

# Extensive Phenotyping of Erythrocyte Antigens in Volunteer Blood Donors in Lubumbashi, Democratic Republic of Congo

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## Abstract

The present study concerns extensive phenotyping of erythrocyte antigens in volunteer blood donors in Lubumbashi, Democratic Republic of Congo. Since blood transfusion is a therapeutic procedure, it presents risks related to immunological responses. These are mainly due to genetic polymorphisms of erythrocyte antigens, leading to anti-erythrocyte alloimmunization, which can lead to transfusion impasse situations. The aim of the study is to determine the frequency of alleles of the blood group system of transfusion interest. This was a cross-sectional observational study. The present study was carried out at the provincial blood transfusion center of Lubumbashi on 211 blood donors during the period from January 2022 to December 2022. Data were collected from the donor's clinical record. Males were the most represented at 94.8%. For the Rhesus system, antigens D, C, E, and e accounted for 98.1%, 10.4%, 14.7% and 39.4% respectively. For the Kell system, 16.6% of donors had the Kell1 antigen. For the MNS, M antigen was predominant at 49.8%. Older age was associated with an increase in blood donations ( $p < 0.001$ ). The use of phenocompatible blood is essential in preventing anti-erythrocyte alloimmunity in polytransfused patients.

## Keywords

Erythrocyte Phenotyping, Blood Donors, Anti-Erythrocyte Alloimmunization

## 1. Introduction

Red Blood Cell (RBC) transfusion is a common practice in the treatment of patients with anemia, Sickle Cell Disease (SCD) and other hematological and malig-

nant disorders. Whether as a simple transfusion or as part of a chronic transfusion program, on one hand it helps reduce the morbidity and mortality associated with the disease, but on the other hand, due to genetic polymorphism, it can provide antigens that the patient does not possess, and which can be the source of immediate or delayed immunological risks. The most frequent and serious risk is erythrocyte alloimmunization [1]-[3].

Globally, the rate of alloimmunization, or the production of anti-erythrocyte antibodies against blood group antigens carried by transfused RBCs that the recipient does not possess and that can destroy recipients' RBCs, is significantly higher (8% - 76%) in patients receiving multiple transfusions, such as sickle cell and thalassemic patients, and increases with repeated transfusions [4]. In Sub-Saharan Africa, this prevalence is estimated at 7.4% in polytransfused patients [5]. However, in Central Africa, where donors and patients are racially more homogeneous, Fasano *et al.* (2019) and Boateng *et al.* (2019) reported an incidence of alloimmunization of 7.4% [5] [6].

In the blood donor population, the prevalence of anti-erythrocyte alloimmunization varies between men and women (multiple pregnancies) and those with a history of transfusion or transplantation. In sub-Saharan Africa, a multicenter study reported an alloimmunization prevalence of 0.9% for 902 blood donors in Cameroon, Côte d'Ivoire and Benin [7]. It depends on the number of transfusions, the immune status of the recipient, and antigenic differences between donor and recipient [8].

The International Society of Blood Transfusion (ISBT) has identified over 300 erythrocyte antigens, listed in 36 blood group systems. These antigens can trigger immunization with the appearance of irregular agglutinins (antibodies) [9]. The classic order of relative immunogenicity of erythrocyte antigens, according to Giblett (1961), is as follows: RH1 (D) > KEL1 (Kell) > RH4 (c) > RH3 (E) > KEL2 (k) > RH5 (e) > FY1 (Fya) > RH2 (C) > JK1 (Jka) > MNS3 (S) > JK2 (Jkb) > MNS4 (s) [10].

Indeed, the antigens currently phenotyped routinely in healthcare facilities are only those of the major ABO and RH groups, and some of the Kell antigen. However, many other antigens are of proven clinical importance, so that routine serology recommended in Europe concerns at least ABO, RH, Kell, Duffy, Kidd and MNS. Unfortunately, in sub-Saharan Africa, few countries systematically test donors and recipients for C, c, e, E and K antigens, exposing the transfused patient to a high risk of alloimmunization [5]. The extension of typing to a larger number of erythrocyte antigens would allow better prevention of immediate or delayed transfusion accidents and alloimmunization, by optimizing compatibility between donor and recipient.

