between 4 and 47% in major sickle cell syndromes [3]. In Chad, findings and data from hospital settings showed that sickle cell disease, a pathology that justifies multiple transfusions, is increasingly being diagnosed. Its management often requires the use of blood transfusion, which is sometimes the only alternative to correct anemia or prevent complications.

In transfusion therapy, the WHO recommends that patients with hemoglobinopathies receive from the start red blood cells compatible with the Kell, RhD and RhE phenotypes, which easily stimulate the production of hemolytic antibodies in recipients [4]. Similarly, in Chad, the decree regulating the donation of whole blood and the processing of blood products recommends that for polytransfused patients, a search for Irregular Antibodies (RAI) be carried out and that these patients, as well as adolescent patients, are transfused, as far as possible, in Rh-Kell isogroup and phenotype [5]. Unfortunately, the common practice in the country is to transfuse ABO and RhD compatible whole blood without any search for irregular antibodies (RAI). Such transfusion practices in the context of a progressive disease expose polytransfused sickle cell patients to alloimmunization risks that can cause adverse effects and compromise the transfusion future of these patients. Add the aim of study here, again. The objective of this work was to contribute to the prevention of anti-erythrocyte alloimmunization in sickle cell patients in Chad.

MATERIALS AND METHODS

Ethical approval

A request for research authorization was sent to the Dean of the Faculty of Human Health Sciences (FSSH), to the Director of the CHU-ME as well as to the head of the sickle cell disease treatment unit and to the director of the CNTS. Verbal consent was obtained from patients or parents before inclusion in the study. The data was anonymous and confidentiality was strictly respected in the data analysis.

Study framework

This was a multicenter study including: the Sickle Cell Disease Management Unit (UPECD) of the University Hospital Center for Mothers and Children (CHU-ME), the Hematology Unit of the National Reference University Hospital Center (CHU-RN), at the National Center for Blood Transfusion (CNTS) in N'Djamena and at the Center for Study and Research in Applied Biology (CERBA) in Paris, France.

Study population

The study, which was descriptive and analytical cross-sectional, focused on a population of sickle cell patients regularly followed at UPECD (CHU-ME), at the Hematology Unit of the CHU-RN and at the CNTS, received during the period from December 2021 to June 2022. The sample consisted of 57 sickle cell patients who consented to participate in this study and whose quantities of blood collected met laboratory standards.

Laboratory tests

The samples were taken from venous blood for each subject, in two dry tubes of 4 mL each. After registering the subject on the tube collection and labeling sheet, the samples were sent to the CNTS laboratory. The samples were then centrifuged, the sera decanted and transferred into two labeled cryovials. These cryovials were then frozen at -80°C and subsequently sent to the Paris Center for Applied Biology Study and Research in France for the research of Irregular Antibodies (RAI). In this laboratory, the RAI were carried out as follows: Screening was based on the use of a range of 3 group O red blood cells (Diacell 123-Biorard) comprising Ag and obligatory phenotypes by haemagglutination. If this step turned out to be negative, the RAI was made negative. On the other hand, if the screening was positive, identification was carried out with a range of 12 or 23 red blood cells-tests depending on the needs (CNRGS and Biorard panels).

Data processing

A pre-established collection sheet was submitted to the patients, aiming to collect socio-epidemiological data, data on transfusion history (number of transfusion episodes and units per episode), as well as on sickle cell phenotypes. Sphinx software version 5.1.0.4 and Microsoft Word and Excel were used for data processing.

RESULTS

Population characteristics

Of the 57 sickle cell patients included, 29 (50.88%) were male. The average age was 8.89 ± 5.40 years (extreme 1 to 22 years) and children under 10 accounted for 64.91%. The sickle cell phenotypes were distributed between 48 SS homozygotes (84.21%), 7 S β -thalassemia (12.28%) and 2 SC composite heterozygotes (3.51%). Patients who had a history of transfusion represented 78.95% (45/57) of the population. Of the 45 (78.95%) patients who had a history of transfusion, 25 (55.56%) had a single episode of transfusion and 20 (44.44%) had received 2 to 4 units of blood. The mean age at the start of transfusion was 5.02 ± 4.49 years.

