Red Blood Cell Alloimmunization in Transfused Patients with Sickle Cell Disease in Sub-Saharan Africa; a Systematic Review and Meta-Analysis

Lilian Antwi Boateng, Alain M. Ngoma, Imelda Bates, Henk Schonewille

PII: S0887-7963(19)30022-7
DOI: https://doi.org/10.1016/j.tmrv.2019.06.003
Reference: YTMRV 50577

To appear in: *Transfusion Medicine Reviews*

Please cite this article as: L.A. Boateng, A.M. Ngoma, I. Bates, et al., Red Blood Cell Alloimmunization in Transfused Patients with Sickle Cell Disease in Sub-Saharan Africa; a Systematic Review and Meta-Analysis, Transfusion Medicine Reviews, https://doi.org/10.1016/j.tmrv.2019.06.003

Red blood cell alloimmunization in transfused patients with sickle cell disease in sub-Saharan Africa; a systematic review and meta-analysis

Lilian Antwi Boateng[a,b], Alain M. Ngoma[c], Imelda Bates[b] and Henk Schonewille[d]

[a]International Public Health, Liverpool School of Tropical Medicine, Liverpool, UK

[b]Medical Laboratory Technology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

[c] Douglas Hospital Research Centre, McGill University, Montreal, Quebec, Canada

[d]Department of Experimental Immunohematology, Sanquin, Amsterdam, Netherlands

Correspondence to:

Lilian Antwi Boateng

Liverpool School of Tropical Medicine

Pembroke Place, L3 5QA, UK

Tel: +44 7459662114

Email: lilian.boateng@lstmed.ac.uk

Abstract

Sickle cell disease (SCD) is the most common monogenic disorder in sub-Saharan Africa (SSA). Blood transfusion to increase the oxygen carrying capacity of blood is vital in the management of many patients with SCD. However, red blood cell (RBC) alloimmunization is a major challenge to transfusions in these patients. Commonly in SSA, pre-transfusion tests only involve ABO D grouping and compatibility without RBC antibody testing. Data on the frequency of RBC alloimmunization in patients with SCD in SSA is limited. We performed a systematic review and meta-analysis on available data on alloimmunization in transfused patients with SCD to determine the published prevalence of RBC alloimmunization in SCD patients in SSA.

Six databases were systematically searched to identify relevant studies, without year or language restrictions. In all, 249 articles were identified and 15 met our selection criteria.

The overall proportion of alloimmunization was 7.4 (95% CI: 5.1 – 10.0), per 100 transfused patients. Antibodies against E, D, C and K antigens accounted for almost half of antibody specificities and antibodies to low and high frequency antigens were also common and represented almost 30% (20% to low frequency antigens and 9% to high frequency antigens) of specificities. Heterogeneity between studies was moderate and meta-analysis found region of Africa as the major contributor to the heterogeneity. We also observed inconsistencies across studies in reporting of factors that may influence alloimmunization.

This review provides an overview of the extent of the alloimmunization problem in SSA and provides a baseline against which to compare the effect of any interventions to reduce the alloimmunization risk.

Key words: Alloimmunization, sub-Saharan Africa, systematic review, sickle cell disease.

Introduction

Sickle cell disease (SCD) is widely disseminated, affecting millions of people worldwide, primarily those of sub-Saharan African (SSA), South and Central American, Arabic, Indian, and Mediterranean descent, because of the protection the sickle cell trait offers against severe malarial infection [1]. It is the most common inherited genetic disorder in SSA, and SSA accounts for over 75% of the global SCD burden and with 50-90% mortality rate, especially among children up to age five [2]. The area of Africa that lies between latitude 15^0 North and latitude 20^0 South have the highest prevalence of the sickle cell trait, ranging from 10%-45% and up to 3% of all children are born with SCD [3-6].

Although, in well-resourced countries, administration of hydroxyurea has improved patients' outcome, blood transfusion therapy remains an important part of disease management to treat or prevent SCD related complications [7-9]. However, in SSA, blood transfusion presents major challenges, due to widespread blood shortages, the high cost and the risk of transfusion-transmitted infections. In view of this, transfusions in patients with SCD in SSA are reserved for life-threatening anemia and acute stroke management, mostly one unit of blood at a time. The number of blood donations in Africa increased by almost 50% between 2008 and 2013 [10] alongside increased effort to reduce transfusion transmitted infections [11,12], which is likely to improve the safety and availability of transfusions for patients with SCD in SSA.

Red blood cell (RBC) antibody formation (i.e. alloimmunization) against foreign RBC antigens, is a major complication in transfused patients with SCD and presents significant challenges in their management. Alloimmunization frequency of up to 76% has been reported

in patients with SCD [13-16]. Risk factors for alloimmunization are multifactorial including patients' age, RBC antigen disparity, age of first transfusion, sex, number of RBC transfusions, race, age of RBCs transfused and patients' genetic and inflammatory status [17-23].

RBC alloimmunization increases the risk of auto-immunization, hyperhaemolysis syndrome and delayed hemolytic transfusion reaction [24-30]. Measures to reduce RBC alloimmunization include transfusing donor blood for which the RBC antigens have been matched to that of the recipients (i.e. RBC antigen matching) and a judicious use of blood transfusions [16,31-33].

Most studies reporting on RBC alloimmunization in patients with SCD have been conducted in well-resourced countries where pre-transfusion antibody screening and identification are routine practices. In resource-limited settings such as SSA, where antibody screening is not routinely performed, data on the prevalence of RBC alloimmunization in patients with SCD are very scarce. We performed a systematic review of the literature and a meta-analysis to determine the published prevalence of RBC alloimmunization in SCD patients in SSA.

Methodology

Inclusion criteria

The review considered all primary peer reviewed studies on transfused patients with SCD in SSA, without restriction to year of publication, study design and language, that reported quantitative data on the prevalence of RBC alloimmunization and alloantibody specificities.

Search strategy and study selection:

The review was conducted following the PRISMA flow diagram (Figure 1) for selection of studies. Three search strategies were used to search for articles published up to February

2019. A search for articles, screening of the articles and data extraction were done independently by two authors (LAB and HS).

The first step involved searching six databases (Medline, CINAHL, Web of Science, Google Scholar, PubMed, African Journals Online (AJOL) with specific search terms sickle cell disease, sickle cell anaemia, SCD, haemoglobin S disease, alloantibodies, allo-antibodies, isoimmunisation, isoantibodies, red cell alloantibodies, unexpected alloantibodies, irregular erythrocyte antibodies, alloimmunisation, alloimmunization, multiple transfusion, blood transfusion, erythrocyte transfusion, "sub-Sahara Africa", Africa, Tanzania, Nigeria, Uganda, Mali, South Africa, Ghana, Cote d'Ivoire, Congo, Mozambique, Namibia, Sierra Leone, Low resource countries and developing countries. References were extracted into Endnote X8 and duplicates were removed. Articles retrieved from the search were screened by their titles to exclude those that were not relevant to the topic. Additional search terms discovered from the first search were used in a second search for articles that were not captured during the initial search. Resulting articles were screened by their abstracts to select articles that met the inclusion criteria. These articles were then assessed for eligibility by reading the full text by LAB, AMN and HS. Finally, references of selected articles were searched for any other articles relevant to the studies.

Data extraction

Data extraction was conducted independently by two investigators (LAB and HS) and discordances were resolved by discussion and consensus. Extracted data included study design, population under study, number of transfused patients, number of alloimmunised patients, and alloantibody specificities (Table 1).

