One third of alloantibodies in patients with sickle cell disease transfused with African blood are missed by the standard red blood cell test panel

Studies on red blood cell (RBC) antibodies in Africa routinely use standard test cells from donors of Caucasian descent. There are no systematic data on alloimmunization against antigens that are almost exclusively present in Africans. We studied the prevalence of antibodies in transfused Ghanaian patients with sickle cell disease (SCD) using standard test cells (representing predominantly antigens more common in Caucasians (Caucasian antigens) and cells expressing antigens more common among Africans (African antigens). Antibodies were present in 16% of 221 patients; 31% of these were directed against African antigens that were not detected with standard test cells. Our findings are not only relevant for an African setting, but also for Western blood banks that are developing strategies to recruit more African donors.

Transfusions in patients with SCD are associated with high rates of red blood cell (RBC) alloimmunization against multiple antigens. Alloantibody screening is performed with standard reagent test cells, mostly from donors of Caucasian descent that lack antigens that are more prevalent in Africans. Alloimmunisation involving antigens that are almost exclusively present in Africans has not been studied systematically in a setting where both patients and donors are Africans. We determined the prevalence of RBC antibodies against Caucasian and African antigens in multi-transfused patients with SCD in Ghana, where pre-transfusion antibody screening and indirect antiglobulin crossmatch are not routine.

Our cross-sectional study recruited patients between July and December 2018, from two tertiary hospitals. Patients were eligible for inclusion if they were at least 2 years of age and had received at least two transfusions at least 6 weeks before study enrolment (to allow time to develop antibodies). Patients were episodically transfused with non-leucoreduced whole blood from African donors. Donors were not screened for sickle cells. Participants' demographics and transfusion history were retrieved from hospital files. Patients or caretakers provided this information, if missing from hospital files, using a standard questionnaire.

Plasma and buffy coat samples, taken at enrolment, were frozen at –80°C and transported to Sanquin, Amsterdam, the Netherlands, for routine antibody testing against a standard three-cell reagent panel (Bio-Rad Laboratories AG, Cressier, Switzerland), not expressing antigens that are more common in Africans, using a low ionic strength solution (LISS) indirect anti-globulin gel column agglutination test. Using the same method, antibody identification was performed with commercial panels of reagent RBC of selected phenotypes and against eight selected cells with antigens that are very rare (<0.01% to 1%) in Caucasians but more frequent (0.5% to 32%) in Africans (*i.e.*, MNS6 [He], MNS25 [Dantu], RH10 [V], RH20 [VS], RH30 [Go^a], RH32, RH43 [Crawford] and KEL6 [Js^a]).[1] These antigens were selected based on the availability of RBC expressing rare antigens archived in the Immunohaematology Diagnostics laboratory at Sanquin. Antibody specificities were confirmed by re-testing with two RBC expressing and two RBC not expressing the target antigens. For patients with anti-D, *RHD* genotyping was done on genomic DNA by Multiplex Ligation-dependent Probe Amplification (MLPA) assay according to the manufacturers' protocol (MRC Holland, Amsterdam, the Netherlands) using a thermocycler (Veriti, Applied Biosystems, Nieuwerkerk aan de IJssel, the Netherlands).[2] When MLPA results were equivocal, DNA sequencing was performed to determine the *RHD* genotype. Sequence products were analyzed on a genetic analyzer (3730xl, Applied Biosystems).

Table 1. Specificities of the 36 red blood cell antibodies in the 35 alloimmunized multi-transfused Ghanaian patients with sickle cell disease.

Blood group system	Antibody specificity, (n)	
	Panel expressing antigens more common in Caucasians	African panel expressing antigens more common in Africans
Rh	E (10); D (7); G (2)	V/VS (2); VS (1); Go^a (1); RH32 (1)
Kell	K (1)	
MNS	s (1)	Dantu (3); He (2)
Unidentified	Pan-reactive (3); Non-specified* (1)	Non-specified* (1)

*The non-specified antibodies were probably against low frequency antigens. One patient had anti C+D.

The study was approved by the Committee on Human Research, Publication and Ethics, Kwame Nkrumah University of Science and Technology, and Korle Bu Teaching Hospital and Liverpool School of Tropical Medicine Institutional Review Boards. Patients or their caretakers gave written informed consent to participate in the study.

Statistical analyses were performed using the SPSS (IBM Corp., Armonk, NY, USA). Results for continuous variables were presented as medians (range) and categorical variables as frequencies (percentages).

We recruited 221 Ghanaian patients (123 women and 98 men; 89% hemoglobin [Hb] SS, 10% HbSC, one HbSD and one HbSβ^0-thalassemia). The median age at enrolment was 17 years (range, 2-66 years). Patients had received a median of three (range, 2-40) whole blood transfusions and the median period between last transfusion and study enrolment was 2.1 years (range, 6 weeks to 55.5 years).

