Red blood cell alloimmunization from an African perspective

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Purpose of review
Red blood cell (RBC) alloimmunization occurs when individuals are exposed to erythrocytes that express blood group antigens different from their own. Consequences of alloimmunization include hemolytic disease of the fetus and newborn and hemolytic transfusion reactions, with potentially serious morbidity and mortality. Patients who formed antibodies showed a four to five times increased risk for additional alloantibodies upon further transfusion exposure and may be at increased risk for transfusion reactions. In view of the main transfusion indications in Africans (e.g. malaria and pregnancy), the lifetime risk of exposure to multiple transfusion events is substantial, stressing the need for information on RBC alloimmunization in African transfusion recipients.

Recent findings
Three cross-sectional studies on RBC alloimmunization from two African countries of Uganda and Malawi showed that 1–6% of transfused patients possessed clinically relevant RBC antibodies.

Summary
Regarding RBC compatibility testing for transfusion, ABO/D typing is mandatory in African settings but complete crossmatches are not routinely performed. Despite the limited data on posttransfusion alloimmunization, a complete crossmatch for patients with past transfusions could be recommended. However, more information on blood group distribution in Africans and RBC alloimmunization rates and specificity is needed to consider immunoprophylaxis for hemolytic disease, pretransfusion testing, and preventive strategies to avoid transfusion-induced alloimmunization.

Keywords
Africa, alloimmunization, hemolytic disease of the fetus and newborn, red blood cell transfusion

Introduction

Red blood cell (RBC) alloimmunization can occur when individuals are exposed, through transfusion, pregnancy, or transplantation, to erythrocytes that express blood group antigens different from their own. The antibodies can cause alloimmune hemolysis presenting as hemolytic disease of the newborn (HDN) or acute and delayed hemolytic transfusion reactions, with potentially serious morbidity and mortality. Incidence and prevalence of RBC alloantibody formation are reported to be less than 1% up to more than 40%, respectively. The frequency of posttransfusion RBC alloimmunization is generally high in patients with hemoglobinopathies, ranging up to 37% in thalassemia and to 65% in sickle cell anemia [1,2]. Factors assumed to influence the rate of alloimmunization are antigen immunogenicity, duration of transfusion therapy, and genetic and environmental factors, but the individual contribution of these factors is not known. Individuals with antibodies against, for example, Fy[a] and K more often carry particular human leukocyte

antigens (HLA), suggesting a role for major histocompatibility complex (MHC) restriction in some RBC antigen presentation [3,4,5•,6•]. Quite consistently, and present across various patient populations with different comorbidity, patients who formed antibodies showed a four to five times increased risk for additional alloantibodies upon further transfusion exposure [7,8]. It is unknown whether initial alloimmunization triggers the formation of more alloantibodies or that a first antibody reveals patients who can be referred to as high responders, possibly through a skewed T-helper 2 type responder status [9••,10]. The observation that preventive matching for the highly immunogenic Rh-K antigens for regularly transfused patients, including sickle cell disease (SCD) patients, seems to protect against antibody formation against other incompatible RBC antigens suggests at least a role for the first possibility [2,11].

In contrast to high-income countries, the majority of transfusions in Africa are life-saving emergency treatments for severe anemia resulting from malaria, obstetric

hemorrhages, and accidents, and about half of transfusion recipients are young patients, prior to or during child-bearing age [12]. Every year, approximately 200 000 newborns on the African continent are born with sickle cell anemia [13]. Currently, many of these infants die but even a modestly improved life expectancy will lead to several millions of patients needing transfusion treatment [13]. Moreover, severe malaria infection with *Plasmodium falciparum* affects the poorest part of the sub-Saharan region and is estimated to be responsible for 30% of mortality in children under the age of 3 years, in particular children with preexisting anemia due to comorbidity such as hemoglobinopathy and HIV [14–17]. In half of these cases, severe anemia is the cause of death [14]. If females survive to childbearing age, they often have several pregnancies, with the risk of massive obstetric hemorrhage, a major cause of maternal mortality [18].