Anti-erythrocyte allo-immunization

It was observed at a prevalence of 8.89% (4/45) exclusively in patients with a history of blood transfusion. The alloantibodies identified (Table I) were anti-RH (85.72%) and anti-Kell (14.28%). In the rhesus system, anti-E was the most represented in 66.66% of cases.

Risk factors for red blood cell allo-immunization

Allo-immunization was more frequent in male patients ($p = 0.041$) (table 2) aged 16 years and older ($p = 0.046$) (Table 3) who received more than one transfusion episode ($p = 0.043$) (Figure 1) and at least 3 units of blood ($p = 0.035$) (Figure 2).

Table 1. Specificity of identified alloantibodies and their frequencies

Antibodies identified	Number	Percentage (%)
HR system	Anti-D	14,28
	Anti-C	14,28
	Anti-E	57,16
Kell system	Anti-K	14,28
Total	7	100

Table 2. Distribution of alloimmunization according to gender

Sex	Result RAI	Positive		Negative		Total	
		n	%	n	%	n	%
Male		4	100	25	47,17	29	50,88
Female		0	0	28	52,83	28	49,12
Total		4	100	53	100	57	100

n=number, %= percentage; RAI = search for irregular antibodies

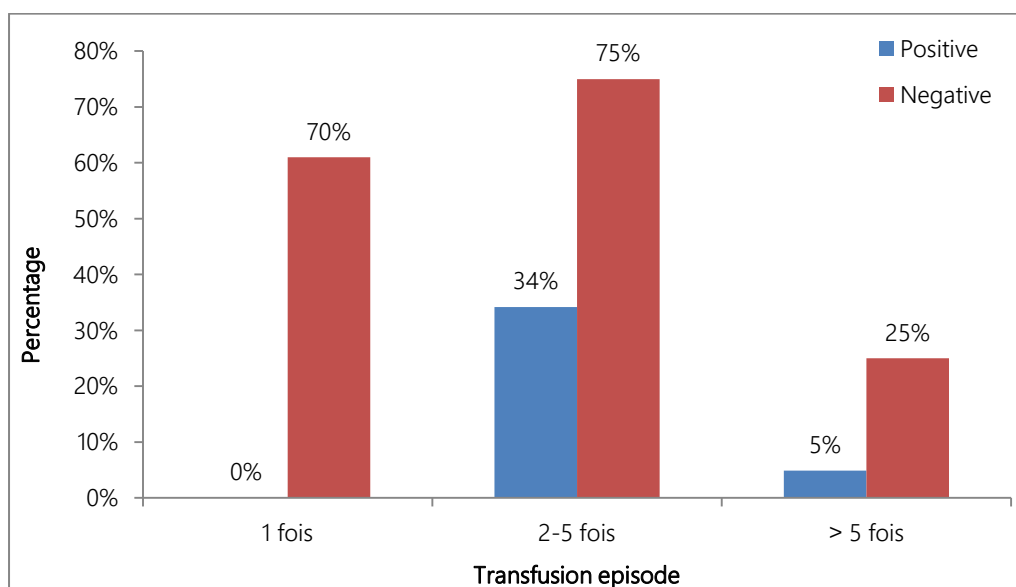


Figure 1. Distribution of allo-immunization according to the number of transfusion episodes.