This study is the first to examine the frequency of different blood group antigens among blood donors in Lubumbashi. In this context, the primary objective of this study was to determine the frequency of certain blood group antigens of transfusion interest (RH, KEL, MNS).

## 2. Methods

### 2.1. Study Design and Setting

A cross-sectional observational study was conducted at the Provincial Blood Transfusion Center of Lubumbashi (PBTC-L) during the period from 05 January 2022 to 24 December 2022. All volunteer blood donors registered at the PBTC-L who had made at least two blood donations were included in the study. Exclusion criteria concerned donors with incomplete records at the center, and those who had made only one blood donation.

For antigenic distribution, we grouped age into deux groups: young adults' donors (18 - 29 years) and adults (>18 years). Ethnicity was grouped into three groups: Grand Katanga, Grand Kasai and Other cities.

### 2.2. Data Collection

Data were collected from the records of recognized blood donors at the PBTC-L. This center regularly organizes voluntary blood donation clinics and holds a register of regular blood donors. Donors were recruited between 05 January 2022 and 30 April 2022. Those who agreed to participate provided oral consent. Sample collection was carried out gradually until 24 December 2022. The data were anonymized to guarantee donor confidentiality, and each donor was assigned a code. The Data collected included age, gender, occupation, ethnicity or province of origin, number of donations, medical history, extended phenotyping of Rhesus, Kell and MNS erythrocyte antigens.

### 2.3. Outcomes Definition

Erythrocyte phenotyping consists of searching for antigens on the surface of red blood cells to define the phenotype of the blood donor. This phenotyping is extended when it corresponds to the search for erythrocyte antigens other than those determined during RH-Kell1 phenotyping (FY, JK, MNS). In the present study, phenotyping was performed for the RH, KELL and MNS blood systems. For the Rh system, we looked for 5 antigens of transfusion interest: D (RH1), C (RH2), E (RH3) and e (RH5). For the KELL system, we have the Kell1 antigen, and for the MNS system, we have the M, N and S antigens [11]. Volunteer blood donors are loyal donors at the Lubumbashi Provincial Blood Transfusion Center.

### 2.4. Immunological Analysis

The phenotypes of Rhesus, Kell and MNS antigens from volunteer blood donors were determined using the agglutination principle on a fully automated immunehematological analyzer. We used Rh subtype antisera for Cypress Diagnostics, Hulshout, Belgium. Normal human erythrocytes possessing the corresponding antigen agglutinate in the presence of the specific antibody directed against the antigen. The lot numbers of reagents were:

- Anti D incomplete: 310120
- Anti Kell: 191019/2

- Anti S: 140319 C
- Anti e: 201119
- Anti M: 25042
- Anti C: 01111912
- Anti N: 230420
- Anti E: 151019/4

For the internal quality-control, we used HEMA CQI (DIAGAST Ref.: 59500), a kit consisting of four 4 ml tubes. It was systematically used during a series of analyses. The absence of anti-c and anti-s reagents is due to the unavailability of these two reagents.

## 2.5. Statistical Analysis

Descriptive analyses were performed on the entire cohort. Results were expressed as means and standard deviations, medians, interquartile and percentage. Chi-square or t-test were used for group comparisons. We evaluated the number of blood donations using multivariate analysis by Poisson regression adjusting to gender, age groups and educational level. Statistical analysis was performed using R software version 4.2.1 was used for analysis. Significance threshold defined by a p-value of less than 0.05 was applied for all analyses.

## 2.6. Ethical Considerations

The study was approved by the Research and Ethical Comity of the University of Lubumbashi.

