UNIVERSITY OF LIVERPOOL

Investigation of post-transfusion red blood cell alloimmunisation in patients with sickle cell disease in Ghana

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Philosophy

By

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Declaration

This work has not been submitted for any other degree or award at this or any other university or place of learning, nor is being submitted concurrently in candidature for any degree or other award. This thesis is being submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy. This thesis is the result of my own independent work/investigation, except where otherwise stated. Other sources are acknowledged by explicit references. The views expressed are my own. I hereby give consent for my thesis, if accepted, to be available for photocopying and for interlibrary loan, and for the title and summary to be made available to outside organisations.

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Dedication

I dedicate this piece of work to the memory of my late father, Mr. James Kwabena Duku, who saw greatness in me at a very young age and desired to train me to any level I aspired to reach despite the challenges to girl child education in Africa. Daddy, your advice, encouragement and sacrifices to see me go higher is still appreciated.

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List of Abbreviations

SCD	Sickle Cell disease
RBC	Red Blood Cell
SSA	Sub Saharan Africa
AHTR	Acute Haemolytic Transfusion Reaction
HIV	Human ImmunodeficiencyVirus
HTLV	Human T-cell Leukaemia Virus
WBC	White Blood Cell
TRALI	Transfuison Related Acute Lung Injury
EDTA	Ethylenediaminetetraacetic acid
HIC	High Income Countries
LMIC	Low and Middle Income Countries
ISBT	International Society of Blood Transfusion
HLA	Human Leucocyte Antigen
MDS	Myelo Dysplastic Syndrome
USA	United States of America
DHTR	Delayed Haemolytic Transfusion Reaction
HS	Hyperhaemolysis Syndrome
DNA	Deoxyribo Nucleic Acid
AQP1	Aqua Phorin 1
SNP	Single Nucleotide Polymorphism
HPA	Human Platelet Antigen
DAT	Direct Antiglobulin Test
APC	Antigen Presenting Cells
TCR	T-like Cell Receptor

MHC	Major Histocompatibility Complex
CD4	Cluster of Differentiation 4
IL	Interleukin
TH	T Helper
IgM	Immunoglobulin M
IgG	Immunoglobulin G
HgA	Haemoglobin A
HbC	Haemoglobin C
HbE	Haemoglobin E
HbS	Haemoglobin S
SCA	Sickle Cell Anaemia
SCT	Sickle Cell Trait
TTI	Transfusion Transmitted Infections
FRD	Family Replacement Donation
VNRD	Voluntary Non-Remunerated Donation
TNFA	Tumour Necrosis Factor A
ILIB	Interleukin 1ß
HDFN	Haemolytic Disease of the Foetus and Newborn
NHSBT	National Health Service Blood and Transplant

Definition of terms:

1. Patients with sickle cell disease (SCD): this term refers to people (children and adults) with the sickle cell disease. For the purposes of this study, Patients with sickle cell disease included those with the Hb SS, Hb SC, Hb SD, Hb $S\beta^{+}$ thalassaemia and Hb $S\beta^{0}$ thalassaemia. They will be called patients with SCD; however, we appreciate that they are not always sick and in hospital.

2. Healthy blood donors are defined as blood donors who are aged 18–65 years; weigh more than 50 kg; have a minimum haemoglobin of 120 g/l; have normal blood pressure (systolic 120–129 mmHg, diastolic 80–89 mmHg) and a pulse rate of 60–100 beats per minute (World Health Organisation, 2012) .

3. Voluntary non-remunerated donors (VNRD) are defined as donors who donate blood by their own free will and without receiving in return any payment in cash or in kind that could be considered a substitute for money (World Health Organisation, 2012; European Blood Alliance, 2016)

4. Family replacement donors (FRD) are defined as donors who donate blood only in response to need by a patient who is known them (Allain *et al.*, 2010)

5. European antigens for the purpose of this study are defined as antigens that are more common in Caucasians.

6. African antigens for the purpose of this study are defined as antigens that are more common in Africans or exclusive to Africans.

