

Red blood cell alloimmunization and minor red blood cell antigen phenotypes in transfused Ghanaian patients with sickle cell disease.

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LB, SH and AA performed the laboratory test and interpreted the results

LB and HS analyzed, interpreted the data and drafted the manuscript

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ABSTRACT

BACKGROUND: The routine pre-transfusion investigations in Southern Ghana involve only ABO-D blood group typing and ABO compatibility testing without screening for irregular red blood cell antibodies. The prevalence and specificities of red blood cell antibodies and frequencies of most minor blood group antigens in transfused patients with sickle cell disease (SCD) in Ghana are not known and are the objectives of this study.

STUDY DESIGN AND METHODS: A cross-sectional study that investigated transfused patients with SCD for the presence of irregular RBC antibodies and Rhesus, Kell, Duffy, Kidd and Ss antigens.

RESULTS: From a total of 154 patients (median age, 9 years), ten patients (6.5%) possessed 13 antibodies, predominantly against D, C and E antigens. In three patients, the antibodies (anti-D, anti-D+C and anti-C+e) were against antigens they possessed by serology. Genotyping showed that two of these patients had variant *RHCE* genes that encode for weak and partial e antigens and one patient had a partial *RHC* gene.

Frequencies of most RBC antigens were comparable with frequencies established among the African American population, however, K-k- and Jk(a-b-) phenotypes were more frequent and were present in 21% and 17% of patients, respectively.

CONCLUSION: The prevalence of RBC alloimmunization in transfused Ghanaian patients with SCD was 6.5% and the majority of antibodies were against antigens of the Rh system. Our findings stress the need to include pre-transfusion testing for RBC antibodies in patients with SCD, to improve transfusion safety.

Key words alloimmunization, sickle cell disease, red blood cell antigens, blood transfusion, Ghana, Africa

Introduction

Sickle cell disease (SCD) is the most common monogenic disorder, with the greatest occurrence in sub-Saharan Africa (SSA). More than 75% of SCD births are currently in SSA and has been predicted to rise to almost 90% by the year 2050.¹ Death rate has been estimated at 50-90% for children with SCD in SSA before the age of 5 years.² In Ghana, 2% of newborns have SCD and up to 30% carry the sickle cell gene.³ Most individuals with SCD in SSA are diagnosed when they present with symptoms (i.e. dactylitis, splenic sequestration) during childhood, at a mean age of two years. Although highly cost-effective, screening newborns for early detection and timely treatment of SCD is, contrary to the United States and many European countries, not routine in Ghana.⁴

Red blood cell (RBC) transfusions in SCD are used to improve oxygen-carrying capacity by correcting anemia, to suppress the production of sickle reticulocytes and to prevent or reverse complications related to vaso-occlusion and hemolysis. RBC transfusion has shown tremendous improvement in patients' wellbeing.^{5,6} However, RBC alloimmunization is a major complication in transfused patients with SCD and the frequency ranges from 2-63%.⁷⁻¹⁴

The pathophysiology of RBC immunization is considered multifactorial. Besides the disparity in RBC antigens between donors and recipients, other contributory factors include sex, age, patients' age at first transfusion, exposure to episodic transfusions, patients' immune regulatory state and genetic status.¹⁵⁻¹⁹

RBC alloimmunization may delay or even prevent blood transfusion, complicate pregnancies (i.e. hemolytic disease of the fetus and newborn) and increases the risk of delayed hemolytic transfusion reactions.^{20,21} In addition, alloimmunized patients have an increased risk of developing additional alloantibodies and autoantibodies.²²⁻²⁴

In Ghana, the main indications for RBC transfusions in patients with SCD are low hemoglobin (resulting mainly from RBC hemolysis and malaria) and acute crisis. There are little or no chronic

transfusion programmes. The routine pre-transfusion investigations involve only ABO-D blood group typing and ABO compatibility testing (immediate spin cross-match) without screening for irregular RBC antibodies. Consequently, the frequency of most blood group antigens other than ABO-D and RBC alloimmunization in transfused patients with SCD in Ghana are not known.

We studied the prevalence and specificities of RBC antibodies and the frequencies of some common minor blood group antigens in transfused patients with SCD at Komfo Anokye Teaching Hospital, Kumasi, Southern Ghana.