Quality assessment

The quality of each included study was independently assessed by two authors (LAB and AMN) discordances were resolved by discussion and consensus. The Standards for Reporting of Diagnostics accuracy (STARD) for diagnostic studies and criteria specific to prevalence studies was used [34]. The tool contained the following items: (1) definition of the outcome; (2) description of the selection criteria; (3) description of the study population; (4) type of study; (5) reference standard; (6) reported prevalence; and (7) reproducibility. The quality for each of the seven criteria was assessed as either 'yes' or 'no'. All 'yes' scores were summed up. Studies were graded as: A, high quality (score of 7), B, moderate quality (score 4 to 6) or C, low quality (score <4) (Supplementary Table 1).

Data synthesis and analysis

For each study, the frequency of RBC alloimmunised patients were calculated as the reported numbers of patients with alloantibodies divided by the total number of patients tested for alloantibodies. Data were analyzed using the metaprop command of the meta package (4.9–2) in R (version 3.5.1) (R Foundation for Statistical Computing, Vienna, Austria) [35]. Random-effects meta-analysis was performed, since variability in frequency estimates from different studies was expected. To minimize the influence from studies with extreme estimates on the overall estimate, proportions were transformed using the Freeman-Tukey double-arcsine transformation, which stabilized the variance of study-specific frequency and pooled within the DerSimonian and Laird using a random-effects model [36]. Heterogeneity in results across the studies was assessed using the I-squared (I^2) statistic, considering as high heterogeneity when I^2 was equal to or greater than 75% [37]. Meta-regression, using the metareg command was done to explore sources of heterogeneity and the univariable models were evaluated from the p-value for the test of moderators. A subgroup meta-analysis was performed based on significant variables. Publication bias was assessed with funnel plots of

study size against log [38]. A linear regression test was used to measure funnel plot asymmetry and a p value less than 0.05 was considered to be statistically significant. A leave-one-out sensitivity analysis was performed to assess if our results were driven by any single study.

Results

Search results

A total of 238 articles were identified in the initial search and 11 in the additional search. After removal of duplicates, 233 articles remained, and of these 16 met the inclusion criteria. Due to methodological flaws of one study [39], 15 studies were included in the review, thirteen were in the English and two in the French language (Figure 1) [40-54].

General study description

The 15 studies were published between 2005 and 2019. Except for the retrospective study by Boma Muteb and collegues[41], all others had a cross-sectional design, with regard to determination of alloimmunization frequency, and all were of moderate quality (i.e. score 4-6). The studies were conducted in nine SSA countries. Between two and four studies were conducted in each of four countries. (Table 2). These ten studies were conducted in eight medical centers from different parts of the four countries. However, two studies from Nigeria [53,54] and the two studies from Cote d'Ivoire [47,48] were from the same medical centers. Although the study periods differed by about four years, patient overlap cannot be excluded.

Prevalence of RBC alloimmunization

Pooling of the 15 studies, with a cumulative sample size of 1994 participants, yielded an overall prevalence of 7.4 (95% CI: 5.1-10.0) per 100 participants (Figure 2). The

heterogeneity between studies was moderate (I^2 = 71.7%; 95% CI, 52.3-83.7). Three studies [47,48,52] reported high prevalences of RBC alloimmunization (18.5-28.6%). Analysis revealed that these studies should be regarded as significant outliers. After excluding these three studies, the overall prevalence was 5.4 (95% CI: 4.2-6.8) per 100 participants and heterogeneity I^2= 24% (95% CI, 0.0-61.2; p=0.21).

Meta-regression subgroup analysis and sensitivity analysis

The meta-regression analysis identified sub-region (West, East and Central Africa) (p<0.01) as the most significant contributor to the heterogeneity between studies. Subgroup analysis, after stratifying studies by sub-regions, showed that there was no heterogeneity in the five studies conducted in East Africa (I^2 = 0%, p = 0.60) and a significant moderate heterogeneity in the eight studies from West Africa (I^2 = 70%, p = 0.01) (Supplementary Table 2). The leave out sensitivity analysis indicated that no single study influenced the overall outcome nor heterogeneity (Supplementary Table 3).

Publication bias

Visually, the funnel plot looked symmetric (Figure 3) and the p value for Egger's linear regression test was 0.86, indicating no evidence of publication bias.

Description of methodologies used in the studies

The sample size per study ranged from 27 to 428 transfused patients. Eight studies included only individuals with the SS genotype [40,41,43-46,53], five had a mixture of the SCD genotypes (i.e. SS, SC, Sβthal) [47-50,54] and the remaining two [42,52] did not report on the SCD genotype of participants. While 13 studies included children and adults, Abbas [43] studied only children and Ugwu [53] only adults. Nine studies used the column gel technique

(CGA) [40,42,46-50,52-53] for antibody testing, three, the tube technique (TT) [43,45,54], one used both TT and CGA [51] and two studies did not report the antibody testing technique [41,44]. All screening and identification reagents were procured from international sources except for the studies performed by Sekongo [47] and Kaliyu [51], which utilized screening and/or identification cells produced at their centre.

Transfusion History

All study participants had received ABO-D compatible, non-leucocyte reduced transfusions. Generally, reports on the transfusion history of study participants were scanty; indication for transfusion, type of blood products (whole blood or RBC concentrates) and source of blood for transfusion (from related or non-related donors) were seldom reported. Some studies did not report mean (with standard deviation) or median (with range) number of transfused units [41,44,50,53]. For the 11 studies that reported these variables, the number of RBC units transfused per patient per study was generally low (mean 2.4 to 9.0 units, median 2-10, and ranged from 1-100). The indication for transfusion was only reported by four studies and anemia was the main indication [41, 42, 48, 54].

Red blood cell antibody specificities

Of the 1994 transfused patients, 136 patients had a total of 206 RBC antibodies. Antibody specificities could not be determined in 15 patients. Of these, three were pan-reactive and identity could not be ascertained for 12. At least, (because not all specificities were known for all patients), 35 (26%) patients had multiple antibody specificities. While antibodies were directed to antigens from ten blood group systems, 49% had D, C, E and K specificity. Antibodies against low frequency antigens (defined as present in <5% of population; i.e. C^w, Go^a, K, Kp^a, M^g, Vw, Lu^a, Di^a and Wr^a) and high frequency antigens (defined as present in

>95% of population; i.e. c, e, k, Kp^b, Js^b and Lu^b) reported for (African-American) Blacks [55] represented 20% and 9% of all specificities, respectively (Table 2).

Discussion

This systematic review identified and analyzed 15 cross-sectional studies on RBC alloimmunization in transfused patients with SCD from nine different SSA countries. The pooled prevalence of RBC alloimmunization was 7.4 (95% CI: 5.1-10.0, I^2 = 71.7%) per 100 transfused patients. Overall, antibodies against E, D, C and K antigens accounted for almost half of antibody specificities and antibodies to low and high prevalence antigens were also common and together represented almost 30% of specificities. Heterogeneity between studies was moderate and meta-regression found region of Africa as the major contributor to this heterogeneity. Data on factors that may influence alloimmunization such as the transfusion history and demographic characteristics of study participants were inconsistently reported across studies.

As far as we know, this is the first systematic review and meta-analysis to provide an overview of the extent of alloimmunization in the transfused SCD population in SSA.

