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Red blood cell alloimmunization and antigen matching in sickle cell disease – the African perspective

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In sickle cell disease (SCD), blood transfusion facilitates improved blood and tissue oxygenation, reduces the propensity to sickling by diluting host cells, and suppresses the production of red blood cells (RBCs) containing sickle haemoglobin (HbS). Delivery of RBC transfusions to patients with SCD varies by method (simple vs. exchange) and frequency (episodic vs. chronic). However, due to the genetic differences between blood donors and recipients, repeated transfusions increase the risk of developing alloantibodies to RBC antigens. The antigens most frequently involved belong to the Rh, Kell, Kidd, Duffy, Lewis, and MNS blood group systems. Consequences of RBC alloimmunization include delays and difficulties in obtaining compatible blood for future transfusions, the occurrence of delayed haemolytic transfusion reactions (DHTRs), the hyperhaemolysis syndrome and autoimmunization. In Europe and USA, RBC alloimmunization rates ranging from 18% to 76% have been reported in SCD while other multiply transfused (OMT) patients had alloimmunization rates of 5% to 20% indicating that SCD patients are at a higher risk of developing RBC alloantibodies. To prevent alloimmunization in SCD patients, the standard practice in Europe and USA is to determine their extended RBC phenotype (ABO, Rh, Kell, Kidd, Duffy, Lewis, MNS) before commencing transfusion therapy and perform antigen matching for C, E and K antigens for patients without prior alloantibody formation. However in Africa, lower RBC alloimmunization prevalence rates of 6–10% have been reported in SCD patients and no differences were observed between SCD and OMT patients. This may be explained by the presumed high phenotypic compatibility between donors and SCD patients who were all Black Africans. Also, a low transfusion load (a median 3 U of blood were transfused) in SCD patients might have led to the poor response to alloantigenic challenge. Anti-K alloimmunization was notably rare among African SCD patients compared to anti-S. In many African countries, pre-transfusion immunohaematologic testing includes neither the detection of RBC alloimmunization nor preventive antigen matching. Most transfusion laboratories are understaffed and underequipped; they perform ABO/D typing plus room temperature saline cross-matches and do not screen for RBC alloantibodies. Hence, immunized SCD patients are not diagnosed and do not have the opportunity of receiving antigen-negative blood. Furthermore, data on the occurrence of DHTRs are lacking. Introducing pre-transfusion RBC alloantibody screening in all African countries will significantly improve the transfusion management of SCD patients. A program of *limited* phenotype matching for C, E and S antigens is recommended to prevent additional alloantibody formation in immunized SCD patients in Africa.

Keywords: Africa, alloimmunization, antigen matching, sickle cell disease.

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Introduction

Sickle cell disease (SCD) was first described by a Chicago physician, Dr James B. Herrick, in 1910 when he reported the case of a 20 year-old black student from the West Indies with severe anaemia characterized by 'peculiar elongated and sickle-shaped red blood corpuscles' [1]. It is an autosomal recessive genetic disorder usually presenting in childhood with chronic haemolytic anaemia punctuated by crises. SCD haemoglobinopathy denotes all genotypes containing at least one sickle gene, in which the sickle haemoglobin (HbS) makes up at least half of the haemoglobin present. The principal genotypes of SCD are homozygous sickle cell anaemia (HbSS), sickle cell haemoglobin C disease (HbSC), sickle cell haemoglobin D disease (HbSD), sickle cell β^0 thalassaemia (HbS/β^0) and sickle cell β^+ thalassaemia (HbS/β^+) [2]. In contrast, the sickle cell trait (HbAS) is a benign condition in which a sickle cell gene is inherited from one parent and a normal gene from another [3].

The distribution of malaria and the sickle cell gene are closely linked. The relative protection from severe falciparum malaria possessed by those with the sickle cell trait explains the reason for the maintenance of a high frequency of the sickle cell gene in sub-Saharan Africa [4]. The sickle cell trait is seen in 10–30% of people in equatorial Africa but is infrequent in northern and southern Africa [5]. The HbS gene is distributed worldwide, occurring around the Mediterranean in Sicily and other parts of southern Italy, northern Greece, along the south-east coast of Turkey, the north African coast, in Saudi Arabia especially the eastern province, Iran, and throughout central India [6]. The incidence of sickle trait in African-Americans is approximately 8% with 1 in 200–500 newborns affected with SCD [7].