## 3. Results

### 3.1. Blood Donor Characteristics

A total of 211 volunteer blood donors were recruited at the Lubumbashi Provincial Blood Transfusion Center. The majority were men (94.8%), compared with women (5.2%). The median age was  $35 \pm 11.1$  years, with a predominance of donors aged under 45 (75%). Over 60% of donors were aged 41 or under, and the average number of donations per donor was 9.5 (min: 2; max: 53). Over 70% of donors (166/211) made between 2 and 13 donations, while almost 15% of donors made more than 20 donations (**Table 1**).

**Table 1.** Blood donor characteristics.

Parameters	No. of donors	%
<b>Age group(years)</b>		
18 - 23	26	12.3
24 - 29	46	21.8
30 - 35	40	19.0
36 - 41	20	9.5
≥42	79	37.4

## Continued

Gender		
Male	200	94.8
Female	11	5.2
Number of donations		
2 - 7	103	48.8
8 - 13	63	29.9
14 - 19	15	7.1
≥20	30	14.2
Educational level		
Primary	3	1.4
Secondary	69	32.7
University	139	65.9

The multi-ethnic character of the donors is demonstrated by the fact that half of them (57.2%) came from Grand Katanga, 29.8% from Grand Kasai, a third proportion (4.8%) from Maniema. A small percentage of blood donors (<3%) came from each of the remaining provinces (Figure 1).

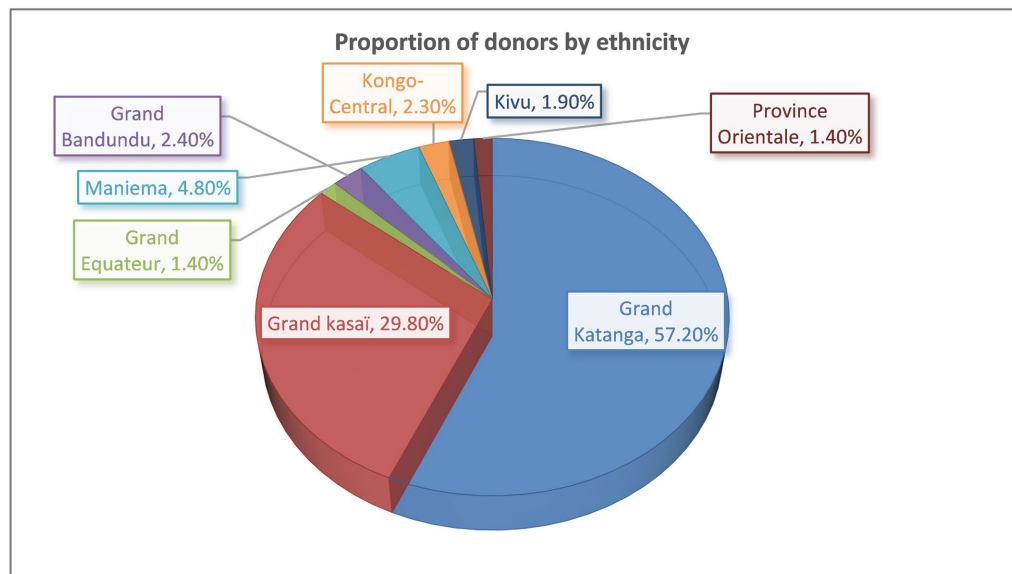


Figure 1. Distribution of donors by ethnicity (province of origin).

### 3.2. Number of Blood Donations

A Poisson regression was performed for the number of blood donations. Adults (more than 30 years) was positively associated with a higher number of blood donations compared to young people under 30 ( $p < 0.001$ ). Compared with women, men were associated with a higher number of donations ( $p < 0.001$ ). University level was positively associated with a higher number of blood donations than non-university donors ( $p = 0.058$ ) (Appendix A, Figure A1).