The appropriate prescription and timely availability of safe transfusions pose a formidable challenge for African blood transfusion services. The WHO African region with a population of over 773 million people yearly requires about 8 million units of blood for its transfusion needs, but currently only a total of 3 million is available [19]. Several African countries are currently in the process of establishing blood banks for the provision of safe blood. However, Africa has the highest prevalence of transfusion transmittable infections. Although an increasing number of African countries have a considerable safe blood supply established through centralized national transfusion services with voluntary, nonremunerated blood donors (e.g. Secondary School and University students), distribution to hospitals may be a problem and many transfusions are still given by hospital-based (paid) family/replacement donors. Recently, similar viral safety of replacement and first-time volunteer donors was shown, while the costs of a blood unit from replacement donors are considerably less [20••,21]. Especially in regions with a low frequency of RhD negatives, scarcity of RhD-negative donors may delay transfusion, whereas the use of first-degree-related donors might increase the chance of finding a RhD-negative donor [22].

In addition to shortage of donor blood, the lack of educated and trained laboratory and blood bank staff can lead to inappropriate prescription, blood preparation and the underuse and overuse of blood products [23]. In many African countries, hemoglobin cannot be routinely measured and pretransfusion testing is limited to ABO/D grouping and a saline room temperature ABO compatibility check. There are few data available on adverse transfusion reactions because of lack of investigation and documentation of adverse events [12]. A 5-year prospective study in Cameroon comprising 40 000 transfusions showed unfavorable outcome, ranging from febrile reactions to death, in more than 50% of transfusions [24]. A much lower incidence of transfusion reactions (9%) was recorded in a small Nigerian study evaluating 462 transfusions [25]. Recently, de Graaf *et al.* [26•] performed an observational study on bedside transfusion practice in a large teaching hospital in Kampala, Uganda and concluded that 'blood transfusion practice is likely to play a role in morbidity and mortality of patients'. What proportion of these adverse events involved irregular RBC alloantibody-induced hemolytic transfusion reactions is unknown. In high-income countries, preventive measures such as RhD immunoprophylaxis and preventive Rh-K matching for women to avoid HDN and for polytransfused patients with hemoglobinopathies are in place. To consider RBC antibody prevention strategies, information is needed on blood group antigen distribution in the various African ethnic groups, alloimmunization rate and specificities, and incidence and causes of HDN. Most of the information on alloimmunization in individuals of African origin comes from European, American, and Canadian studies and only recently have such observations been reported from some African countries such as Uganda and Malawi [27,28•,29].

Blood group antigens

Blood group antigens are inherited polymorphic structures located on mostly nonpolymorphic proteins, glycoproteins, and glycolipids on the red cell membrane, accessible for antibodies. Because individuals with null phenotypes lacking all antigens are healthy in most cases, other molecules are likely capable of carrying out the same function. The S-s-U-, Le(a-b-), and Fy(a-b-) phenotypes are more common in blacks compared to whites [30].

Blood group antigen frequency differs depending on the ethnic and environmental background and can be separated into common antigens in the ABO, RH, FY, JK, and MNS blood group systems (Table 1) [31], high frequency antigens in the RH, KEL, FY, and MNS blood groups, and low frequency antigens in the RH and KEL blood groups (Table 2) [31]. Infectious diseases are probably the selective force driving blood group expression. The O blood group and FY-negative phenotype provide a selective advantage against severe malaria, explaining their high frequencies in individuals of African origin [32,33••].

Risk of red blood cell alloimmunization in blacks against clinically relevant blood groups

The risk of receiving an incompatible transfusion (or to experience an incompatible pregnancy) depends on the frequency distribution of blood groups in the population. Based on differences in these frequencies between American blacks and whites, blacks have a theoretically

Table 1 Frequency of clinically relevant blood groups and population immunization risk in African–Americans compared to whites

Blood groups[a]	Blood group frequency (%)[b]		Risk of alloantigen exposure (%)[c]		
	African–Americans	Whites	African–Americans	Whites	Ratio[d]
D	92	85	NA	NA	
C	27	68	19.7	21.7	0.91
E	22	29	17.2	20.6	0.83
c	96	80	3.8	16.0	0.24
K	2	9	2.0	8.2	0.24
Fy^a	10	66	9.0	22.4	0.40
Fy^b	23	83	17.7	14.1	1.26
Jk^a	92	77	7.4	17.7	0.42
Jk^b	49	74	25.0	19.2	1.30
S	31	55	21.4	24.8	0.86
s	94	89	5.6	9.8	0.57