Blood transfusion therapy in sickle cell disease

Blood transfusion is an important therapeutic tool in the management of SCD patients. It facilitates improved blood and tissue oxygenation, reduces the propensity to sickling by diluting host cells, and temporarily suppresses the production of RBCs containing HbS [8]. In SCD, transfusions have been shown to be effective in the management of severe anaemia [9], the prevention of recurrent strokes [10,11], the prevention of painful crises [12], and in lowering of maternal morbidity and perinatal mortality when used prophylactically during pregnancy [13]. Other indications of transfusion therapy in SCD patients include acute chest syndrome, heart failure, multi-organ failure syndrome, splenic sequestration and aplastic crises [14].

Regimens include episodic (acute simple) and chronic transfusions. Episodic transfusions are indicated when increased oxygen carrying capacity is desired in cases of anaemia or other clinical complications, but no significant decrease in HbS is required. In contrast, some clinical situations (e.g. prevention of stroke) require long-term suppression of circulating sickle cells and chronic transfusion therapy usually achieves this goal. Chronic transfusions are performed primarily on a scheduled basis (every 3––4 weeks) to prevent complications or their progression; they may be further classified as prophylactic or therapeutic and include simple and exchange transfusions [15]. However, SCD patients who are repeatedly exposed to transfusions with allogeneic donor blood have an increased risk of developing complications such as transmission of viral infections (e.g. hepatitis B, hepatitis C and HIV), iron overload (which requires chelation therapy with desferrioxamine), and alloimmunization to RBC antigens.

RBC alloimmunization in sickle cell disease

Alloimmunization against minor RBC antigens is a major problem for SCD patients who receive frequent transfusions [16,17]. It is due to the genetic differences between blood donors and recipients, and in general, the risk increases with the number of blood transfusions, although many patients become alloimmunized early during transfusion therapy [18]. In Europe and the United States, most of the blood available for transfusion comes from people of European descent while the majority of SCD patients are of African ancestry. Factors implicated in RBC alloantibody formation include recipient sex and age, history of pregnancy, number and timing of blood transfusions, recipient clinical diagnosis and treatment, genetic factors related to the antigenic response, and racial differences between donors and recipients [18–20]. The antigens most frequently involved belong to the Rh, Kell, Kidd, Duffy, Lewis and MNS blood group systems [17,18]. RBC alloimmunization rates ranging from 18% to 76% have been reported in transfused patients with SCD [17,21–23] while other multiply transfused (OMT) patients had alloimmunization rates of approximately 5–20% [24–26] indicating that patients with SCD are at a significantly higher risk of developing alloantibodies to RBC antigens unless preventive strategies are in place.

Consequences of post-transfusion RBC alloimmunization include delays and difficulties in obtaining compatible blood for future transfusions and the occurrence of delayed haemolytic transfusion reactions (DHTRs) [21,27]. About 25% of the clinically significant RBC alloantibodies become undetectable over time, potentially confounding future transfusions and placing the patient at risk of an anamnestic antibody production and severe DHTRs [17,28].

Approximately 40% of SCD patients who are alloimmunized have or will experience a DHTR [29]. Importantly, DHTRs can mimic or trigger various complications of SCD and should be suspected when patients present with appropriate symptoms (e.g. pain, fever, accelerated haemolysis) after a recent transfusion. Another complication of alloimmunization in SCD patients is the hyperhaemolysis syndrome which is characterized by destruction of both donor and recipient RBCs with associated severe anaemia and reticulocytopenia. The haemoglobin level often drops to below pre-transfusion levels approximately 1 week post-transfusion [30]. The exact pathophysiologic mechanism of this syndrome is not well understood. However, transfusion suppression of erythropoiesis and a bystander haemolysis mechanism have been proposed [31,32]. In many cases, the haemolysis occurs in the absence of a positive direct antiglobulin test (DAT), no new RBC alloantibodies are detectable and even after the transfusion of compatible RBC units. Subsequent transfusion may further exacerbate the haemolysis, which can become life-threatening and may be fatal. It has been suggested that transfusion be withheld in severe haemolytic episodes [33]. Also, there is evidence that RBC alloimmunization may lead to the production of autoantibodies that may be pathologic [34,35]. Castellino *et al.* [36] reported the frequency of autoantibody formation as approximately 7·6% in multiply transfused children with SCD and a strong association between autoantibody formation and the presence of RBC alloantibodies was noted. In contrast, alloimmunization in patients with autoimmune haemolytic anaemia (AIHA) seems to be less frequent. According to Garratty [37], transfusion-induced alloimmunization is commonly associated with autoantibody formation, although AIHA is rare.