### 3.3. Phenotyping of Erythrocyte Antigens

Rhesus antigens taken separately showed that the most immunogenic Rhesus antigen, RhD+, was in the majority in 98.1% of donors, versus 1.9% who were RH-1 (D-). As the Kell system is important in blood transfusion and obstetrics due to the immunogenicity of the Kell1 antigen, it was present in 16.6% of blood donors and 83.4% lacked it. Although less immunogenic in the MNS system, M, N and S antigens were present in 49.2%, 36.5% and 2.8% respectively (**Appendix B, Table B1**). In the MNS system, the most frequent phenotypes were M-N+S+ and M+N+S+ with 30.8% respectively.

For the extended Rhesus system, we analyzed the 3 main antigens C, E and e with 10.4%, 14.7% and 39.4% respectively. Among RhD+ donors, the dominant phenotypes were D+C-E-e- and D+C-E-e+ with 49.7% and 25.6% respectively. Among Rh-donors, only the D-C+E+e-phenotype was present at 1.9% (**Appendix C, Table C1**).

By analyzing the frequency of antigens in relation to demographic characteristics, such as age, sex, and ethnicity, the distribution of different antigens is greater in men than in women. This trend is also observed in adults compared to young adults. A greater antigenic distribution is also observed in Greater Katanga and Greater Kasai. However, there is no significant difference between antigenic distribution and age, sex, or ethnicity (**Appendix C, Table C2**).

## 4. Discussion

In the present study, 211 blood donors were followed up with a median age of 35 years. More than half of the blood donors in our population (52.8%) were young adults under 35 years of age. Several studies have reported a median age similar to ours around 36 years (25 - 45 years) among blood donors including Sekongo *et al.* (2019) who found in West Africa an average age of 37.5 years [11]-[13]. In the DRC, Kabemba *et al.* (2017) found a similar mean age of  $37.6 \pm 1.8$  years among blood donors in Tanganyika province [14]. However, with 37.5% of donors aged over 40, efforts still need to be made to sensitize more young people to donate blood.

In terms of gender, 94.8% were men vs. 5.2% women. This large predominance was reported in low- and middle-income countries in Sub-Saharan Africa, including Cameroon (78.3%), Mauritania (92.2%) and Tanzania (83.7%) [15]-[17]. This finding has been corroborated by the WHO, which shows that less than 10% of donations are made by women [18]. Apart from certain physiological conditions that may account for this difference, such as menstrual periods, pregnancy and breast-feeding, the cultural context may also be a factor of exclusion. While it is interesting to note that awareness of blood donation within the population does not explain this gender difference, the literature highlights that men donate blood more frequently than women, regardless of the number of invitations to donate issued by different means of communication [19].

In this study, the individual antigens show that the most immunogenic D anti-

gen is present in 98.1% of blood donors, and 1.9% lack it. According to ethnic origin, Dean (2005) reported 92% of blacks Rh+, 99% of Asians Rh+ and 85% of Caucasians Rh+ [20]. In sub-Saharan Africa, Siransy *et al.* (2014) in Cote d'Ivoire, Sidy Diallo (2019) in Mali, Etura *et al.* (2020) in Nigeria and Angounda *et al.* (2024) in Republic of Congo found similar results among blood donors: 92.93%, 92.2%, 97.7% and 94.86% respectively [21]-[24]. In the DRC, these results intersect with those of Kabemba *et al.* (2017), who found 98.4% Rh+ and 1.6% Rh- in blood donors [14].

In the extended Rh system, we did not look for the c antigen but it's generally more frequent than C in African populations (96%) [20]. However, the e antigen was the most frequent at 39.4%, and the C and E antigens were present at 10.4% and 14.7% respectively. In contrast to white blood donors (USA and Europe) who have the highest frequencies of C, E, the majority of blacks are devoid of C and E antigens (73% and 78% respectively). Thus, several studies report results similar to ours and thus a lower prevalence of alloimmunization when blood donor and patients with major sickle cell syndrome share greater antigenic similarity [20] [25].