In a recent review, performed in transfused patients in SSA, of which 31% were patients with SCD, the pooled prevalence of alloimmunization was 6.7% (95% CI: 5.7-7.8) which is comparable to our prevalence of 7.4% [56]. In well-resourced countries, the prevalence of alloimmunization has shown to be higher in patients with SCD than the other transfused population, partly because of patients' specific inflammatory status and the likelihood of more RBC transfusions in their lifetime, associated with more allo-antigen exposure [13,23]. The latter may not hold true for patients with SCD from less well-resourced countries, because the majority of transfusions are reserved for severe life-threatening anemia in all patients, with and without SCD. In addition, because transfusion in SCD is common in well-

resourced countries, antibody tests are performed more frequently, increasing the likelihood of antibody detection.

In well-resourced countries, patients with SCD often receive transfusions for which RBC antigens are matched between patients and donors [57]. There have been proposals to apply this policy for patients with SCD in SSA, [43,47,51,53-54]. At present, this may not be feasible because 1) most transfusions are given in emergency situations, 2) the limited availability of donor blood in SSA and 3) the cost. However, since patients with SCD are likely to be multiply transfused a more pragmatic approach to increase safety would be to start with routine testing for RBC antibodies and indirect antiglobulin cross matching to select compatible units for transfusions. Patients who have already made antibodies could undergo additional matching, at least for Rh antigens, since these are the most immunogenic.

RBC alloantibody specificity

The observation that anti-E, -D, -C and -K represented almost 50% of antibody specificities in our review is comparable to alloimmunization rates reported among the general multi transfused patients in SSA [56] and in non-African countries, despite the frequent Rh and K antigen matched transfusions in the latter [58].

Even though matching for D antigen is routine in SSA, anti-D was present in our study in 22 (10.7%) patients. While most studies did not report on the event (for example, pregnancy or previous transfusion) that led to anti-D production or the D-status of patients with anti-D, Natukunda et al., found that most of their Ugandan patients with anti D had *RHD* variants [45]. *RHD* variants have been reported to be common in individuals of African descent [59]. Patients with *RHD* variants (such as DAU3, DIIIa and DIVa) that are D-positive with serology, will receive D positive blood and are at risk of making anti D [60], although the

actual risk in these patients is unknown. In daily practice, the presence of *RHD* variants are most often found when D+ patients made anti-D.

Twenty-one (15% of immunized) patients had anti K and 20 of these were from East-African studies. This may be explained by the variations in the frequency of the K-antigen in different parts in SSA and the associated risk for anti-K. Akasha found the K antigen in 5.6% of 500 random samples from a Sudanese population (East Africa) [61] whilst, studies in Nigeria and Cote d'Ivoire, which are both in West Africa, found lower frequencies, ranging from zero to 2% [62-66].

The remarkably high percentage of antibodies against low and high frequency antigens observed in this review suggests that antigens categorized as low and high frequency in the Blacks, also mainly based on African American population, may not be readily extrapolated to the Black Africans [49]. In contrast to antibodies against high frequency antigens, antibodies against low frequency antigens are unlikely to cause transfusion problems with regard to finding compatible donors. However, because low-frequency antigens are generally not present on antibody testing cells, many antibodies will remain undetected and if clinically relevant, could cause hemolytic transfusion reactions [67,68]. Therefore, establishing the frequency of RBC antigens in Black Africans is imperative since it may influence the selection of antigens for RBC antibody testing panels for Africa.

Overall, the variability in the prevalence and specificities of antibodies, highlights the need for more studies on blood group antigen diversity among different ethnic groups in SSA.

Then, establishing a database of antigen typed blood donors would assist in the local manufacture of reagent red cells for antibody testing which would aid in the detection of antibodies to African antigens [69]. A locally manufactured reagent red cells would also decrease the dependence on internationally prepared reagent red cells which are not generally affordable in resource-limited SSA, thereby, providing an appropriate, sustainable and

economical means of RBC antibody testing in patients with SCD and other multi-transfused patients.

Strengths and limitations of the review

The literature search was exhaustive with no database, publication year or language restrictions and was performed independently by two researchers. In addition, the review identified studies from a range of countries.

However, the findings of the studies should be interpreted in light of some limitations. First, 14 of 15 studies were cross sectional, which probably under reported the frequency. At the time of sampling, probably less than one third of antibodies might have been detected, because, some antibody titres might have fallen not have reached, or may have fallen below, detectable levels [70]. Most of the studies had incomplete descriptions of sampling methods so it was not possible to tell whether studies involved a biased, and therefore potentially unrepresentative, population. The paucity of data (i.e. pregnancy, transfusion history, type and source of blood, period between last transfusion and antibody testing, etc) in many studies prohibited the analyses for risk factors for alloimmunization.

Implication for transfusion practice and future research

Consideration should be given to the incorporation of RBC antibody testing into routine pre-transfusion screening procedures for patients with SCD and for blood banks to always perform complete (indirect antihuman globulin) cross-match prior to blood transfusion.

Authors speculate that inconsistencies in reporting of patient characteristics in the included studies may be due to inadequate documentation at the participating hospitals. We therefore suggest the need for healthcare facilities in SSA to improve documentation of patients' records to allow researchers to obtain accurate and up to date information on patients with

SCD. Major teaching hospitals in SSA could consider upgrading from manual entry of patients' records to the use of a medical computerized program that electronically captures and manages patients' records. This would not only help promote research in SSA but improve patient management.

Studies are needed which report information on parameters that have been shown to influence alloimmunization risk such as sex, age of patients, number of transfusion events before alloimmunization occurred, history of pregnancy, age at first transfusion and age of RBCs transfused.

The RBC antigen profile of blood donors and patients with SCD across different ethnic groups in SSA should be established. Then, studies to explore the feasibility of incorporating RBC antibody screening and identification tests using locally prepared reagent red cells (expressing 'African antigens') would be essential to identify the potential challenges to the incorporation of RBC antibody testing in SSA. Testing for RBC antibodies, preferably at fixed time points after transfusion, would aid in establishing the incidence and specificity of alloimmunisation in SSA more precisely.

Strengthening of haemovigilance systems in SSA are important, to accurately document the incidence of post transfusion complications including acute and delayed hemolytic transfusion reactions [12,71]. This would help quantify the extent of the clinical consequences of alloimmunization in patients with SCD.

Conclusion

Findings of this review provide evidence that patients with SCD receiving blood transfusion in SSA are burdened with the development of RBC alloantibodies, although lower compared to those in well-resourced countries, where transfusions are given more frequently. However, in resource-limited settings such as SSA, implementation of RBC antigen matching for all

patients would be difficult. A practical approach would be to screen patients with SCD for RBC antibodies and perform a complete crossmatch prior to RBC transfusion to aid in the detection of potentially dangerous antibodies. Alloimmunised patients should be given blood that is antigen negative, at least for the antibodies they possess. Given that most transfusions are for acute situations, a database of antigen typed donors would be essential, for timely selection of compatible units.

Competing interests

The authors have no competing interests.

All authors critically revised the manuscript for important intellectual content and gave final approval of the version to be published.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] Piel FB, Patil AP, Howes RE, Nyangiri OA, Gething PW, Williams TN, et al. Global distribution of the sickle cell gene and geographical confirmation of the malaria hypothesis. Nat Commun 2010; 1:104. doi: 10.1038/ncomms1104

[2] Grosse SD, Odame I, Atrash HK, Amendah DD, Piel FB, Williams TN. Sickle cell disease in Africa: a neglected cause of early childhood mortality. Am J Prev Med 2011; 41(6):S398-S405. doi: 10.1016/j.amepre.2011.09.013

[3] Agasa B, Bosunga K, Opara A, Tshilumba K, Dupont E, Vertongen F, et al. Prevalence of sickle cell disease in a northeastern region of the Democratic Republic of Congo: what impact on transfusion policy? Transfus Med 2010;20(1):62-5. doi: 10.1111/j.1365-3148.2009.00943.