Alloimmunization among SCD patients in Africa

SCD patients in Africa generally receive acute simple RBC transfusions which are not leukocyte depleted and not matched for common minor blood group antigens. Also, they are not heavily transfused compared to their counterparts in the developed world who may be on chronic transfusion programs. Most of the literature on RBC alloimmunization in SCD patients comes from European and American studies and only recently have such observations been reported from some African countries such as Uganda and the Democratic Republic of Congo (DRC). Although higher rates of RBC alloimmunization have previously been reported among transfused SCD patients in developed countries of Europe and USA [23,34], recent reports have shown lower prevalence rates in the post-transfusion anti-RBC alloimmune response in SCD patients in Africa [38,39]. In fact, there was no difference in the RBC alloimmunization rates between SCD and OMT patients in Uganda: both groups of transfusion recipients had an equal alloimmunization frequency of 6·1% [38,40] while the alloimmunization rate for SCD patients in the DRC was 10%. The low rate of RBC alloantibody formation observed among Ugandan and Congolese SCD patients may be explained by the presumed high phenotypic compatibility between blood donors and SCD patients who were Black Africans in both cases. This proposition is supported by findings from a Jamaican study in which a cohort of transfused SCD patients had a low alloimmunization rate of 2·6% due to the high racial homogeneity among blood donors and the patients [23]. Also, RBC alloimmunization rates in chronically transfused SCD patients reportedly decreased from a historic 3% per unit to 0·5% per unit with the use of phenotypically matched units of RBCs in the Stroke Prevention Trial in the USA with C, E and Kell matching [41]. However, transfused SCD patients in Uganda may have responded poorly to the alloantigenic challenge presumably because of the low transfusion load (a median 3 U of blood were transfused). Due to the shortcomings of a cross-sectional design, some RBC alloantibodies might have been missed since they have been reported to disappear with time [28,42] and our timing of serological investigations was by no means optimal.

Prevention of RBC alloimmunization – the role of antigen matching in Africa

In order to prevent alloimmunization in SCD patients in Europe and USA, the standard practice is to determine their extended RBC phenotype (ABO, Rh, Kell, Kidd, Duffy, Lewis, MNS) before commencing transfusion therapy and perform antigen matching for C, E and K antigens for patients without prior alloantibody formation [15,41]. A permanent record of the phenotype results is maintained in the transfusion laboratory to optimize future matching and a copy of the record given to the patient or family. Whereas anti-C and -E alloantibodies were among the most common specificities found in African SCD patients, we observed that anti-K alloimmunization was rare compared to anti-S which was more frequent [38]. Given that more than 98% of Black Africans have been found to be K negative [43–45] and about 30% possess the S antigen [45], a program of *limited* phenotype matching for C, E and S antigens is proposed so as to prevent additional alloimmunization in SCD patients in Africa who have already formed an alloantibody. Due to the presumed racial homogeneity between blood donors and SCD patients, costly e*xtended* phenotype matching is presently unnecessary in the African setting.

Current pre-transfusion immunohaematologic tests in many African countries include neither the detection of RBC alloimmunization nor preventive minor antigen

matching. RBC alloantibody screening and identification tests are only carried out in a few countries on the continent, i.e. Egypt, Namibia and South Africa. Most transfusion laboratories do not screen for RBC alloantibodies as part of pre-transfusion serologic testing and local transfusion services do not have phenotyped donors beyond ABO/D. The practice is to perform ABO/D typing plus room temperature (RT) saline cross-matches only. Thus, immunized SCD patients are not detected and they do not have the opportunity of receiving antigen-negative blood. A recent survey in twenty African countries by this author indicated that indirect antiglobulin test (IAT) cross-matches are rarely performed and DHTRs are seldom recognized as well as poorly investigated (unpublished data). Furthermore, transfusion laboratories are understaffed and underequipped, reagents are insufficient and records of testing results are poorly kept. Uniform pre-transfusion testing guidelines for Africa should be formulated to inform local transfusion practice. Introducing RBC alloantibody screening tests in all African countries will significantly improve the transfusion management of SCD patients. In future, availability of high-throughput molecular immunohaematologic techniques in Africa will hasten *RHD* and *RHCE* genotyping and aid in the detection of Rh variants in SCD patients [46,47] and hence the prevention of alloimmunization.

Conclusion

Despite the racial homogeneity between blood donors and SCD patients in Africa, there is significant alloimmunization to RBC antigens. With improvements in health care, more SCD patients will be exposed to frequent blood transfusions and others will be enrolled into chronic transfusion programs leading to an increased risk of RBC alloimmunization. There is a need for Transfusion Medicine practitioners to prevent alloimmunization and its consequences by improving the quality and quantity of pre-transfusion immunohaematologic testing in both blood centres and hospitals in Africa. Regional blood group reference laboratories should be established to handle unusual antigen–antibody problems. Resource constraints notwithstanding, *limited* antigen matching (C, E and S antigens) is recommended to prevent additional alloantibody formation in immunized SCD patients.

Disclosure

The author declares no potential conflict of interest.

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