Materials and Methods

Patient recruitment

In a cross-sectional study, patients with SCD were recruited, between January to November 2016, at the sickle cell clinic of the Komfo Anokye Teaching Hospital, (KATH), Kumasi, with approval from the Committee on Human Research, Publication and Ethics, Kwame Nkrumah University of Science and Technology. KATH is a 1200-bed hospital with approximately 17,000 transfusions annually, of which 80% is transfused as whole blood. Blood is sourced from both voluntary (80%) and (family) replacement donors (20%). Blood is neither irradiated nor leucoreduced before transfusion. The sickle cell clinic treats and transfuses approximately 5000 and 150 SCD patients annually, respectively. It has about 1000 transfused patients with SCD of which 600 have received up to four RBC transfusions.

The study inclusion criteria were: patients with SCD of any age and SCD genotype, with at least one RBC transfusion event and the last transfusion at least two weeks before enrollment into the study. Patients with the last transfusion at least two weeks before the time of enrollment were selected to allow a time window within which (new or boosted) antibodies might develop following

transfusion. Informed consent was obtained from participants of 18 years and older and from guardians of those under 18 years of age.

Data collection

Participants' basic demographic characteristics, number of previous transfusions, age at first transfusion and indication for transfusion were retrieved from patients' hospital files and recorded on clinical record forms. Patients or their caretakers provided us with this information and transfusions in hospitals other than KATH, if missing from the hospital file. However, because patients' (or guardians') memory may not be exactly accurate, the number of transfusions were categorized as 1, 2-4 and ≥ 5 RBC units.

Sample processing and laboratory investigation

Patients' blood samples were separated into RBC with buffy-coat and plasma. RBC antigen typing was performed at the Haematology Unit of KATH. Frozen RBC and plasma samples were transported to the University of Michigan Reference Laboratory, Michigan, for antibody screening and identification tests and molecular genotyping. Genotyping was performed when antibodies were present against antigens the patient possessed with serology.

Red blood cell antigen and antibody investigations

Serologic blood group antigen (D, C, c, E, e, K, k, Fy^a, Fy^b, Jk^a, Jk^b, S and s) typing was performed with commercially available antisera (Immucor, Inc., Norcross, GA; Ortho Clinical Diagnostics, Inc., Raritan, NJ), using the conventional tube method, according to the manufacturer's instructions.

RBC antibody screens were performed with the indirect antiglobulin technique (IAT), using a LISS gel test and a two-cell screen panel (Ortho Clinical Diagnostics, Inc., Raritan NJ). Antibody

identification was performed on samples with a positive screen using a 12-cell panel by the same technique. Antibody specificities that could not be identified with the LISS technique were subjected to further gel column agglutination testing with enzyme (ficin) treated panel cells.

Frozen RBC samples from patients with antibodies to antigens they typed positive for by serology were sent to Grifols Immunohematology Center, USA for genotyping using the BLOODchip ID CORE XT v.4.0, BID XT software, Allele-Specific PCR and Sanger DNA sequencing.²⁵

Statistical analysis

Median and range described non-normally distributed continuous variables. Univariate logistic regression was used to determine the association of patient characteristics, i.e. sex, age at enrollment (continuous), age at first transfusion (categorized as ≤ 1 , 2-5, 6-9 and ≥ 10), SCD genotype (SS and other), ethnicity (Akan and other) and number of transfused units (categorized as 1, 2-4 and ≥ 5), with the presence of antibodies. Results are presented as odds ratios (OR) with 95% confidence intervals (CI). A p-value < 0.05 was considered statistically significant. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc, Chicago, IL, USA).

Results

A total of 154 patients with SCD (male to female ratio, 1.3; median age 9; range 1-50 years) were recruited. Patients were mainly from the Akan tribe (81%) and 87% had the SCD SS genotype. The transfusion history revealed that 32% of patients had received one RBC unit, 55% received 2-4 units and 12% more than four RBC units. While 23% of patients received the first RBC transfusion during the first year of live, only 7% did so at or after age ten (Table 1).

In ten patients (6.5%; 95% CI, 3.3-11.9%), antibody screening was positive and identification revealed 13 RBC antibodies. Nine antibodies were against antigens in the Rh system, three against the

M antigen and one against a low frequency antigen of unknown specificity. Three patients had multiple antibodies against Rh system antigens (Table 2). In three patients, the antibodies (anti-D, anti-D+C and anti-C+e) were against antigens they possessed by serology. Genotyping showed that the two serologically D+ male patients had *RHD*deletion* and variant *RHCE* genes that encode for weak and partial RHe antigens. The patient with anti-C+e had partial *RHC* and normal *RHe* genes (Table 2).