NA, not applicable.
[a] Blood group frequencies present in <2% and >98% in both populations are not included.
[b] Adapted from [31]; the frequencies of blood groups may differ between African populations reaching 0 or 100%, for example, D, c, K, Fy^a, Fy^b, and Jk^a.
[c] Frequency of antigen-negative recipients multiplied by the frequency of antigen-positive donors.
[d] Risk alloantigen exposure in African–Americans divided by the risk in whites.

lower risk for alloimmunization against clinically relevant RBC antigens when they receive transfusions from donors of the same background (Table 1).

Anti-D formation, however, is more frequent in D+ individuals of African descent than in Europeans, which is probably the result of the high frequency of aberrant *RHD* alleles belonging to the three African D clusters, for example, DIVa, weak D type 4, and DAU in some African populations [34••]. Similarly, partial C antigens [*(C)ce^s* and *R^N* haplotypes], which are not uncommon in Africans, increase the risk for alloanti-C formation [35••]. Despite a relatively high frequency of FY-negative phenotypes, protecting against invasion of *Plasmodium vivax*, anti-Fy^b is an uncommon alloantibody in blacks because the mutated GATA box represses erythroid-specific Fy^b expression, but not that on other body tissues [36].

RBC antibodies are, depending on the specificity, only considered clinically relevant in the case of a subsequent

Table 2 Some examples of blood group antigens more or less common in blacks compared to whites

Blood group system	Antigen	Blacks (%)	Whites (%)
RH	f	92	65
	Ce	27	68
	V	30	1
	VS	32	<0.01
	STEM	6	<0.01
KEL	Js^a	20	<0.01
FY	Fy3	32	100
MNS	He	Only in blacks	Absent

Adapted from [31].

transfusion event or pregnancy. A recent study showed that 15% of pediatric transfusion recipients with malaria had been exposed to a previous transfusion event [37]. Multiple pregnancies are common in African women, and because it is the second most frequent transfusion indication, it can be anticipated that African women have a high risk of experiencing multiple transfusion events. In view of the main transfusion indications in Africans (e.g. malaria and pregnancy), the lifetime risk of exposure to multiple transfusion events is substantial.

Hemolytic disease of the fetus and newborn
Maternal IgG antibodies against paternal antigens on fetal RBCs can pass across the placenta and hemolyse fetal RBCs, resulting in severe fetal anemia, hydrops fetalis, and fetal death. In western countries, antibodies to the highly immunogenic RhD antigen are the main cause of hemolytic disease of the fetus and newborn (HDFN).

According to the 'Sixth report on perinatal care in South Africa', the perinatal mortality rate was 38 per 1000 births [38]. Although this report did not mention HDFN as a possible cause for fetal death, being RhD-negative was the most frequent cause of a high-risk pregnancy in a cross-sectional survey of the pattern of high-risk pregnancies in Nigeria [39].

The incidence of RhD immunization is related to the frequency of D-negative individuals in a population. Depending on the ethnic population, less than 1–6% of African pregnant women are D-negative [22]. According to paternal D-positive phenotypes, the mean 50% risk for anti-D immunization is comparable to D-negative white women. Recently, a cross-sectional study on Cameroonian women of reproductive age revealed anti-D immunization in 4.0% of women [40]. However, aberrant *RHD* alleles, amounting to 30% in some African populations and some of which are known for anti-D immunization, can increase the risk for anti-D HDN, but data other than casual data on its incidence are lacking [41,42].