Antibody screening, using standard test cells, was positive in 24 patients (10.9%) and revealed 25 antibody specificities (Table 1). Although D antigen matching was routine in Ghana, anti-D was present in seven patients. *RHD* genotyping of these patients revealed that three women and two men had only *RHD*-null alleles (three *RHD*01N.01/RHD*01N.01*, one *RHD*01N.01/RHD*01N.03* and one *RHD*08N.01/RHD*08N.01*). In the three D negative (D-) women, anti-D could have been induced by a D positive (D+) pregnancy. For the two D- men, errors in blood group typing or mistakenly transfusing patients with D+ blood are possible causes for the anti-D. The presence of RH variants expressing weak D among D- African donors might have contributed to these mismatched transfusions. Two patients possessed variant *RHD* genes (*RHD*03.04* and *RHD*04.01*) associated with D+ serology and carriers of these variants can make anti-D after D-antigen exposure.[3,4]

The nine patients with D, G and s antibodies and the three patients with pan-reactive antibodies, were not tested against the African antigens because African test cells lacking these antigens were not available. Of the

remaining 209 patients, eleven (5.3%) patients who did not have alloantibodies against the standard three-cell screen panel, had a total of eleven antibodies against African antigens; three anti-Dantu (for one, confirmation by a second Dantu+ test cell was not performed, due to lack of Dantu+ cells), two anti-He, two anti-V/VS, one anti-VS, one anti-RH32, one anti-Go^a and one antibody probably against a low frequency antigen but no plasma was available for further investigation (Table 1).

Overall, 31.4% of alloimmunized patients had antibodies against African antigens that were not detected during standard antibody screening. Other studies in patients with SCD have reported antibodies against African antigens[5-13] but these patients were transfused in the US and Europe and thus not exclusively with blood from African donors. Furthermore, these antibodies were encountered during complex antibody workup, crossmatch incompatibilities and hemolytic transfusion reactions rather than through systematic screening. The antibody frequency against African antigens in these studies ranged from 0.7% to 13.3%, with anti-V/VS, anti-Js^a and anti-Go^a representing 90% of specificities. So far, only one study in France systematically screened a heavily transfused patient cohort with SCD using selected African red cells. After receiving a mean of 144 RBC transfusions (11.5% from donors of African descent) 5.2% of 211 patients had antibodies against African-specific antigens (MNS6, RH10, RH20, RH23, RH30 and KEL6).[14] However, unlike our study, these patients received blood matched for major Rh and K antigens, which precludes insight in differences in immunogenicity of African and the classical 'major' blood group antigens. Surprisingly, in our study seven of 36 (19%) antibodies were against low frequency African antigens (*i.e.*, Go^a 2%, RH32 1%, He 1% and Dantu 0.5% in Africans).[1] This suggests that these antigens are immunogenic in Ghanaians and may be more immunogenic than antigens for which we test in the European setting. The results from these studies stress the importance of testing against African antigens when patients receive transfusions from African donors.

Antibodies in patients with SCD are notorious for their high evanescence rate, which may be up to 60-80%.[15] Because our study had a cross-sectional design, it is likely that antibody frequency was underestimated. In the French study, none of the eight previously detected African antibodies, were detectable at study enrollment, suggesting that African antibodies also have a high evanescence rate.[14] Furthermore, African antibodies have been implicated in severe hemolytic transfusion reactions upon receipt of transfusions with the cognate antigens.[12]

Our study was limited by the scarcity of test cells expressing African antigens, so antibodies against other African antigens, such as RH49 (STEM), GE6 (Ls^a) and CROM3 (Tc^b), might have been missed.[1] Furthermore, antibodies against antigens that are more frequently expressed on RBC of Ghanaian donors might have been missed, because we did not specifically test Ghanaian RBC.

Since we found African antibodies in African patients transfused with African blood, it is also quite likely that similar antibodies may be elicited in Caucasian patients receiving blood from African donors and through pregnancy. These antibodies cannot be detected during pre-transfusion antibody screening using standard test cells and without performing an indirect antiglobulin crossmatch with donor cells (and many countries only perform type and screen). Our findings are relevant for blood banks in Western countries since many are developing strategies to recruit more African donors. Unless they can supply antigen compatible blood, African antibodies may increasingly be missed as the pool of African donors expands.

This is the first study to systematically screen for RBC antibodies against African antigens in a large cohort of transfused patients with SCD who were exclusively transfused with blood from African donors. In order to increase the availability of test cells with African antigens, African donors should be typed, for relevant antigens and then retain them as repeat donors for the preparation of test cells. As long as antigens commonly expressed in Africans are not incorporated on the RBC testing panel, transfusions for patients with SCD should be cross-matched using an indirect antiglobulin test, even when they do not have RBC antibodies using the standard three-cell screen panel.