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Abstract

Blood transfusion in patients with sickle cell disease (SCD) is associated with the development of antibodies against the foreign transfused red blood cell (RBC) antigens, i.e. RBC alloimmunisation. Very few studies have reported on RBC alloimmunisation in patients with SCD in sub-Saharan Africa (SSA), where antibody testing and RBC matching beyond blood groups ABO and Rh D are not done in most centres. Even when antibody testing is done, it is performed with standard reagent test cells mostly from donors of Caucasian descent that lack antigens that are more prevalent or exclusively present in Africans. This may miss antibodies to the antigens that are predominantly present in Africans, putting patients at risk of haemolytic transfusion reactions upon subsequent transfusions with antigens to which patients have made antibodies.

The overall goal of this project was to investigate post transfusion RBC alloimmunisation and adverse transfusion events in patients with SCD in Ghana and to explore ways of optimising transfusion practices to detect alloimmunisation to clinically relevant RBC antigens and, thereby, prevent transfusion reactions.

To achieve this:

1. a systematic review of published literature and a meta analysis were performed to determine the estimated frequency of RBC alloimmunisation in SSA.
2. A cross-sectional study was performed and this was in two parts:
   - first, to determine the prevalence, specificities, and risk factors for RBC alloantibodies against a standard Caucasian antigen panel and eight selected antigens predominantly present in Africans (frequency 0.5% to 32%) and adverse transfusion events assessed by patient recall in multi-transfused patients with SCD.
   - second, to determine the frequency of 24 clinically relevant RBC antigens- routinely included in commercially available antibody screening panels- among patients with SCD and/or blood donors in Ghanaians of different ethnicity.

The systematic literature review and meta-analysis included 15 studies of RBC alloimmunisation in patients with SCD from nine SSA countries. The overall proportion of alloimmunisation in patients with SCD in SSA was 7.4% (95% confidence interval (CI) 5.1-10.0). Antibodies E, D, C and K accounted for almost 50% of the specificities. Antibodies to uncommon and common antigens accounted for 20% and 9% respectively of the antibody specificities.

The first part of the cross-sectional study recruited 226 patients with SCD. In these patients antibodies were present in 36 (16%); 25 patients (11.1%) with 26 antibody specificities were positive with the standard Caucasian panel and 11 patients (5.3%) with 11 antibody specificities were positive with the selected African antigens. Receiving the first transfusion after the age of three years (aOR 3.28) and the number of transfusions (aOR 2.00) were positively associated with alloimmunisation. Adverse transfusion reactions were recalled by 68 patients (30%), of which 23 patients had signs suggestive of haemolytic reactions. Adverse transfusion reactions were positively associated with the number of transfusions (aOR 2.06).

The second part of the cross-sectional study performed RBC antigen typing for 24 antigens; antigens A, B, D, C, E, c, e, Jk^a, Jk^b, M, N, S, s, Fy^a, Fy^b by serology and low prevalent antigens C^w, K, Kp^a, Js^a and Lu^a and high prevalent antigens k, Kp^b, Js^b and Lu^b by genotyping. The numbers of samples tested per antigen ranged from 117 to 505. Among patients and donors, the antigens and antigen phenotypes frequencies did not differ, except for the C antigen, and the ccddee phenotype. Participants belonged to 26 different ethnic groups and this was categorized into four groups namely Akan, Ga, Ewe and Other. Among the four ethnic groups, frequency variations were observed for some antigens with the Ewe population showing the highest number of antigens (A, B, E, and Lu^a) and antigen phenotypes (ccDEe, ccDee and Ccddee) differences compared to the other ethnicity groups. The Ga population also differed from the other ethnic groups in the frequency of the Js^a antigen and the Js(a+b+) phenotype.

Overall, my research shows that in Ghana patients with SCD do devleop RBC antibodies (16%) following blood transfusions to 'foreign' RBC antigens present in the donor blood, and some of these antibodies result in haemolytic transfusion (10%) reactions upon subsequent transfusions. Antibody testing to identify these antibodies and providing appropriate units lacking their corresponding antigens would improve transfusion safety in these patients. Furthermore, since these antibodies are mainly towards the major Rh antigens and some African antigens, standard test cells, which are generally of Caucasian origin, will fail to detect the antibodies to the African antigens, putting the patients at risk for haemolytic transfusion reactions.
For effective management of anaemia requiring transfusion in SCD in Ghana, my findings support the routine testing for RBC antibodies using test cells that express the immunogenic African antigens V, VS, Go^a, Dantu and Henshaw. Until Africa-specific red cell screening panel are available, in LMICs in sub-Saharan Africa with limited resources, all patients with SCD should be screened for RBC antibodies with the standard Caucasian panel prior to RBC transfusions and indirect antiglobulin crossmatched to select the best suited blood for transfusion.