[4] Ama V, Kengne AP, Nansseu NJ, Nouthe B, Sobngwi E. Would sickle cell trait influence the metabolic control in sub-Saharan individuals with type 2 diabetes? Diabet Med 2012; 29(9):e334-7. doi: 10.1111/j.1464-5491.2012.03620.x

[5] Delicat-Loembet LM, Elguero E, Arnathau C, Durand P, Ollomo B, Ossari S, et al. Prevalence of the sickle cell trait in Gabon: a nationwide study. Infect Genet Evol 2014; 25:52-6. doi: 10.1016/j.meegid.2014.04.003.

[6] McGann PT. (2016) Time to invest in sickle cell anemia as a global health priority. Pediatrics 2016; 137(6): e20160348. Doi: 10.1542/peds.2016-0348.

[7] Wang WC, Morales KH, Scher CD, Styles L, Olivieri N, Adams R, et al. Effect of long-term transfusion on growth in children with sickle cell anemia: results of the STOP trial. J Pediatr 2005; 147(2):244-7. doi: https://doi.org/10.1016/j.jpeds.2005.02.030

[8] Lee MT, Piomelli S, Granger S, Miller ST, Harkness S, Brambilla DJ, et al. Stroke Prevention Trial in Sickle Cell Anemia (STOP): extended follow-up and final results. Blood 2006; 108(3):847-52. doi: https://doi.org/10.1182/blood

[9] Wahl S, Quirolo KC. Current issues in blood transfusion for sickle cell disease. Curr Opin Pediatr 2009; 21(1):15-21. doi: 10.1097/MOP.0b013e328321882e.

[10] WHO 2017 blood safety and availability https://www.who.int/news-room/fact-sheets/detail/blood-safety-and-availability accessed on 16th December, 2018

[11] Bloch EM, Vermeulen M, Murphy E. Blood transfusion safety in Africa: a literature review of infectious disease and organizational challenges. Transfus Med Rev 2012; 26(2):164-80. doi: 10.1016/j.tmrv.2011.07.006

[12] Weimer A, Tagny C, Tapko J, Gouws C, Tobian A, Ness P et al. Blood transfusion safety in sub-Saharan Africa: A literature review of changes and challenges in the 21st century. Transfusion 2019; 59(1):412-27. doi: 10.1111/trf.14949

[13] Olujohungbe A, Hambleton I, Stephens L, Serjeant B, Serjeant G. Red cell antibodies in patients with homozygous sickle cell disease: a comparison of patients in Jamaica and the United Kingdom. Br J Haematol 2001; 113(3): 661-5.

[14] Aygun B, Padmanabhan S, Paley C, Chandrasekaran V. Clinical significance of RBC alloantibodies and autoantibodies in sickle cell patients who received transfusions. Transfusion 2002; 42(1):37-43.

[15] Sakhalkar V, Roberts K, Hawthorne L, McCaskill D, Veillon D, Caldito G, et al. Allosensitization in patients receiving multiple blood transfusions. Ann N Y Acad Sci 2005; 1054(1):495-9.

[16] Lasalle-Williams M, Nuss R, Le T, Cole L, Hassell K, Murphy JR, et al. Extended red blood cell antigen matching for transfusions in sickle cell disease: a review of a 14-year experience from a single center (CME). Transfusion 2011; 51(8):1732-9. doi: 10.1111/j.1537-2995.2010.03045

[17] Sins JWR, van den Bersselaar SM, Heijboer H, von Ronnen FB, Fijnvandraat K, Biemond BJ, et al. Early occurrence of red blood cell alloimmunization in patients with sickle cell disease. Am J Hematol 2016; 91(8):763-9. doi: 10.1002/ajh.24397

[18] Murao M, Viana MB. Risk factors for alloimmunization by patients with sickle cell disease. Braz J Med Biol Res 2005, 38(5):675-82. doi.org/10.1590/S0100-879X2005000500004

[19] 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. doi: 10.1111/trf.13037

[20] Desai PC, Deal AM, Qaqish B, Pfaff ER, Hebden LM, Park YA et al. Alloimmunization is associated with older age of transfused red blood cells in sickle cell disease. Am J Hematol 2015; 90(8):691-5. doi: 10.1002/ajh.24051.

[21] Oliveira VB, Dezan MR, Gomes FCA, Levi JE, Mendrone-Junior A, Dinardo CL, et al. -318C/T polymorphism of the CTLA-4 gene is an independent risk factor for RBC alloimmunization among sickle cell disease patients. Int J Immunogenet 2017; 44(5):219-24. doi: 10.1111/iji.12334

[22] Sippert EÂ, Gilli SCO, Addas-Carvalho M, Saad STO, Costa FF, Castilho L, et al. Red blood cell alloimmunization in patients with sickle cell disease: correlation with HLA and cytokine gene polymorphisms. Transfusion 2017; 57(2):379-89. doi: 10.1111/trf.13920

[23] Fasano RM, Miles M, Luban NLC, Meier ER, Booth GS, Du L, et al. Red blood cell alloimmunization is influenced by recipient inflammatory state at time of transfusion in patients with sickle cell disease. Br J Haematol 2015; 168(2):291-300. doi: 10.1111/bjh.13123.

[24] Garratty, G. (2004) Autoantibodies induced by blood transfusion. Transfusion 2004; 44(1): 59.

[25] Win N. Hyperhaemolysis syndrome in sickle cell disease. Expert Rev. Hematol 2009; 2(2):111-5. doi: 10.1586/ehm.09.2.

[26] Banks M, Shikle J. Hyperhaemolysis Syndrome in Patients with Sickle Cell Disease. Arch Pathol Lab Med 2018; 142(11):1425-7. doi: 10.5858/arpa.2017-0251-RS.

[27] Garratty G. Severe reactions associated with transfusion of patients with sickle cell disease. Transfusion 1997; 37(4):357-61.

[28] Scheunemann LP, Ataga KI. Delayed hemolytic transfusion reaction in sickle cell disease. Am J Med Sci 2010; 339(3):266. doi: 10.1097/MAJ.0b013e3181c70e14

[29] de Montalembert M, Dumont MD, Heilbronner C, Brousse V, Charrara O, Pellegrino B, Piet al. Delayed hemolytic transfusion reaction in children with sickle cell disease. Haematologica 2011; 96(6):801-7. doi: 10.3324/haematol.2010.038307

[30] Friedman D, Kim H, Manno C. Hyperhemolysis associated with red-cell transfusion in sickle-cell disease. Transfusion 1993; 33S:14

[31] 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):684-90

[32] Yazer MH, Jackson B, Beckman N, Chesneau S, Bowler P, Delaney, et al. Changes in blood center red blood cell distributions in the era of patient blood management: the trends for collection (TFC) study. Transfusion 2016; 56(8):1965-73. doi: 10.1111/trf.13696

[33] Tormey CA, Hendrickson JE. Transfusion-related red blood cell alloantibodies: induction and consequences. Blood 2019; doi: 10.1182/blood-2018-08-833962. [Epub ahead of print]

[34] Hlela C, Shepperd S, Khumalo NP, Taylor GP. The Prevalence of Human T-Cell Lymphotropic Virus Type 1 in the General Population is Unknown. AIDS Rev 2009; 11(4):205-14.