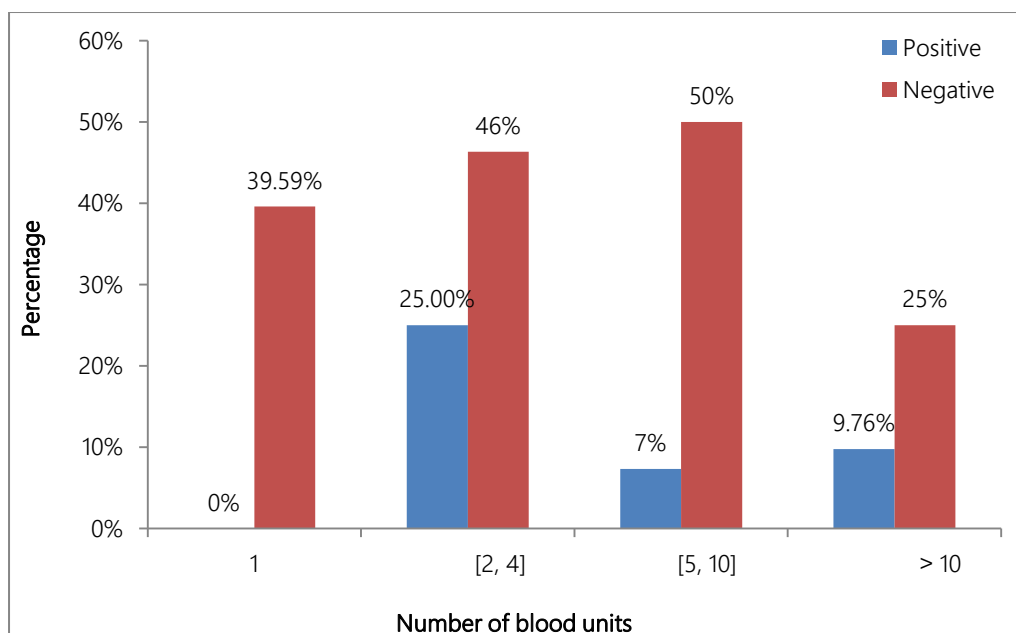


Figure 2. Distribution of allo-immunization according to the number of units of blood received.

DISCUSSION

Epidemiology

Regarding age, the average in our study population was 8.89 ± 5.40 years. This average age was lower than that of [Rodrigues et al. \[6\]](#) in Brazil in 2020 and that of [Elalfy et al. \[7\]](#) in Egypt in 2021 who respectively report 11.2 ± 2.84 years and 13.5 ± 2.81 years. These differences in results could be explained by the different geographical situations or by a selection bias. Considering the sex ratio (Male/Female), it was 1.03. This result was close to the results of [Rodrigues et al. \[6\]](#) in 2020 in Brazil and [Elalfy et al. \[7\]](#) in 2021 in Egypt, which reports a sex ratio of 1.18 and 1.63 respectively. Our results are contrary to those of [AIDawood \[8\]](#) in 2022 in Saudi Arabia and [Tuono et al. \[9\]](#) in 2022 in Douala, Cameroon, which report 0.97 and 0.70 respectively. These differences have no statistical significance because the transmission of sickle cell disease is purely autosomal recessive.

Regarding the sickle cell phenotype, SS homozygotes accounted for 84.44% of cases. Our results were superimposable to those of [Adewoyin \[10\]](#) in 2021 in Lagos, Nigeria and [AIDawood \[8\]](#) in 2022 in Saudi Arabia which report 87.5% and 84.4% respectively. This predominance of SS homozygosity could be explained by the geographical location of the country in the LEHMAN sicklemic belt.

Transfusion

Regarding the frequency of blood transfusion, it was 78.95%. This result was close to that of [AIDawood \[8\]](#) in 2022 in Saudi Arabia (72.5%) and [Conrath](#) in 2021 in Guyana (66%). These results could be explained by the constant use of blood transfusion in the treatment of sickle cell disease.