1.0 Chapter 1: INTRODUCTION

1.1 Background

Sickle cell disease (SCD) causes a chronic haemolytic anaemia that is often exacerbated into life-threatening severe anaemia by infections and crises episodes. Blood transfusion forms an integral part of the management of SCD, in that it increases the oxygen-carrying capacity of the blood by increasing the circulating haemoglobin and decreasing the percentage of haemoglobin S by dilution (Wayne, Kevy and Nathan, 1993). Despite its usefulness in reducing morbidity and mortality, transfusion is not without risk. Notable among the deleterious effects are the risks of transfusion-transmissible infections, iron overload, and red blood cell (RBC) alloimmunisation.

RBC alloimmunisation is defined as the development of RBC antibodies against foreign antigens in transfused blood. The prevalence of RBC alloimmunisation has been reported to range from 18-76% prior to the start of matching of RBC antigens (that is beyond crossmatching for major blood group antigens ABO and Rh D) between blood donors and recipients (RBC antigen matching) (Orlina, Unger and Koshy, 1978; Rosse *et al.*, 1990; Vichinsky *et al.*, 1990; Aygun *et al.*, 2002; Castro *et al.*, 2002; Sakhalkar *et al.*, 2005; Ameen, Al Shemmari and Al-Bashir, 2009) and 7-47% (Aygun *et al.*, 2002; Sakhalkar *et al.*, 2005) after the introduction of RBC antigen matching. The main reason ascribed to this finding is antigen disparity between Caucassian donors and SCD recipients who are mainly Black (Vichinsky *et al.*, 1990).

Most studies that have reported on RBC alloimmunisation in SCD were conducted in well-resourced countries, where antibody screening/identification are often routine practices. Very few studies have reported on RBC alloimmunisation in patients with SCD in sub-Saharan Africa (SSA) which harbours over 75% of the global SCD burden and where antibody screening, along with indirect antiglobulin crossmatching, and RBC matching beyond ABO and Rh D are not done for patients with SCD in most centres (Natukunda, 2012). This is likely to put transfused patients with SCD at risk of developing RBC antibodies and haemolytic transfusion reactions with subsequent transfusions.

A report on RBC alloimmunisation in multi transfused patients (mostly patients with disorders other than SCD) in Ghana found a prevalence of 9.4% (Boateng *et al.*, 2014). This study, together with studies in other African countries (Diarra *et al.*; Batina Agasa *et al.*, 2010; Natukunda *et al.*, 2010; Abbas *et al.*, 2013; Meda *et al.*, 2014; Eldour *et al.*, 2015; Mangare *et al.*, 2015; Ugwu *et al.*, 2015; Adewoyin, 2016; Boma Muteb *et al.*, 2017; Siransy *et al.*, 2018) were performed with the standard reagent test cells, mostly from donors of Caucasian descent that lack antigens that are more prevalent or exclusively present in Africans. This is likely to miss antibodies to the antigens that are exclusively present in Africans, leading to the underestimation of alloimmunisation and more importantly putting the patients at risk of haemolytic transfusion reaction upon subsequent transfusions with the cognate antigens to which patients have made antibodies.

Studies on non-infectious adverse transfusion events in patients with SCD in SSA are scarce. Only two cross-sectional studies, both in Nigeria, reported on acute transfusion reactions in patients with SCD in SSA, which occurred in 5.8% and 23.8% of patients (Kangiwa *et al.*, 2015; Ugwu *et al.*, 2015).

1.2 Research aim and objectives

1.2.1 Aim

The overall goal of this project was to investigate RBC alloimmunisation and adverse transfusion events in transfused patients with SCD in Ghana and to explore the possible ways of optimising transfusion practice to detect RBC alloimmunisation to clinically relevant RBC antigens and prevent transfusion reactions.

1.2.2 Research objectives

The research had the following objectives in relation to blood donors and transfusion recipients with SCD in Ghana.

- To determine the burden of RBC alloimmunisation in patients with SCD in SSA.
- To determine the prevalence, and specificities of of RBC antibodies and adverse transfusin events in multi-transfused patients with SCD in Ghana.