Univariate logistic regression revealed that age at enrollment, age at first transfusion, SCD genotype and ethnicity were not associated with the presence of antibodies. Alloimmunization showed a trend to be lower in females compared to males (OR, 0.30; 95% CI 0.06-1.48; p=0.14) and was significantly associated with the number of transfused units (OR 2.87; 95% CI 1.02-8.08; p=0.046) (Table 3). The frequency of patients with antibodies increased from 2% in the 50 patients who had received only one unit, to 7% in 85 patients with 2-4 units and 16% in 19 patients with more than four RBC units. In multivariate analysis, including sex and number of transfusion, only the number of transfusions was associated with the presence of antibodies (aOR 2.87; 95% CI 1.02-8.08; p=0.046).

Due to limited reagent availability, serologic RBC antigen typing was performed in 78 to 133 patients. The D, c, e and s antigens were present in more than 90% of patients, k and Jk^a in about 75%, A, B, C, E, Jk^b and S in 24-47% and Fy^a, Fy^b and K antigens in less than 5% of patients (Table 4). The Fy(a-b-) phenotype was present in 92% of the 116 Fy^a and Fy^b typed patients and the S-s- phenotype in 0.9% of 107 S and s typed patients. The K-k- phenotype was present in 21% of 71 K and k typed patients and the Jk(a-b-) phenotype in 17% of 126 Jk^a and Jk^b typed patients (Supplemental Table 1, including an overview of studies on RBC antigen phenotypes in other Ghanaian ethnicities and published in African Americans). None of the patients with the K-k- and the Jk(a-b-) phenotypes were known family members.

Discussion

This cross-sectional study, conducted in Southern Ghana, which recruited 154 transfused patients with SCD, predominantly children from the Akan tribe, revealed an alloimmunization prevalence of 6.5%. The number of transfused units was associated with the presence of antibodies. The majority of antibodies were against antigens from the Rh blood group system. To the best of our knowledge, this is the first study reporting on the prevalence and nature of RBC antibodies in transfused patients with SCD in Ghana. In our previous study, 9.4% of 106 patients, predominantly transfused adults with other diseases from the same area in Ghana, were alloimmunized.²⁶ A meta-analysis comprising eleven studies from SSA, and mainly in transfused patients of various ages with SCD, showed that 6.7% (95% CI 5.7-7.8%) had clinically significant RBC alloantibodies. Antibodies against Rh antigens were the most prevalent, comparable with our results.²⁷ The alloimmunization prevalence observed in our study is consistent with the 2.9-6.1% established in other studies, that were performed in (predominantly) children with SCD from other SSA countries.^{7-10,28}

The frequency of patients with antibodies in our study increased from 2% after one RBC unit to 16% in patients who had received more than four units. Previous studies showed a higher risk for alloantibodies with increasing number of transfused RBC units in patients with and without SCD.^{12,29-}

³¹ Since patients with SCD are lifelong transfusion dependent, preventive extended RBC antigen matching, especially for C and E antigens, should be considered. A study exploring Rh blood group antigen frequencies among 1533 blood donors from the Akan tribe, found C and E antigens less frequent compared to our patients with SCD (19% vs 37% and 17% vs 28%, respectively).³² The ccee phenotype was present in 65% and 82% of the D+ and D- donors, respectively, suggesting that matching for C and E antigens is feasible. However, considering that 1) homozygous serologic expression of C or E antigens was only present in none and two of our patients, respectively, 2) the frequent presence of *RH* variant alleles in Blacks and 3) most of our patients received transfusion for

unplanned emergencies, a more pragmatic approach would be to transfuse all patients with SCD with C and E negative RBC.^{33,34} This will require a pool of C and E antigen typed donors, which will be challenging in Ghana.