Data on RBC alloantibody-complicated pregnancies in African countries are scarce. A study involving 3000 women in Zimbabwe showed RBC antibodies in 50 (1.7%) cases, of which seven (four anti-D, two anti-E, and one anti-Js^a) were clinically relevant for HDFN and responsible for three fetal deaths and two babies with jaundice [43]. RhD alloimmunization and pregnancy outcome was studied in 67 RhD-negative Nigerian women and revealed that six (9%) babies were severely affected by maternal D immunization, with three fetal deaths and three had an exchange blood transfusion [44]. During a 5-year period involving 22 493 infants in Zimbabwe, 25 anti-D and three anti-K cases of HDFN

Table 3 Red blood cell antibody frequency and specificity in African patients from Malawi and Uganda

	M'baya et al. [29], Malawi	Natukunda et al. [27], Uganda	Natukunda et al. [28●], Uganda
Number of patients	1000	214	428
Diagnosis	General and antenatal	General	SCD
Percentage transfused	10	100	100
Immunized patients (n, %)	11 (1.1)	13 (6.1)	26 (6.1)
Antibody specificities			
D	2	1	7
Rh Non-D	0	6	13
K	0	1	1
Fy^a	0	0	1
Jk^a	0	0	2
S	2	3	4
M	6	0	1
Other	1	1	3

SCD, sickle cell disease.

were reported [45]. The prevalence of clinically relevant non-ABO alloimmune HDFN from these studies is 0.12–0.16%, but may be as high as 9% in D-negative pregnant women.

In well resourced countries, RhD immunization prevention programs have greatly reduced the incidence of maternal anti-D formation, but such programs are routine in only a few African countries, such as Egypt and South Africa.

Posttransfusion red blood cell alloimmunization

The prevalence and specificity of RBC antibodies after transfusion in an African population has been the subject of only three published cross-sectional studies conducted in Uganda and Malawi. Overall, a total of 742 transfusion recipients were tested for the presence of RBC antibodies, and 43 antibody specificities were found in 40 patients [5.4%; 95% confidence interval (CI) 4–7%; Table 3] [27,28●,29]. This frequency of immunized patients is comparable to whites after a similar amount of RBC transfusions, but may be an underestimation due to the cross-sectional study design employed. The time between the last RBC transfusion and antibody investigation ranged from 0 to 49 years in the studies by Natukunda et al. [27,28●] and many antibodies may have become undetectable over time [46,47].

Ranking antibody specificities showed that anti-E was the most frequently encountered antibody, comparable to its frequency in whites. Anti-K was less common and anti-S more frequent as compared to most studies on whites. The lower frequency of anti-K is most likely the result of the low K-antigen frequency (e.g. 0.6% in the Malawi study). The relatively high frequency of anti-S was unexpected, because 37% of 500 Malawi donors were S-positive resulting in an equal immunization risk as in whites (Table 1). Anti-D was found in eight patients and D-genotyping revealed that six patients had partial D

(4 D^{Va}, 1 D^{IIIb}, and 1 R$_0^{Har}$), one patient had a probable D pseudogene, and one was D-negative. The latter two patients (aged 5 and 11 years, respectively) had most likely received D+ RBC transfusions. These data underscore the need for D typing sera recognizing variant D groups and, hence, the indication to perform a pretransfusion antibody screening and a complete crossmatch in previously transfused patients.

Conclusion

The WHO African Region program on Blood Transfusion Safety covers four major components for blood safety:

(1) Establishment of blood transfusion services with quality systems.
(2) Collection of blood only from voluntary nonremunerated blood donors from low-risk populations.
(3) Screening of all donated blood for transfusion transmittable infections (TTI), blood grouping, and compatibility testing.
(4) Improving effective clinical use of blood and blood components.

Although much progress has been made over the last few years, the above components are not yet in place in all African countries and they still remain a formidable challenge.

With regard to RBC compatibility testing, ABO and D RBC typing is mandatory, but a crossmatch including an antiglobulin phase is not routinely performed, which may be indicative of the nonavailability of reagents and trained staff in local hospital transfusion services. Despite the limited data available on posttransfusion alloimmunization, showing 1–6% immunization, complete crossmatching for patients with a history of past transfusions could be considered. Before recommendations on RBC antibody prevention can be formulated, information is needed on blood group distribution, including D variants, in the various black subpopulations,

alloimmunization rate and specificities, and incidence and causes of HDN.

Acknowledgements

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