- To determine the pattern of RBC antigen distribution among patients with SCD and blood donors of different ethnic background in Ghana.

1.3 Thesis structure

Chapter 1 presents a brief introduction describing the background, aims, objectives and the study sites of the project. **Chapter 2** is a review of the relevant literature focussing on identifying relevant publications that contained information on SCD, blood transfusion, RBC antigens and RBC alloimmunisation. The major purpose of the literature review was to discover what is already known about SCD and alloimmunisation and to identify gaps in knowledge. **Chapter 3** describes objective one which was a systematic review on RBC alloimmunisation in patients with SCD and a meta analysis to determine the estimated burden of RBC alloimmunisation in patients with SCD in SSA.

Chapter 4 describes objective two which was a cross sectional survey to determine the prevalence and specificities, and risk factors for, RBC antibodies against European and African antigens and adverse transfusion events in multi-transfused patients with SCD in Ghana.

Chapter 5 outlines objective three which described the pattern of RBC antigen distribution among patients with SCD and blood donors**.**

Chapter 6 summarises the research findings, discusses the challenges, the next steps, strengths and limitations of the project, and concludes with some recommendations. All resources and references are duly cited in the reference section.

1.4 Study timelines

The project started in October 2017. The first three months were used to develop and fine tune the project proposal and to plan the timing for the various activities that would be carried out during the study. Between February and August, 2018, all four ethical approvals (appendix IX) required from the sponsor University, Liverpool School of Tropical Medicine and the three sites/facilities (Korle Bu Teaching Hospital, Komfo Anokye Teaching Hospital and The National Blood Service, Ghana) where

participants recruitment would take place were obtained. Within this same period literature review was performed. The literature review included the traditional literature review and a systematic literature search with specific search terms to obtain relevant literature for a systematyic review and meta analysis. The systematic review and meta analysis was completed in December 2018 and published in 2019. The traditional litrerature review continued until the thesis of the project was handed in. Preparations for fieldwork to recruit study participants and obtain blood samples for the project commenced in January 2018. The preparatory stage which comprised of the design of participant information sheet, participant informed consent form, data capture sheet for the patients and donors lasted for six months. Field work in Ghana to recruit and collect data and blood samples from patients and donors started in July 2018 and ended in December 2018. The blood samples were shipped to Sanquin, Amsterdam, Netherlands, in January 2019. Part one laboratory work, which included all laboratory tests for objective two, started in February till mid-April 2019. Due to technical problems it was necessary to repeat the blood sample collection in Ghana from patients and donors between July and September 2019 and samples were shipped to Sanquin, in September, 2019. Part two laboratory work, which comprised of the laboratory tests for objective three, was carried out between November, 2019 and January, 2021 in Sanquin. Thesis write up which already commenced in January, 2018 continued and the completed thesis was submitted to the University of Liverpool in June, 2021.

1.5 Description of project sites

This was a multicentre project that was carried out in two hospitals and one stand alone blood centre in Ghana: Korle Bu Teaching hospital (KBTH), Accra, Komfo Anokye Teaching hospital (KATH), Kumasi and the Southern Area Blood Centre (SABC) of the National Blood Service (NBSG), Accra. From KATH sickle cell clinic and KBTH sickle cell clinic, patients with SCD were recruited and the blood donors were recruited from the Transfusion Medicine Unit of KATH and SABC of NBSG.

Ghana is located in West Africa, lying in the centre of the coast of the Gulf of Guinea. It covers a total area of about 238,535 square kilometres. Ghana shares boundaries to

the north with Burkina Faso, east with Togo, west with Cote d'Ivoire and south with the Gulf of Guinea and the Atlantic Ocean (figure 1.1). Administratively, Ghana is divided into 16 regions and 260 local districts. The projected population of Ghana as of May 2020 was 30.9 million (Ghana Statistical Service, 2020).

Ghana is a multi ethnic country with over 70 ethnic groups, each with its own distinct language and culture. These 70 ethnic groups originate from a common ancestory belonging to the Niger-Congo language family of Africa. The six major ethnic groups in Ghana in decreasing order of size are Akan, Mole-Dagbani, Ewe, Ga-Adangbe, Gurma and Guan.

Figure 1. 1: Map of Ghana showing the boundaries, the 16 regions and Kumasi and Accra metropolis where Komfo Anokye and Korle Bu Teaching hospitals and the Southern area blood centre are located.