Three patients had anti-M, which is often naturally occurring, usually of IgM type, relatively common in children (with and without SCD), may appear in response to an infection and is often clinically insignificant.^{35,36} However, transfusion reactions due to anti-M reactive at 37 °C have been reported and patients with warm-reacting anti-M should receive M- RBCs.^{37,38}

One patient had anti-D and one anti-C+D, but tested both D+ by serology. Genotyping revealed *RHD*deletions*, absence of C-alleles and variant e-alleles (ce(48C) and ceAG)) in both, the latter frequently present in African-Americans.³⁹ A number of variant Rhce proteins such as ceCF, ceRT and ceSL carry D-specific amino acids or express D-like epitopes that can react with some monoclonal anti-D.⁴⁰ For ceAG, this has not been described, so incorrect serologic D typing cannot be ruled out. The C+e+ patient with anti-C and auto- or e-like antibody had a *RHC*-variant allele, serologically detectable by some anti-C reagents.

Frequencies of most RBC antigens in our patients were comparable to those published in a text book for the Black, mainly African American, population.⁴¹ Frequencies of k (78% and 100%), Fy^b (4% and 23%) and Jk^a antigens (75% and 92%) were markedly lower, reflecting differences in the frequencies of some RBC antigens between predominantly Akan patients with SCD and the Black population. Our finding that 17% of our patients had the Jka(a-b-) phenotype is in accordance with the study by Acquaye who reported a Jk(a-b-) phenotype in 13% of 121 Southern Ghanaian donors from Ewe ethnicity.⁴² In addition, this phenotype was found in 38% of 158 Ghanaian donors from Ga ethnicity and in 87% of 162 pregnant women from various ethnicities in Western Nigeria, further stressing that blood group prevalence can vary substantially among African ethnicities.^{43,44} So far, more than 40 variant Kidd alleles have been described in many different ethnic groups. These variants

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can silence protein expression or produce weak or partial antigens, hampering serologic typing.⁴⁵ The high frequency of the K-k- phenotype in our patients is a novel finding. Similar to Kidd, more than 40 variant *KEL* alleles lead to the K₀ phenotype or reduced expression of Kell glycoprotein, termed Kmod phenotype.⁴⁶ Only a few studies determined the frequency of Kell system blood groups in SSA populations and almost all were limited to the K-antigen. The frequency of k-antigen is largely unknown, but presumed to reach almost 100%. Blood group antigen frequencies quoted for Blacks are often based on African Americans and obviously do not reflect distributions in African regions. For instance, the Kp^b antigen from the Kell blood group system is, like k-antigen, presumed a high frequency antigen almost universally present on RBCs. However, a study from Cote d'Ivoire showed that 17% of 651 blood donors had the Kp(a-b-) phenotype.⁴⁷ These novel findings deserve further explorations, including genotyping to determine the molecular basis of these phenotypes, but financial restraints prohibited this in our study.

Pre-transfusion investigations in Ghana are limited to ABO-D blood group typing and ABO compatibility testing. Little is known on the risk of transfusion reactions in patients with SCD receiving not completely cross-matched transfusions in SSA and transfusion reactions were not recorded in our study. However, in a one-year study in our hospital in 372 patients without SCD, the prevalence of acute (within 24 hours after transfusion) hemolytic transfusion reactions was 9.3 per 1000 transfusions.⁴⁸ None of the reactions were attributable to ABO incompatibility, but the presence of irregular RBC antibodies was not investigated and cannot be ruled out as having been implicated.

Our study had several limitations. First, despite our thoroughness of investigation, demographic and transfusion information were not completely available for all patients, due to sub-optimal hospital documentation. In addition, transfusion history may be impaired because patients' (or guardians') memory may not be exactly accurate. Second, the optimal period after transfusion for antibody detection is largely unknown, therefore our antibody screening might have been too soon

after the last transfusion to detect new antibodies or too late resulting in evanescence of antibodies.⁴⁹ Both result in an underestimation of antibody frequency. Also, because the actual number of RBC units before antibodies were formed were not known, - cross-sectional study design and patients might have been transfused in other hospitals -, the precise immunization rate per transfused unit could not be determined. Lastly, the study consisted of patients predominantly from the Akan tribe and results may not be generalizable for patients with SCD from other ethnic groups in Ghana.

In conclusion, the prevalence of RBC alloimmunization in transfused patients with SCD in Southern Ghana was 6.5% and alloantibodies were in the majority of cases against D, C and E antigens.