[35] Schwarzer G. Meta: Meta-Analysis with R. Available: http://cran.r-project.org/web/packages/meta/

[36] Fleiss JL. The statistical basis of meta-analysis. Stat Methods Med Res1993; 2:121–45

[37] Higgins J, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002; 21:1539–58

[38] Hunter JP, Saratzis A, Sutton AJ, Boucher RH, Sayers RD, Bown MJ. In meta-analyses of proportion studies, funnel plots were found to be an inaccurate method of assessing publication bias. J Clin Epidemiol 2014; 67(8):897–903. doi: 10.1016/j.jclinepi.2014.03.003.

[39] Akre DP, Seka-Seka J, Dasse SRKple-Faget P, Hien S, N'Guessan K, et al. alloimmunisation anti´erythrocytaire post transfusionnelle chez les dr´epanocytaires au CHU de Cocody Abidjan. Int J Pharma Bio Sci 2008; 9(2):64–70

[40] Batina Agasa S, Dupont E, Kayembe T, Molima P, Malengela R. Kabemba S, et al. Multiple transfusions for sickle cell disease in the Democratic Republic of Congo: the importance of the hepatitis C virus. Transfus Clin Biol 2010; 17(4):254-9.

[41] Boma Muteb P, Kaliula Mamba JFJ, Muhai Pfutila P, BiloV, Panda Mulefu JD, Diallo DA. Effectiveness, safety, and cost of partial exchange transfusions in patients with sickle-cell anemia at a sickle cell disease center in sub-Saharan Africa. Med Sante Trop 2017; 1:27(4):387-91. doi: 10.1684/mst.2017.0719. doi: 10.1684/mst.2017.0719

[42] Mangare C, Mbugua A, Maturi P, Rajab J, Blasczyk R, Heuft HG. Red cell allo- and autoimmunisation in transfused sickle cell and cancer patients in Kenyatta National Hospital, Nairobi, Kenya. Afr J Lab Med 2015; 4:297. doi:10.4102/ajlm.v4i1.297

[43] Abbas M, Bolad A, Jiefri N, Mergani A. Red Blood Cell Alloimmunization among Sudanese Homozygous Sickle Cell Disease Patients. Am J Med Med Sci 2013; 3(4):61-7. doi: 10.5923/j.ajmms.20130304.02

[44] Eldour AAA, Ismail ME, Osman T, Younis KMS, Babker AMAAA. Red cell alloimmunization in blood transfusion dependent Patients with Sickle Cell Disease in El-Obied city, Sudan. IOSR-JDMS 2016; 14(12):137-41. doi: 10.9790/0853-14124137141

[45] Meda E, Magesa PM, Marlow T, Reid C, Roberts DJ, Makani J. Red Blood Cell Alloimmunization in Sickle Cell Disease Patients in Tanzania. East Afr J Public Health 2014; 11(2):775-80.

[46] Natukunda B, Schonewille H, Ndugwa C, Brand A. Red blood cell alloimmunization in sickle cell disease patients in Uganda. Transfusion 2010; 50(1):20-5. doi: 10.1111/j.1537-2995.2009.02435.x

[47] Sekongo YMV, Kouacou AP, Kouamenan GS, Kassogue KL, N'Guessan P, Danho NC, et al. Anti-Erythrocyte Allo-Immunization to Sickle Cell Disease Patients Followed in Transfusion Therapy Unit of the National Blood Transfusion Center of Abidjan Côte D'Ivoire'. Int J Immunol 2017; 5(1):1-4. doi: 10.11648/j.iji.20170501.11

[48] Siransy LK, Romualde DS, Yeboah R, Kouacou PA, Adou H, Hien S. (2018) Immune Status and Red Cell alloimmunization among SCD Patients in Côte d'Ivoire. J Clin Exp Immunol 2018; 3(1):1-6.

[49] Boateng, LA, Campbell AD, Davenport RD, Osei-Akoto A, Hugan S, Asamoah A, Schonewille, H. Red blood cell alloimmunization and minor red blood cell antigen phenotypes in transfused Ghanaian patients with sickle cell disease. Transfusion doi: 10.1111/trf.15197 Epub ahead of print

[50] Diarra AB, Guindo A, Kouriba B, Dorie A, Diabaté DT, Diawara SI, et al. Sécurité transfusionnelle et drépanocytose à Bamako, Mali. Séroprévalence des infections à VIH, VHB, VHC et allo-immunisation anti-Rh et Kell chez les drépanocytaires. Transfus Clin Biol 2013; 20(5/6):476-81.

[51] Kuliya-Gwarzo A, Akanmu AS, Dutse AI. Prevalence of red cell alloantibodies in multi-transfused patients with sickle cell anemia in Nigeria. Africa Sanguin 2005; 8(1):1-4.

[52] Kangiwa U, Ibegbulam O, Ocheni S, Madu A, Mohammed N. Pattern and prevalence of alloimmunization in multiply transfused patients with sickle cell disease in Nigeria. Biomark Res 2015; 3:26. DOI 10.1186/s40364-015-0050-3. doi: 10.1186/s40364-015-0050-3

[53] Ugwu NI, Awodu OA, Bazuaye GN, Okoye AE. Red cell alloimmunization in multi-transfused patients with sickle cell anemia in Benin City, Nigeria. Niger J Clin Pract 2015; 18(4):522-6. doi: 10.4103/1119-3077.154204

[54] Adewoyin S. Erythrocyte Transfusion and Alloimmunization Patterns among Sickle Cell Disease Patients, Benin City, Nigeria. Br J Med Med Res 2016; 11(10):1-8. doi: 10.9734/BJMMR/2016/21755

[55] Reid ME, Lomas-Francis CL, Olsson M. The blood group antigen factsbook. 3rd ed. London: Elsevier Academic Press, 2012

[56] Ngoma AM, Mutombo PB, Ikeda K, Nollet KE, Natukunda B, Ohto H. Red blood cell alloimmunization in transfused patients in sub-Saharan Africa: A systematic review and meta-analysis. Transfus Apher Sci 2016; 54(2):296-302.

[57] Fasano RM, Meyer EK. Branscomb J, White MS, Gibson RW, Eckman JR. Impact of red blood cell antigen matching on alloimmunization and transfusion complications in patients with sickle cell disease: a systematic review. Transfus Med Rev 2019; 33(1):12-23. doi: 10.1016/j.tmrv.2018.07.003.

[58] da Cunha Gomes EG, Machado LAF, de Oliveira LC, Neto JFN. (2018) The erythrocyte alloimmunization in patients with sickle cell anemia: a systematic review. Transfus Med 2018; doi:10.1111/time.12543. [Epub ahead of print]

[59] Noizat-Pirenne F, Tournamille C. Relevance of RH variants in transfusion of sickle cell patients. Transfus Clin Biol J Société Fr Transfus Sang 2011; 18:527–35. doi: 10.1016/j.tracli.2011.09.001

[60] Chou ST, Jackson T, Smith-Whitley K, Friedman DF, Vege S, Westhoff CM. High prevalence of red blood cell alloimmunization in sickle cell disease despite transfusion from Rh-matched minority donors. Blood 2013;122(6):1062-1071. doi: 10.1182/blood-2013-03-490623.

[61] Akasha AS. (2012) The frequency of kell red cell antigens (K, k) among the major Sudanese tribes. Recent Res Sci Technol 2012; 4(1):44-45.

[62] Adewoyin A, Lee G, Adeyemo T, Awodu O. Rh and Kell blood group antigen prevalence in a multi-ethnic cohort in Nigeria: implications for local transfusion service. Immunohematology 2018; 34(2):61-65.