The capital of each region is in bracket

Adapted from https://www.ghanamissionun.org/map-regions-in-ghana/

1.5.1 Korle Bu Teaching hospital (KBTH)

KBTH is the largest hospital in Ghana located in Accra, the capital city of the country. It was established in 1923 as a general hospital, with 200 bed capacity, to manage the health of the indigenes. Through population growth and quality of services provided by the hospital, its patronage had escalated so that a recommendation was put forward in 1953 for its expansion. This compelled the then government to expand the facility to a 1200 bed capacity. The hospital was promoted to a teaching hospital following the establishment of the University of Ghana Medical School, where it partnered with the school in training the students in the health sciences and performing research.

KBTH is currently a 2,000-bed capacity and sees about 1500 patients every day with admission cases of 250 patients daily. It has 17 clinical and diagnostic departments and continues to serve as the training and research centre for the College of Health Sciences, University of Ghana.

It is the largest referral centre with patients from all regions across Ghana and neighbouring countries such as Togo, Burkina Faso and Nigeria.

The hospital had only an adult sickle cell clinic that was governed by the hospital management team but has recently established a paediatric sickle cell clinic under the auspices of the Ministry of Health, Ghana. It has about 26,000 registered patients with SCD and sees 40-50 of these patients a day.

KBTH used to collect and screen its own blood until the establishment of the NBSG in 1973, which has taken over the screening and supply of ready to use blood to the facility.

Figure 1. 2: The front view of the Korle Bu Teaching Hospital building

Copied from https://propertytrendsonline.com/korle-bu-teaching-hospital-launches-innovation-summit-2020/

1.5.2 The Southern Area Blood Centre (SABC) of the National Blood Service Ghana (NBSG)

The SABC of the NBSG is located in Accra, in close proximity to the KBTH. It is the only stand alone blood centre in Ghana. It has a total of 1,405 square meters of laboratory space, with modern equipment. The SABC provides blood services to the entire Greater Accra, part of Central and Eastern Regions. The centre collects about 34,000 donations annually from both VNRD and FRD within the coverage area (National Blood Service Ghana, 2017). It has seven stationary (fixed) blood collection centres. Six of these are within five hospitals and one polyclinic; Korle-Bu Blood Centre, Tema General, La General, LEKMA Ridge Regional Hospitals, and the Maamobi Polyclinic, where predominantly FRD donations are collected. The remaining centre collects VNRD donations and this is situated in the Accra Shopping Mall. The centre also has three mobile donation teams that undertake weekly mobile donation sessions. The mobile collection sites are strategically positioned, either in the city center or further deep into the rural areas within the catchment area, to be easily accessible to different groups of people and/or organisations.

Figure 1. 3: The Front view of the Southern Area Blood Centre of the National Blood Service Ghana

Copied from Asamoah -Akuoko, L., (2019), Thesis submitted to the University of Liverpool https://core.ac.uk/download/pdf/186329381.pdf

1.5.3 Komfo Anokye Teaching hospital (KATH)

KATH is located in Kumasi, the capital city of the Ashanti region, Ghana. It started as a small hospital in the 1940s and grew to achieve a teaching hospital status in 1975; serving as a partner to the School of Medical Sciences, Kwame Nkrumah University of Science and Technology in training medical and other allied health students.

It is the second largest tertiary hospital in Ghana providing specialised services in various health disciplines. Because of its tertiary status and strategic location, it receives referrals from regions that share boundaries with the Ashanti Region and further away; particularly from the three Northern Regions of Ghana (Northern, Upper West, Upper East), Brong Ahafo, Central, Western, Eastern and parts of Volta Region.

KATH has 14 directorates and a 1200 bed capacity. An average of 1000 patients access the hospital daily with a daily admission of about 200 patients.

KATH runs the second largest sickle cell clinic in the country under the child health directorate. It is made up of mainly paediatric patients with SCD. Adult patients with SCD are seen in a different place in the hospital called consulting room 8. There are about 15,000 registered patients and up to 20 patients are seen in a day.