Compared to the age of transfused patients, the mean was 9.33 ± 5.44 years. The patients were younger than those in [Elalfy et al \[7\]](#) in Egypt in 2021 (13.5 ± 2.81 years) and [Rodrigues et al. \[6\]](#) in 2020 in Brazil (11.5 ± 2.74 years) but older than those of [Tebuka \[12\]](#) in Tanzania in 2020 (4.5 years). This fact could be explained by the method of patient recruitment. Indeed, Elalfy and Rodrigues mainly recruit young adults and adolescents while Tebuka mainly recruits children under 5 years old and this study recruited much more subjects aged 5-12 years. Considering the age at the start of transfusion, the average was 5.02 ± 4.49 years. The patients had received their first transfusion earlier than those in [Aldakheel \[13\]](#) in 2022 in Saudi Arabia (8 years) but later than those in [Allali et al. \[14\]](#) in 2017 in France (3 years). These differences could be explained by differences in transfusion procedures in these countries.

Regarding the number of blood units received by patients, there were 45 patients transfused with a total of 172 units (3.82 units per patient). Those who had received a single unit of blood accounted for 33.33% of cases against 44.44% who had received 2 to 4 units and 22.22% who had received more than 4 units. This result was close to that of [Boateng et al. \[15\]](#) who reports 32%, 55% and 12% respectively. [Tebuka et al. \[12\]](#) reports that 88.5% of patients were transfused with 2 to 4 units of blood with an average of 3 units per patient. The highly pediatric age of the patients, the unavailability of blood units in blood banks and the difficulty in finding family donors in time in our context are among other factors that may explain the differences observed in the present study.

Allo-immunization

Regarding the prevalence of allo-immunization, it was 8.88% in the transfused population. This prevalence was lower than the prevalence of [Rodrigues et al \[6\]](#) in Brazilian children with sickle cell disease in 2020 (11.1%), [Allali et al \[14\]](#) in French children with sickle cell disease in 2017 (13.7%) and [Elalfy et al \[7\]](#) in children with sickle cell disease in Egypt in 2021 (16%). This difference could be explained by the very young age of our subjects. Most of our subjects had had their last episode of transfusion more than 4 months ago. To this end, some alloantibodies could already disappear at the time of the study, explaining this low frequency. The lack of racial difference between blood donors and recipients in our context could also explain the low frequency of allo-immunization in our series. Compared to the specificity of the antibodies developed, we had identified a total of seven irregular antibodies, and these Abs were directed against the Ag of the RH and KELL systems respectively in the order of 85.72% and 14.29%. . This result was comparable to those of [Regalado-Artamendi et al \[16\]](#) in 2021 in Spain, who reports in his series 54.6% of Abs directed against the RH and Kell systems as well as those of [Akpan et al. \[17\]](#) in Nigeria in 2022 which reports 66.67% of Ab directed against the Ag of the Rhesus system and 33.3% of Ab directed against the Ag of the Kell and Lutheran (LU) systems combined. This could be explained by the strong immunogenicity of these blood group systems.

Allo-immunization risk factors

Regarding the age-related risk of immunization, the present study showed that immunization was frequent from the age of 16 ($p = 0.046$). The results of [Fekri et al \(2018\)](#) showed more frequent immunization from the age of 14 [18]. These results proved with those of other authors that the risk of immunization increases with age [8, 10].

In relation to gender and allo-immunization, only men were allo-immunized ($p = 0.041$). This result was contrary to that of [Karafin et al. \[19\]](#) and [Jalali et al. \[20\]](#) who found that women were more immunized than men; result justified by the exposure of women to many immunizing events such as pregnancy and transfusions. Other authors, on the other hand, found no statistically significant relationship between gender and allo-immunization [7, 8, and 10]. This result could be explained by the highly pediatric age of our population, thus eliminating the risk of immunization of fetomaternal origin or by simple chance.

Speaking of the risk of allo-immunization linked to the sickle cell phenotype, only homozygous patients were immunized without statistical significance ($p = 0.667$). This result was similar to that of [Rodrigues \[6\]](#). [Elalfy \[7\]](#) reports that immunization was more frequent in $S\beta$ +Thalassemia subjects. [Muraio et al. \[21\]](#) reports that SC composite heterozygous patients were statistically the most immunized ($p = 0.048$). [Fekri et al. \[18\]](#) showed for his part that $S\beta$ -Thalassemia patients were the most immunized but without statistical significance ($p = 0.73$). Other researchers [8, 10], on the other hand, find no statistically significant relationship between allo-immunization and the sickle cell phenotype. These controversial results show that whatever the sickle cell phenotype, the transfusion context remains an important factor in the genesis of alloantibodies.