[63] Ugboma, HA, Nwauche CA. Kell blood group antigen in Port Harcourt, Nigeria: a pilot study. Port Harcourt Med J 2009;4.

[64] Kulkarni AG, Ibazebo RO, Dunn DT, Fleming AF. Some red cell antigens in the Hausa population of northern Nigeria. Hum Hered 1985; 35:283–7

[65] Osaro E, Ladan MA, Zama I, Ahmed Y, Mairo H. Distribution of Kell phenotype among pregnant women in Sokoto, North Western Nigeria. Pan Afr Med J 2015; 21:301.

[66] Siransy Bogui L, Dembele B, Sekongo Y, Abisse, Konaté S, Sombo M. Phenotypic profile of Rh and Kell blood group systems among blood donors in Cote d'Ivoire, West Africa. J Blood Transfus 2014; 1-4 http://dx.doi.org/10.1155/2014/309817

[67] Daneils G. Low frequency antigens in Human Blood Groups, 3rd Edition, Wiley-Blackwell 2013; 495-499

[68] Larson PJ, Lukas MB, Friedman DF, Manno CS. Delayed hemolytic transfusion reaction due to anti-Goa, an antibody against the low-prevalence Gonzales antigen. Am J Hematol 1996; 53(4):248-250

[69] Mattos LCD. Molecular polymorphisms of human blood groups: a universe to unravel. Rev Bras Hematol Hemoter 2011; 33(1):6-7. doi: 10.5581/1516-8484.20110005

[70] Stack G, Tormey C. Detection rate of blood group alloimmunization based on real-world testing practices and kinetics of antibody induction and evanescence. Transfusion 2016; 56:2662-67. doi: 10.1111/trf.13704

[71] Barro L, Drew VJ, Poda GG, Tagny CT, El-Ekiaby M, Owusu-Ofori S, et al. Blood transfusion in sub-Saharan Africa: understanding the missing gap and responding to present and future challenges. Vox Sang 2018; 113:726-36 doi: 10.1111/vox.12705.

Figure 1: Summary of data extraction history

Figure 2: Forest plot of proportion estimates of RBC alloimmunisation in transfused patients with sickle cell disease, ranked from low to high number of total patients per study

Figure 3: Funnel plot of sample size against log odds

Table 1: Characteristics and outcomes of the 15 eligible cross-sectional studies from sub-Sahara Africa on red blood cell alloimmunization in transfused patients with sickle cell disease.

First author (year of publication)[r]	Country (region)	N of Patients/ Transfuse	Male to femal	Patient age (SD;	RBCs transfused	Antibody test method	N of Patients with allo-	N of allo-antibodies :
Batina Agasa (2010)[40]	Congo (Central	144 / 127	1.26†	Mean 15.5	Mean 5.3 (6.6; 1-	CGA-LISS	13 (10.2;	17: 3D, 3C, 2E,
Boma Muteb (2017)[41]	Congo (Central	39 / 39	0.77	Mean 8.6	82% >2 units	NR	1 (2.6; 0.1-	1: 1K
Mangare (2015)[42]	Kenya (East)	137 / 137	0.99	Mean 8	Mean 2.4 (NR; 1-8)	CGA-NaCl/LISS	4 (2.9; 0.9-	4: 1C^{w}, 1S, 1M, 1Co^{b}
Abbas (2013)[43]	Sudan (East)	100 / 100	1.33	Median 2-8	Mean 6.7; Median 5	TT NaCl/LISS	4 (4.0; 1.3-	4: 2K, 1C, 1E
Eldour (2015)[44]	Sudan (East)	210 / 210	1.44	Median 2-5	≥2 units (NR; NR)	NR	9 (4.3; 2.1-	10: 5K, 2E, 1c,
Meda (2014)[45]	Tanzania (East)	365 / 365	0.81	Median 16 (NR; 0.5-	Mean 3.2; Median 2 (NR; 1- 40)	TT-NISS	15 (4.1; 2.4- 6.8)	63: 12K, 9Le^{a}, 5Co^{b}, 4Fy^{b}, 4Kp^{a}, 3D,
Natukunda (2010)[46]	Uganda (East)	428 / 428	1.04	Median 12	Median 3 (NR; 2-	CGA-LISS	26 (6.1; 4.1-	32: 10E, 7D, 4S,
Sekongo (2017)[47]	Cote d'Ivoire	42 / 42	1.00	Mean 24.5	Mean 9, Median 6-	CGA-LISS	12 (28.6; 16-	14: 6E, 4C, 1D, 1e, 1S,
Siransy (2018)[48]	Cote d'Ivoire (West)	31 / 27	0.82†	Mean 25.8 (NR; 7-55)	Mean 5.7§ (NR; 1- >10)	CGA (E-NR)	5 (18.5; 7.0-39)	3: 1C, 1E, 1Le^{a} (Specificity was determined in two patients)

Boateng (2019)[49]	Ghana (West)	154 / 154	1.30	Median 9	Median 2-4 (NR; 1->10)	CGA-LISS	10 (6.5;	13: 3D, 3M, 2E, 2C, 1e, 1C^{w}, 1UI
Diarra (2013)[50]	Mali (West)	133 / 90	0.73†	Mean 21†	NR	CGA (E-NR)	4 (4.4;	4: 2C, 1D, 1c
Kuliya-Gwarzo (2005)[51]	Nigeria (West)	135 / 68	1.52	Mean 14 (6.5;	Median 1-5 (NR; 1-10)	TT-Alb and CGA	6 (8.8; 3.6-	11: 2D, 2E, 1Kp^{b}, 1Js^{b}, 1Wr^{a}, 1M^{g}, 1Vw, 1Di^{a}, 1Go^{a}
Kangiwa (2015)[52]	Nigeria (West)	120 / 80	0.78	Median 21†	Mean 3 (NR; 2-25)	CGA-LISS	15 (18.8;	13: 2E, 2c, 2e, 1D, 1Fy^{a}, 1Fy^{b}, 1k, 1Kp^{b},
Ugwu (2015)[53]	Nigeria (West)	86 / 86	1.10	Mean 26	≥2 units (NR; 2-NR)	CGA-LISS	8 (9.3;	11: 3E, 2C, 1D, 1e, 1k, 1Kp^{a}, 1Js^{b}, 1Lu^{b}
Adewoyin (2016)[54]	Nigeria (West)	55 / 41	1.39†	Mean 23†	Mean 4.5; Median 2	TT (E-NR)	4 (9.8;	6: 2C, 2E, 1k, 1Le^{a}

N, number; SD, standard deviation; CI, confidence interval; CGA, column gel agglutination; LISS, low ionic strength solution; TT, tube technique; NISS, normal ionic strength solution; NR, not reported; E-NR, enhancer not reported; Alb, Albumin; UI, antibodies whose specificity could not be identified; PAN, pan-reactive antibody; †Including the non-transfused patients because data for transfused patients were not reported separately; ‡Kidd specificity was not determined; §Mean number of transfusions per year.

Table 2: Specificities of the 206 red blood cell antibodies in transfused patients with sickle cell disease in sub-Sahara Africa.