In Rh+ donors, the D+C-E-e- and D+C-E-e+ phenotypes were predominant in 48.3% and 25.6% of donors respectively. Compared with other continents, the D+C+E-c+e+ phenotype is more frequent in Europe and the D+C-E-c+e+ phenotype is more frequent in sub-Saharan Africa, whereas it is less than 2% in individuals of European origin [20]. The latter phenotype has been confirmed in (65.12%), Mali (69.2%) and Nigeria (46.2%) [21]-[23]. As a result, the majority of donors lack antigens C, E. With an order of  $E > c > e > C$ , their presence in donors contraindicates any transfusion in Rh+ recipients lacking these antigens. Anti-C, anti-E or anti-c antibodies may form, leading to a delayed hemolytic transfusion reaction or hemolytic disease of the fetus and newborn during pregnancy involving anti-D and anti-c [20]. Given that one of the causes of post-transfusion anti-erythrocyte alloimmunization is incompatible antigenic differences between donor and recipient, these results reinforce the hypothesis that ethnic or racial homogeneity between donors and recipients is one of the safety measures to prevent this alloimmunization, especially in sickle cell patients polytransfused [26].

In the Kell system, 83.4% of donors lacked the Kell antigen and 16.6% had it. With a similar sample, Soumaila (2005) found a total absence of Kell antigen and Traoré (Mali, 2002) found a lower frequency of 2.4% or 5/208 of Kell+ donors [7] [27]. More than 10 years after these studies, this is in concordance with Angounda *et al.* (2024) shows a lower frequency of K positive antigen (0.86%) and 99.14% of K negative antigen among 350 blood donors in Republic of Congo [24]. Yusuf Olawale Nurudeen *et al.* (2024) showed that Kell (K) antigen was found positive in 6% blood donors and negative in 94% out of 287 donated blood units in Nigeria [28]. In comparison with these results and considering the 2% overall prevalence of Kell antigen in blacks [29], our results found call for further investigation or genotyping in our at-risk donors to prevent alloimmunization in polytransfused

patients and women of childbearing age.

In the MNS system, M and N antigens were frequent among blood donors at 49.8% and 36.5% respectively. These results are consistent with the high frequency of M and N antigens found in Caucasians (78%) and Blacks (74%). The S antigen is less common than the s antigen in African populations. A study reported a frequency of approximately 30% in Black individuals, while the s antigen is present in about 88.7% of individuals [20]. In our study, we found a lower frequency (2.8%) of the S antigen. The M+N+S+ phenotype was most frequent in 30.8% of blood donors. We did not test for s antigen, but the literature shows a 98% frequency of s antigen in blacks [20]. The M+N+S+s+ phenotype is more frequent in Caucasians (24%) and 13% in blacks [20]. Patients lacking these antigens can develop anti-M, anti-N and anti-S antibodies. Although less immunogenic, these antibodies can interfere with pre-transfusion testing, but are considered clinically insignificant for most patients, as they generally do not cause any acute or delayed hemolytic reaction, except in patients with sickle cell anemia, in whom they can cause hemolysis, or even precipitate hyperhemolysis [20] [30]. Similarly, anti-S antibodies can cause severe hemolysis in hemolytic disease of the newborn [20]. For these patients with SCD, units of RGCs (red blood cells) devoid of M and S antigens should be transfused.

Without more complete information on donors' medical history, it is difficult to assess the true burden of RBC alloimmunization and its effect on the demand for phenotyped RBCs. Further studies are needed to document the profile of at-risk blood donors in a larger, more diverse sample. However, these data on the prevalence of RBC antigens in blood donors are essential for understanding what can happen in the community and how blood supply planning is adapted accordingly.

Despite better understanding of the pathophysiology of anti-erythrocyte alloimmunization and better execution of serological matching of D, C, E or C/c, E/e antigens, and Kell antigen, high rates of alloimmunization persist even in blood donors due to Rh genetic diversity in individuals of African ancestry. Rh variant antigens are difficult to distinguish serologically and require RH genotyping to be identified. In Africa, this diversity of erythrocyte antigens is higher and its knowledge is essential to ensure blood compatibility during transfusions, thus minimizing transfusion reactions and alloimmunizations. Genetic factors play an important role in the distribution of erythrocyte antigens in Africa [31]. Our results having shown a high frequency of antigens in the Rh and Kel systems, RH-KEL1 phenotyping therefore appears both as a transfusion safety measure and, in women, as a measure to prevent hemolytic disease of the newborn.