Lilian A. Boateng,[1,2] Henk Schonewille,[3] Peter C. Ligthart,[3] Ahmad Javadi,[3] Barbera Veldhuisen,[3] Alex Osei-Akoto,[4,5] Yvonne Dei-Adomakoh,[6,7] Imelda Bates[1] and C. Ellen van der Schoot[3]

[1]Center for Capacity Research, International Public Health, Liverpool School of Tropical Medicine, Liverpool, UK; [2]Medical Diagnostics, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana; [3]Experimental Immunohaematology, Sanquin, Amsterdam, the Netherlands; [4]Child Health, School of Medicine and Dentistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana; [5]Directorate of Child Health, Komfo Anokye Teaching Hospital, Kumasi, Ghana; [6]Hematology, University of Ghana Medical School, University of Ghana, Accra, Ghana and [7]Ghana Institute of Clinical Genetics, Korle Bu Teaching Hospital, Adult Sickle Cell Clinic, Accra, Ghana

Correspondence:

LILIAN A. BOATENG - lilian.boateng@lstmed.ac.uk

doi:10.3324/haematol.2021.278451

Received: January 28, 2021.

Accepted: March 11, 2021.

Pre-published: April 1, 2021.

Disclosures: the authors declare no competing financial interests.

Contributions: LB, HS, AO, YD, EVS and IB conceived the idea and designed the study; LB, PL, AJ and BV performed the laboratory test and interpreted the results; LB, HS, IB and EVS analyzed and interpreted the data; LB and HS drafted the manuscript.

Acknowledgments: the authors would like to thank the staff, patients and donors at the sickle cell clinics and blood banks in KATH and KBTH, Ghana, for their contributions in obtaining blood samples for the research project.

Funding: this work was supported by grant GHCA-2017-18 to LB, from the Commonwealth Scholarship Commission, UK and from Sanquin Blood Supply, The Netherlands.

References

1. Reid ME, Lomas-Francis C, Olsson ML. The blood group antigen factsbook. London, UK: Academic Press Limited; 2012.
2. Haer-Wigman L, Veldhuisen B, Jonkers R, et al. RHD and RHCE variant and zygosity genotyping via multiplex ligation–dependent probe amplification. Transfusion. 2013;53(7):1559-1574.
3. von Zabern I, Wagner FF, Moulds JM, Moulds JJ, Flegel WA. D category IV: a group of clinically relevant and phylogenetically diverse partial D. Transfusion. 2013;53(11pt2):2960-2973.
4. Wagner FF, Frohmajer A, Ladewig B, et al. Weak D alleles express distinct phenotypes. Blood. 2000;95(8):2699-2708.
5. Rosse WF, Gallagher D, Kinney TR, et al. Transfusion and alloimmunization in sickle cell disease. Blood. 1990;76(7):1431-1437.
6. Telen MJ, Afenyi-Annan A, Garrett ME, Combs MR, Orringer EP, Ashley-Koch AE. Alloimmunization in sickle cell disease: changing antibody specificities and association with chronic pain and decreased survival. Transfusion. 2015;55(6 Pt 2):1378-1387.

7. Castro O, Sandler SG, Houston-Yu P, Rana S. Predicting the effect of transfusing only phenotype-matched RBCs to patients with sickle cell disease: theoretical and practical implications. Transfusion. 2002;42(6):684-690.
8. Karafin MS, Field JJ, Gottschall JL, Denomme GA. Barriers to using molecularly typed minority red blood cell donors in support of chronically transfused adult patients with sickle cell disease. Transfusion. 2015;55(6):1399-1406.
9. Yee ME, Josephson CD, Winkler AM, et al. Red blood cell minor antigen mismatches during chronic transfusion therapy for sickle cell anemia. Transfusion. 2017;57(11):2738-2746.
10. Campbell-Lee SA, Gvozdjan K, Choi KM, et al. Red blood cell alloimmunization in sickle cell disease: assessment of transfusion protocols during two time periods. Transfusion. 2018;58(7):1588-1596.
11. Miller ST, Kim H-Y, Weiner DL, et al. Red blood cell alloimmunization in sickle cell disease: prevalence in 2010. Transfusion. 2013;53(4):704-709.
12. Coleman S, Westhoff CM, Friedman DF, Chou ST. Alloimmunization in patients with sickle cell disease and underrecognition of accompanying delayed hemolytic transfusion reactions. Transfusion. 2019;59(7):2282-2291.
13. Chou ST, Evans P, Vege S, et al. RH genotype matching for transfusion support in sickle cell disease. Blood. 2018;132(11):1198-1207.
14. Floch A, Gien D, Tournamille C, et al. High immunogenicity of red blood cell antigens restricted to the population of African descent in a cohort of sickle cell disease patients. Transfusion. 2018;58(6):1527-1535.
15. Harm SK, Yazer MH, Monis GF, Triulzi DJ, Aubuchon JP, Delaney M. A centralized recipient database enhances the serologic safety of RBC transfusions for patients with sickle cell disease. Am J Clin Pathol. 2014;141(2):256-261.