KATH has an excellent transfusion medicine unit equipped with modern facilities for testing and processing of blood and a strong transfusion management team. It is responsible for collecting and screening its blood for transfusion. Due to the cosmopolitan nature of Kumasi, their blood comes from a pool of donors of different ethnic backgrounds. Their main sources of blood are from VNRD and FRD in a ratio of 4:1. The VNRD is achieved by the vibrant blood donor organising team which organises regular mobile blood donations sessions to visit senior high schools and appeal to eligible students and staff to donate blood. KATH also serves as the centre where smaller district and private hospitals contact for blood supply when they experience blood shortages.

Figure 1. 4: The side view of the Komfo Anokye Teaching Hospital building

Copied from https://citinewsroom.com/2018/04/bece-candidate-referred-to-komfo-anokye-after-alleged-gang-rape/komfo-anokye-teaching-hospital/

2.0 CHAPTER 2: LITERATURE REVIEW

2.1 Blood group antigens

2.1.1 Human blood group antigens

Blood group antigens were discovered by Landsteiner in the early 1900s (Sulek, 1968), when he observed that plasma of certain individuals agglutinated red cells from other people.

Human blood group antigens are polymorphic structures uniquely inherited and identified by specific antibodies. They are either proteins or carbohydrate molecules that present as integral proteins or glycoproteins of the red cell membrane or as membrane glycolipid. Blood group antigens are mostly synthesised on the red cell surface membranes; however, some antigens, the Lewis and Chido\Rodger blood group antigens, are adsorbed from plasma onto the red cell surface. The red cell membrane has three types of protein which carry the blood group antigens. These are the single-pass proteins, multi-pass proteins and glycosylphosphatidylinositol- linked proteins (Dean, 2005) (Figure 2.1)

Some antigens are expressed solely on the extracellular surface of RBCs, while others are found both on RBC surfaces and on tissues or organs. Occasionally, some humans may express the antigens elsewhere in their body but not on the RBC surface. An example of this is the Duffy antigen in individuals with the GATA-box silencing mutation; individuals with this mutation express the Duffy antigens in cellular locations such as the epithelial cells of the kidney, purkinje cells of cerebellum and endothelial cells lining blood vessels, but do not express on RBC surfaces. They thus appear as negative for Duffy antigens during serologic RBC antigen testing.