Also, the knowledge of the diverse distribution of red cell antigens in the population reinforces the need for expanded WBC phenotyping in healthcare facilities to create a more diverse and compatible blood supply. This is particularly important for patients with rare blood types. This expanded phenotyping for certain at-risk groups requires the use of advanced technologies such as red cell genotyp-

ing to identify the rare blood types.

### Limitations of Our Study

This study did not evaluate other blood group systems that are also incriminated in delayed transfusion reactions. The small size of this sample means that the results cannot be extrapolated to the entire donor population in our setting. Restricting this sample to donors who have made at least two donations may result in underrepresentation of certain donor groups, particularly new donors or those who donate less frequently. This could affect estimates of the frequency of antigens encountered in the blood donor population.

### 5. Conclusion

This study has enabled us to study the extensive phenotype of volunteer blood donors, highlighting a distribution of certain antigens requiring particular attention during transfusions. The best way to prevent transfusion-related accidents is, of course, to prevent anti-erythrocyte alloimmunization by prescribing phenocompatible blood in the Rhesus, Kell, Duffy, Kidd and MNSs blood group systems during transfusions.

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### Conflicts of Interest

The authors declare no conflicts of interest in the publication of these results.

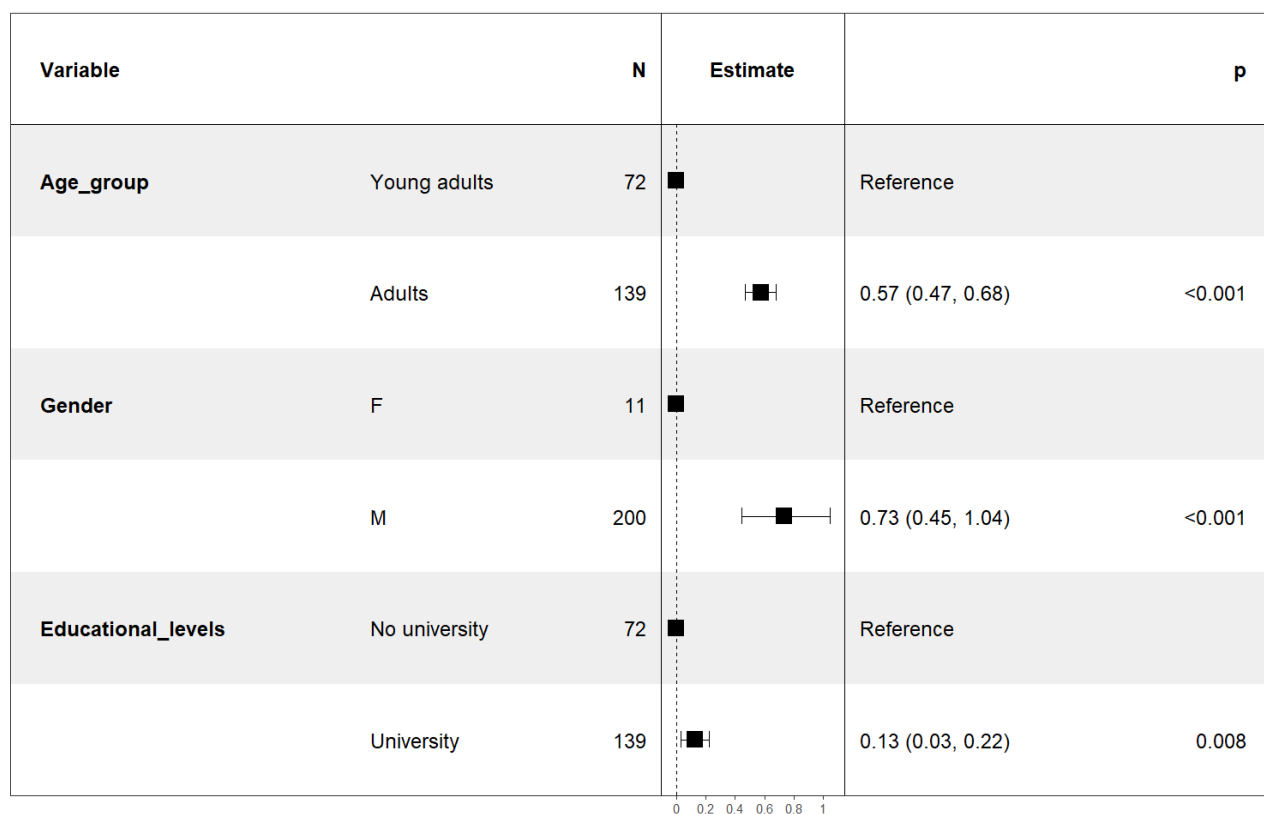
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## Appendix A