Blood group system (N, %)†	RBC antigen	N (%)	Blood group system (N, %)	RBC antigen	N (%)
Rhesus (95; 47.0)	E	36 (17.5)	Lewis (15; 7.4)	Le^a	12 (5.8)
	D	22 (10.7)		Le^b	3 (1.5)
	C	21 (10.2)	Duffy (7; 3.5)	Fy^b	5 (2.4)
	C^w	6 (2.9)		Fy^a	2 (1.0)
	e	5 (2.4)	Kidd (6; 3.0)	Jk^a	3 (1.5)
	c	4 (1.9)		Jk^b	2 (1.0)
	Go^a	1 (0.5)		Kidd‡	1 (0.5)
Kell (31; 15.3)	K	21 (10.2)	Colton (6; 3.0)	Co^b	6 (2.9)
	Kp^a	5 (2.4)	Lutheran (5; 2.5)	Lu^a	4 (1.9)
	k	3 (1.5)		Lu^b	1 (0.5)
	Js^b	3 (1.5)	Globoside (2; 1.0)	P	2 (1.0)
	Kp^b	2 (1.0)	Wright (1; 0.5)	Wr^a	1 (0.5)
MNSs (19; 9.4)	S	8 (3.9)	Diego (1: 0.5)	Di^a	1 (0.5)
	M	6 (2.9)			
	s	2 (1.0)	Unidentified (IUI)		12 (5.8)
	N	1 (0.5)	Pan-reactive (PAN)		3 (1.5)
	M^g	1 (0.5)			
	Vw	1 (0.5)			

†N, total number of antibodies against antigens from the blood group system; %, with total number of antibodies (n=206) as denominator.

‡The Kidd antibody specificity was not reported.

Highlights

1. The pooled proportion of alloimmunisation in SCD in SSA is 7.4 (95% CI: 5.1–10.0)
2. Almost 50% of antibody specificities were against D, C, E and K antigens
3. Antibodies to low and high frequency antigens accounted for 29% of antibodies
4. Studies did not consistently report factors that influence alloimmunisation
5. SCD patients in SSA should be screened for RBC antibodies and IAT crossmatched

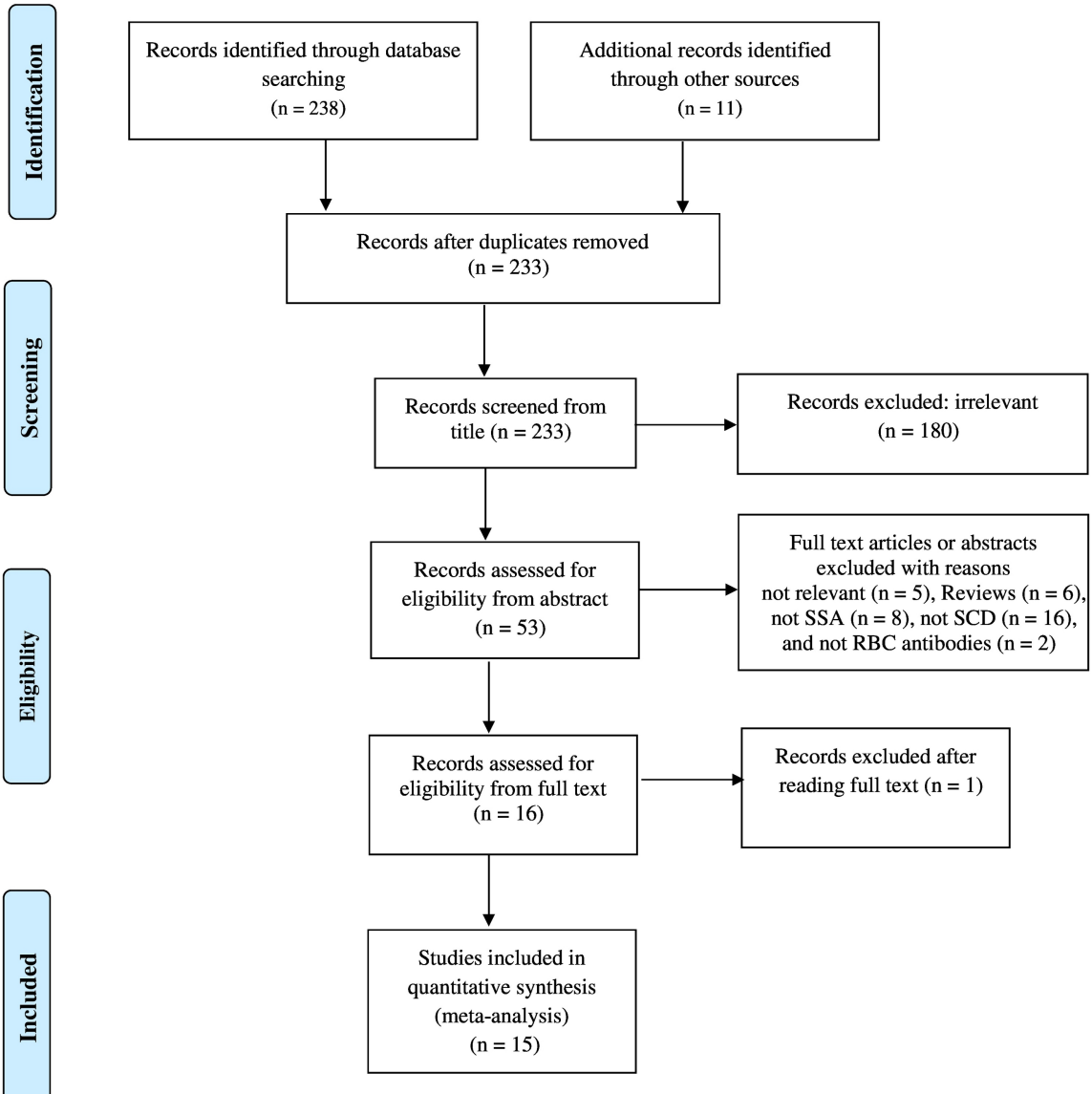
Identification
Screening
Eligibility
Included
Records identified through database searching (n = 238)
Additional records identified through other sources (n = 11)
Records after duplicates removed (n = 233)
Records screened from title (n = 233)
Records excluded: irrelevant (n = 180)
Records assessed for eligibility from abstract (n = 53)
Full text articles or abstracts excluded with reasons not relevant (n = 5), Reviews (n = 6), not SSA (n = 8), not SCD (n = 16), and not RBC antibodies (n = 2)
Records assessed for eligibility from full text (n = 16)
Records excluded after reading full text (n = 1)
Studies included in quantitative synthesis (meta-analysis) (n = 15)