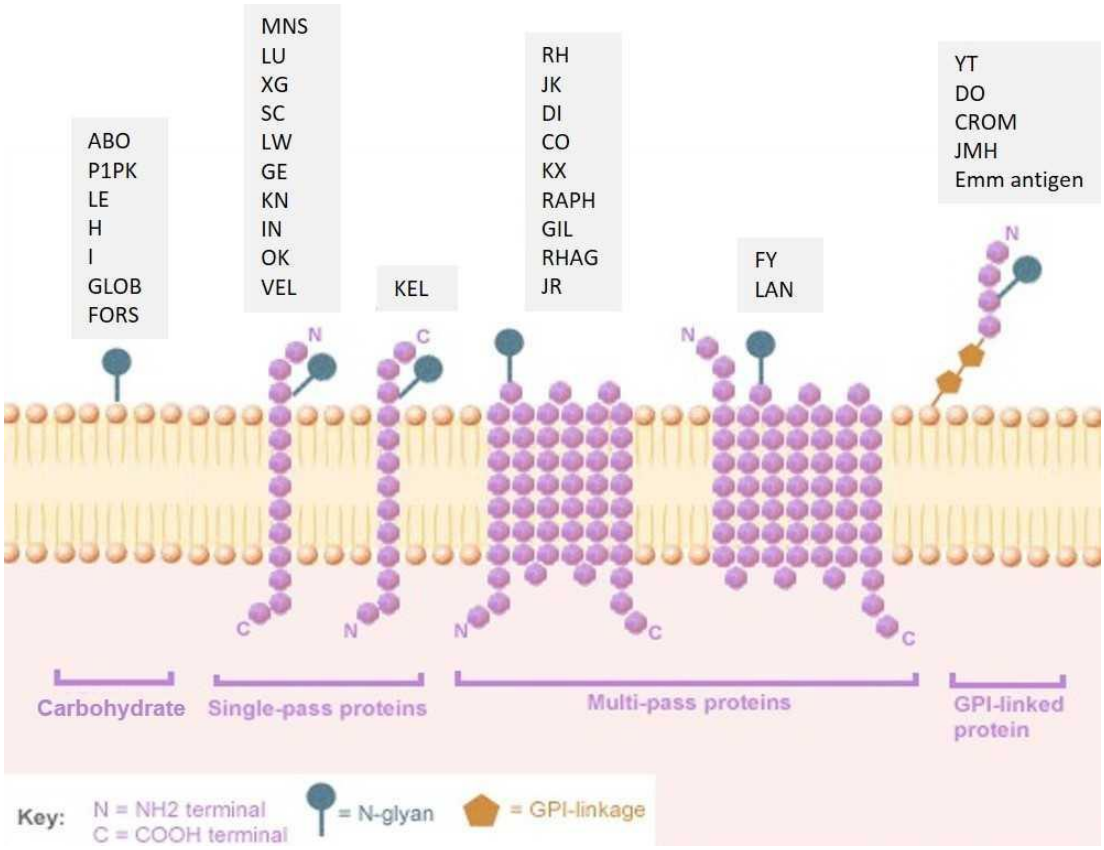


Figure 2. 1: Pictorial representation of the red blood cell membrane with some of the blood group antigens attached to it

Adapted from (Dean, 2005)

2.1.2 Classification of human blood group antigens

Generally, blood group antigens are organised into one of four classifications; systems, collections (also known as 200 series), high incidence antigens (also known as 901 series) and low incidence antigens (also known as 700 series). According to the International Society for Blood Transfusion (ISBT), a blood group system consists of one or more antigens that are controlled by a single gene locus or by two or more very closely linked homologous genes with minute or no observed recombination between them (Daniels *et al.*, 2004). This makes each blood group system genetically different. If antigens lack adequate genetic evidence and do not fulfil the requirements for classification into a system, they are put into the collections. The collections consist of serologically, biochemically and genetically related antigens which do not fulfil the criteria for a system. Antigens that do not meet the criteria for a system or collection are either put into the 901 series or the

700 series. Antigens are ascribed into the 901 series, if they are expressed in more than 90% of individuals within most populations or 700 series, if they are expressed in less than 10% of individuals within most populations (Daniels *et al.*, 2009).

To date, more than 350 blood group antigen specificities have been identified, named and assigned by the ISBT Working Party; about 324 have been assigned into one of 38 blood group systems. Each system accommodates from one up to 55 antigens. (Table 2.1). The remaining antigens are classified into one of five collections, the 901 series and the 700 series (Storry *et al.*, 2016).

Number	System Name	System symbol	Number of antigens	HGNC gene symbol(s)	Chromosomal location	structure	CD number
001	ABO	ABO	4	*ABO*	9q34.2	Carbohydrate	
002	MNS	MNS	49	*GYPA, GYPB, GYPE*	4q31.21	Glycoprotein	CD235a/b
003	P1PK	PIPK	3	*A4GALT*	22q13.2	Carbohydrate	
004	Rh	RH	55	*RHD, RHCE*	1p36.11	Protein	CD240D/CE
005	Lutheran	LU	25	*BCAM*	19q13.2	Glycoprotein	CD239
006	Kell	KEL	36	*KEL*	7q33	Glycoprotein	CD238
007	Lewis	LE	6	*FUT3*	19p13.3	Carbohydrate	
008	Duffy	FY	5	*DARC*	1q21-q22	Glycoprotein	CD234
009	Kidd	JK	3	*SLC14A1*	18q11-q12	Glycoprotein	
010	Diego	DI	22	*SCL4A1*	17q21.31	Glycoprotein	CD233
011	Yt	YT	5	ACHE	7q22	Glycoprotein	
012	Xg	XG	2	XG, CD99	Xp22.32/Y	Glycoprotein	CD99
013	Scianna	SC	7	ERMAP	1p34.2	Glycoprotein	
014	Dombrock	DO	10	ARTA	12p13-p12	Glycoprotein	CD297
015	Colton	CO	4	AQPI	7p14	Glycoprotein	