**Figure A1.** Factors influencing the number of blood donations (Poisson regression).

## Appendix B

**Table B1.** Frequency of antigens in blood donors in different systems.

Extended Rh antigens (Phenotype)	Frequency (%)					
	Our study (n = 211)	DRC (2017) [14]	Cote d'Ivoire (n = 651) [21]	Nigeria (n = 130) [23]	Mali (n = 332) [22]	Caucasians [28]
<b>Extended Rh D</b>						
D+ (RH1+)	98.1	98.4	92.93	97.7	92.2	85
D- (RH-1)	1.9	1.6	7.70	2.3	7.8	
C+ (RH2+)	10.4	-	21.97	30.7	15.1	68
E+ (RH3+)	14.7	-	13.82	39.2	10.2	29
e+ (RH5+)	39.4	-	99.85	95.4	99.7	98
<b>Kell (Kell1)</b>						
K+	16.6	-	0.92	-	0	0.2
<b>MNS</b>						
M+	49.8	-	-	-	62.5	78
N+	36.5	-	-	-	77.4	72
S+	2.8	-	-	-	13	55

## Appendix C

**Table C1.** Distribution of phenotypes in the extended Rh system in blood donors.

Rhesus Phenotypes	Frequency (%)					
	Our study (n = 211)	Mali (n = 332) [22]	Cote d'Ivoire (n = 651) [21]	Africa (blacks) [28]	Caucasians [28]	Asian [28]
D+C-E-e+	25.6	69.2	65.12	44	-	-
D+C+E-e-	5.2	-	-	-	-	-
D+C-E-e-	49.7	-	2.1	-	-	-
D+C-E+e-	6.2	0.3	1.3	-	-	-
D+C+E+e-	0.9	-	0.17	-	-	-
D+C+E-e+	4.7	11.1	13.2	-	42	70
D+C-E+e+	7.6	16.7	18.3	-	-	-
D-C-E-e+	1.9	8.3	5.7	8	15	1

**Table C2.** Distribution of antigens according to sociodemographic parameters.

Antigen	Age group		Gender		Ethnicity			P value
	Young adults (%)	Adults (%)	Male (%)	Female (%)	Grand Katanga (%)	Grand Kasai (%)	Other cities (%)	
D	34.3	65.7	94.7	5.3	57.4	29.4	13.2	ns
C	34.6	65.4	94.7	5.3	59.1	28	12.9	ns
E	36.1	63.9	93.9	6.1	56.7	29.8	13.5	ns
e	37.5	62.5	93	7	55.9	30	14.1	ns
Kel1	31.8	68.2	89.5	10.5	58.6	30	11.4	ns
M	32	68	95.3	4.7	57.1	30.1	12.8	ns
N	33.6	66.4	93.3	6.7	54.1	30.1	15.8	ns
S	34.1	65.9	94.6	5.4	57.9	29.2	12.9	ns