Figure 1

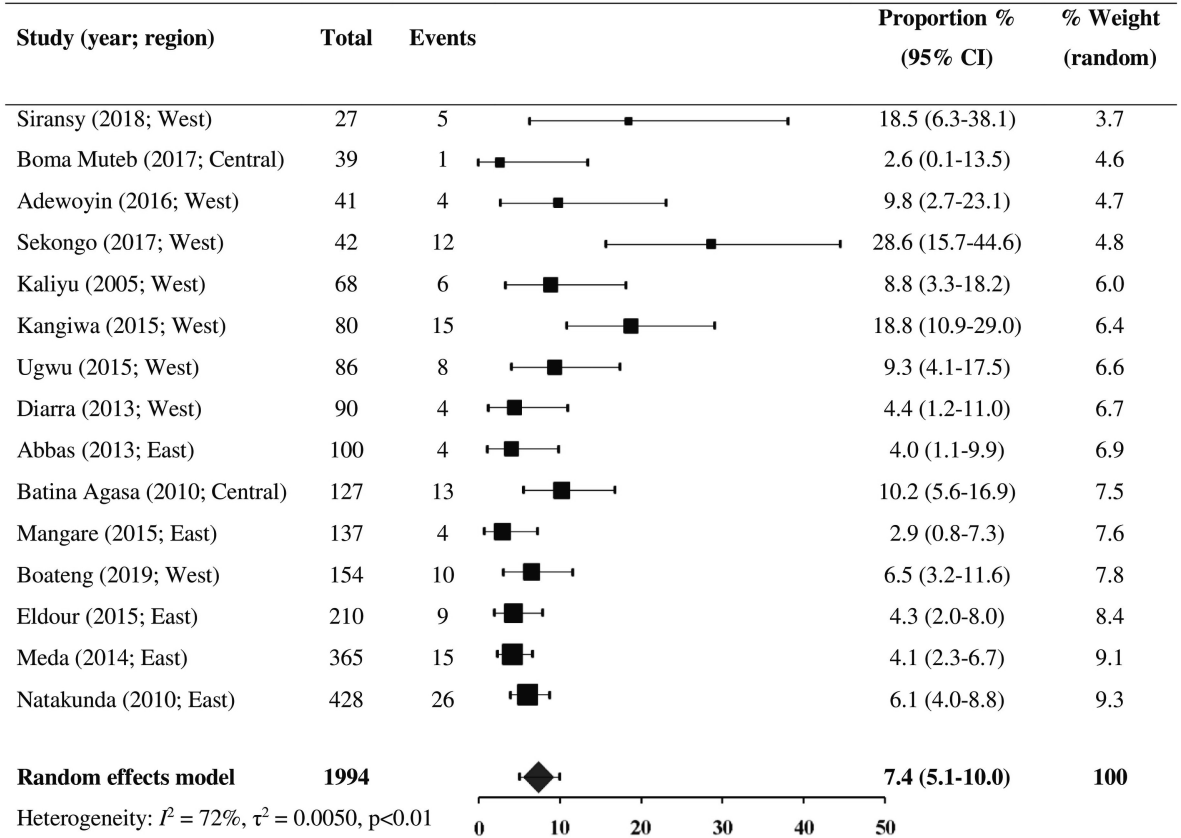
Study (year; region)
Total
Events
Proportion % (95% CI)
% Weight (random)
Siransy (2018; West) 27 5 18.5 (6.3-38.1) 3.7
Boma Muteb (2017; Central) 39 1 2.6 (0.1-13.5) 4.6
Adewoyin (2016; West) 41 4 9.8 (2.7-23.1) 4.7
Sekongo (2017; West) 42 12 28.6 (15.7-44.6) 4.8
Kaliyu (2005; West) 68 6 8.8 (3.3-18.2) 6.0
Kangiwa (2015; West) 80 15 18.8 (10.9-29.0) 6.4
Ugwu (2015; West) 86 8 9.3 (4.1-17.5) 6.6
Diarra (2013; West) 90 4 4.4 (1.2-11.0) 6.7
Abbas (2013; East) 100 4 4.0 (1.1-9.9) 6.9
Batina Agasa (2010; Central) 127 13 10.2 (5.6-16.9) 7.5
Mangare (2015; East) 137 4 2.9 (0.8-7.3) 7.6
Boateng (2019; West) 154 10 6.5 (3.2-11.6) 7.8
Eldour (2015; East) 210 9 4.3 (2.0-8.0) 8.4
Meda (2014; East) 365 15 4.1 (2.3-6.7) 9.1
Natakunda (2010; East) 428 26 6.1 (4.0-8.8) 9.3
Random effects model 1994 7.4 (5.1-10.0) 100
Heterogeneity: I^2 = 72%, τ^2 = 0.0050, p<0.01
0
10
20
30
40
50

Figure 2

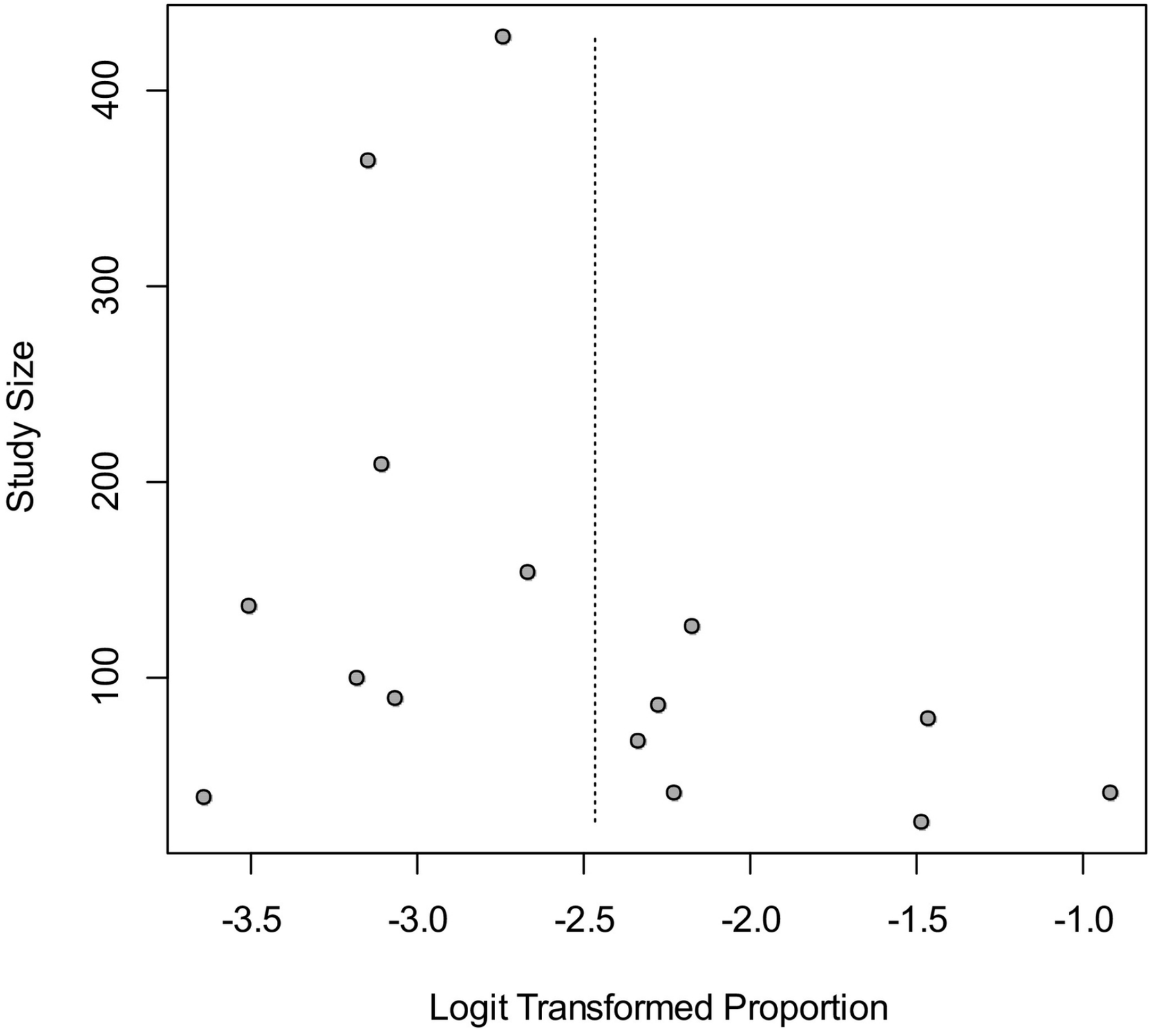
Study Size
100
200
300
400
-3.5
-3.0
-2.5
-2.0
-1.5
-1.0
Logit Transformed Proportion

Figure 3