Regarding the risk related to the history of transfusion, although all allo-immunized patients were transfused, there was no statistically significant relationship between transfusion and allo-immunization ($p = 0.284$). This result was corroborated by [Adewoyin et al. \[10\]](#) ($p = 0.135$). The small size of our sample could explain this.

Regarding the risk linked to the number of episodes of transfusion received by the patients, the present study had shown that the production of irregular antibodies was statistically linked to the number of episodes of transfusion of the patients ($p = 0.03$). The mean number of transfusions was higher in allo-immunized subjects (4.75 ± 2.22 times) than in non-allo-immunized (2.13 ± 1.75 times). This result was corroborated by studies carried out by other authors [9, 11, 22] thus proving that the number of stimulations constitutes an important risk factor for anti-erythrocyte allo-immunization.

Regarding the number of blood units received and alloimmunization, statistical significance was found in this study ($p = 0.035$). Allo-immunized patients received more units of blood (8.50 ± 5.92 bags) than non-allo-immunized patients (mean: 3.40 ± 3.79 bags). This association was found in most studies [7, 9, 10, 12, 15, and 22]. Few units of blood were sufficient to immunize our patients, certainly because of the transfusion with whole and non-phenotyped blood that they had received, unlike the others who had received phenotyped RCCs. This result confirmed once again that the number of exposures to antigens as well as the transfusion of non-phenotyped blood constitutes an important risk factor for anti-erythrocyte alloimmunization.

Regarding the risk of alloimmunization related to the age of the first transfusion, no correlation was found ($p = 0.577$). Alloimmunized patients received their first transfusion earlier (4.50 ± 3.87 years) compared to non-immunized patients (5.17 ± 4.60 years). [Aldawood \[8\]](#) finds that alloimmunized patients were statistically ($p = 0.037$) older (22.7 years: 14.4-31.1) than non-immunized (20.9 years: 7.8-27.5). This fact was justified by the fact that most of the subjects were transfused elsewhere at an early age. Other studies [10, 13, and 17] found no statistically significant relationship between the age at the start of transfusion and alloimmunization corroborating our results. The small size of our sample could also explain this result.

Speaking of the date of last transfusion as a risk factor for alloimmunization, no statistically significant relationship was found ($p = 0.512$) although the last transfusion received by our immunized patients dated back 4 months or more. [Adewoyin et al. \[10\]](#) finds a result similar to ours with a statistically significant relationship between the age of last transfusion and allo-immunization ($p = 0.043$) if the last transfusion episode was more than 4 weeks ago but less than 12 weeks ago. . No relationship was found if the last episode dated back more than 12 months ($p = 0.215$).

The kinetics of alloantibodies could explain this phenomenon. Indeed, some antibodies disappear very quickly after about 3 months after the last episode of transfusion [1]. Most of our alloimmunized subjects (3/4) had received their last transfusion more than 4 months ago; period sufficient for certain Ab to no longer be detectable.

CONCLUSIONS AND RECOMMENDATIONS

Anti-erythrocyte alloimmunization is a reality in the Chadian sickle cell population. Although the prevalence found in this study is within the range of literature data, it is high when taking into account the sample size, blood transfusion procedures that do not meet WHO standards, and local recommendations governing the act of transfusion. The results prove once again that the strong immunogenicity of the Rhesus and Kell systems, as well as the more frequent exposure to erythrocyte antigens through multiple blood transfusions, are strongly involved in the onset of alloimmunization. It is therefore essential to put into practice the national and international

recommendations relating to good transfusion practices in order to guarantee the safety of blood transfusions for this category of the population which is constantly increasing.

DECLARATIONS

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

All authors have read and approved the final version of the manuscript.

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