Our findings stress the need to test for the presence of RBC antibodies in SCD patients before transfusion, preferably using a standardized red cell-panel of 'African origin' (i.e. expressing V, VS and Js^a antigens), but at least by performing an indirect antiglobulin compatibility test with donor RBC and patient serum to improve transfusion safety. The latter is probably cheaper and easier to implement in Ghana. However, to effectively improve safety, knowledge on blood group antigen frequencies in ethnic groups in SSA, RH genotyping and (limited) antigen matching may be essential in the future.

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TABLE 1. Demographic and transfusion characteristics of the 154 transfused patients with sickle cell disease in Southern Ghana.

Characteristic	Alloimmunized	Not alloimmunized
Number of patients (%)	10 (6.5)	144 (93.5)
Males / Females	80 / 20*	55 / 45
Median age at enrollment in years (range)	8 (2-22)	9 (1-50)
Sickle cell disease genotype: SS / other	90 / 10	87 / 13
Ethnicity: Akan tribe / Others	80 / 20	81 / 19
Age at first transfusion [†] : ≤1 / 2-5 / 6-9 / ≥10	30 / 30 / 30 / 10	23 / 46 / 24 / 7
Number of transfused units: 1 / 2-4 / ≥5	10 / 60 / 30	34 / 55 / 11

Date expressed as percentages, unless stated otherwise.

*The two females with antibodies were 6 and 13 years of age.

[†]Data available for 127 of 144 (88%) not alloimmunized patients.

TABLE 2. Red blood cell alloantibody specificities and Rh antigen serology and genotyping results in the ten alloimmunized patients with sickle cell disease in Southern Ghana

Antibody		D, C, c, E and e pheno- and genotypes		
specificity	N	Serology	Genotyping	Predicted phenotype
M	3	n.a.	n.a.	n.a.
D	1	D-, C-, c+, E-, e+	n.a.	n.a.
D	1	D+, C-, c+, E-, e+	Homozygous <i>RHD*deletion</i> , Homozygous <i>RHCE*ceAG</i>	D-, C-, E-, c+, partial-e
E	1	D+, C- c+, E-, e+	n.a.	n.a.
D+C	1	D+, C+, c+, E-, e+	Homozygous <i>RHD*deletion</i> <i>RHCE*ce(48C)</i> and <i>RHCE*ceAG</i>	D-, C-, E-, c+, weak-e/partial-e
C+e-like	1	D+, C+, c+, E-, e+	<i>RHD*deletion</i> and <i>RHD*r's</i> <i>RHCE*ce</i> and <i>RHCE*ce[733G,1006T]</i>	D-, partial-C, E-, c+, e+
E+C ^w	1	D+, C-, c+, E-, e+	n.a.	n.a.
LFA†	1	n.a.	n.a.	n.a.

N, number; n.a., not applicable.

†Antibody against an unknown low frequency antigen.

TABLE 3. Univariate analysis of variables associated with the presence of RBC antibodies in 154 transfused patients with sickle cell disease in Southern Ghana.

Characteristic	OR	95% CI	p-value
Sex (male* vs female)	0.30	0.06-1.48	0.14
Sickle cell disease genotype (SS* vs other)	0.73	0.09-6.10	0.77
Ethnicity (Akan* vs other)	1.08	0.22-5.39	0.92
Age at first transfusion† (≤ 1 , 1-5, 6-9 and ≥ 10)	1.07	0.51-2.42	0.86
Age at enrollment (continuous)	1.01	0.91-1.12	0.82
Number of transfused units (1, 2-4 and ≥ 5)	2.87	1.02-8.08	0.046

*The reference.

†Data was available for 127 of 144 (88%) not alloimmunized patients.

In multivariate analysis, including sex and number of transfused units, only the number of transfused units was associated with the presence of antibodies (aOR 2.87; 95% CI 1.02-8.08; p=0.046).

TABLE 4. Frequencies of minor red blood cell antigens in patients with sickle cell disease from Southern Ghana.

RBC antigen	Number of patients tested	Antigen frequency (%)	RBC antigen	Number of patients tested	Antigen frequency (%)
A*	129	33	K	105	0
B*	129	24	k	78	78
D	132	96	Fy ^a	116	4.3
C	133	37	Fy ^b	133	3.8
E	132	28	JK ^a	132	75
c	133	100	JK ^b	127	47
e	133	99	S	118	39
			s	116	94

*ABO blood group frequencies were: O 50%, A 26%, B 17% and AB 7%.