016	Landsteiner-Weiner	LW	3	ICAM4	19p13.2	Glycoprotein	CD242
017	Chido-rodgers	CH/RG	9	C4A, C4B	6p21.3	Glycoprotein	
018	H	H	1	FUT1	19q13.33	Protein	
019	Kx	XK	1	XK	Xp21.1	Glycoprotein	CD236
020	Gerbich	GE	11	GYPC	2q14-q21	Glycoprotein	CD55
021	Cromer	CROM	20	CD55	1q32	Glycoprotein	CD35
022	Knops	KN	9	CR1	1q32.2	Glycoprotein	CD44
023	Indian	IN	6	CD44	11p13	Glycoprotein	CD147
024	Ok	OK	3	BSG	19p13.3	Glycoprotein	CD151
025	Raph	RAPH	1	CD151	11p15.5	Glycoprotein	CD108
026	John Milton Hagen	JMH	6	SEMA74	15q22.3-q23	Glycoprotein	
027	I	I	1	GCNT2	6p24.2	Carbohydrate	
028	Globoside	GLOB	2	B3GALT3	3q25	Carbohydrate	
029	Gill	GIL	1	AQP3	9p13	Glycoprotein	
030	RHAG	RHAG	3	RHAG	6p12.3	Glycoprotein	CD241
031	Forssman	FORS	1	GBGT1	9 q34.13-q34.3	Carbohydrate	
032	JR	JR	1	ABCG2	4q22.1	Glycoprotein	

033	Lan	LAN	1	ABCB6	2q36	Glycoprotein	
034	Vel	VEL	1	SM1M1	1p36.32	Glycoprotein	
035	CD59	CD59	1	CD59	11p13	Glycoprotein	CD59
036	Augustine	AUG	4	SLC29A1	6p21.1	Glycoprotein	
037	KANNO	KANNO	1	PRNP	20p13	protein	CD230
038	Sid	SID	1	B4GALNT2	17q21.32	Carbohydrate	

Table 2. 1: Table of the 38 blood group systems identified and assigned by the ISBT Adapted from the International Society of Blood Transfusion Red Cell Immunogenetics and Blood Group Terminology and Hoffbrand et al., 2016

2.1.3 Blood group genetics and polymorphisms

Although environmental factors can affect blood group antigen expression, they are mostly inherited as Mendalian characters. There are 43 genes in the 38 blood group systems, with all genes being autosomal except for *XK*, *XG* and *CD99*. *XK*, *XG* are located on the X chromosome while *CD99* is found on both X and Y chromosomes. All the genes have been cloned and sequenced. Most of the blood group systems (34) are controlled at a single genetic locus. Xg, Rh, Chido-Rogers have 2 loci each and MNS, 3 loci (Jill R. Storry and Olsson, 2004; Storry *et al.*, 2011).

With 55 and 49 antigens, respectively, Rh and MNS are classified as the most complex and highly polymorphic blood group systems. Most polymorphisms in the blood group systems are single nucleotide polymorphisms (SNP) resulting in amino acid substitution in the extracellular domain of the RBC surface protein or a glycosyltransferase (Daniels, 2005). Gene deletion or inactivation may lead to absence of a whole protein from the membrane resulting in a null phenotype. All polymorphisms, together with the genes coding for the blood group systems, have been identified.

2.1.4 Functions of the human blood group antigens beyond blood transfusion

The presence of blood group antigens on surfaces other than RBC implies that their role is not limited to transfusion medicine but also organ development and function. Cartron and Colin divided the blood group antigens into 5 main functional categories. These are transporters/channels, enzymes, adhesion molecules, receptor and structure. For example; the Kidd and Diego (Band 3) antigens are urea and membrane transporters respectively, the Colton blood group antigen, aquaphorin1 (AQP1), a water channel. RhAG, is a probable gas channel. ABO H and Lewis also have enzymatic functions. Landsteiner-Weiner, Lutheran, Xg, Ok and Indian act as ligands. The Duffy and knops antigens are chemokine and complement receptors respectively. Some antigens may display multiple functions and thereby may fit into more than one category, as in the case of protein Band 3 which is a membrane structure and transporter (Cartron and Colin, 2001; Telen, 2005).

2.1.5 RBC antigen typing

RBC antigen phenotyping has over the years been achieved using serological testing, a method that utilises an antibody-antigen interaction, hemagglutination technique. This technique has adequate specificity and sensitivity required for the clinical care of patients who need blood transfusion. It is easy to perform and requires less equipment compared to other haematology and chemistry tests. However, hemagglutination technique has the following limitations:

- antisera for some antigens are not commercially available, so, some donor antigens cannot be tested.
- typing of recently transfused patients and patients with sensitised RBC's (that is patients whose RBC are already coated with antibodies) is difficult.
- lacks precision in indicating RHD zygosity in D positive individuals.

Technological advancement in transfusion medicine over the last decades has led to the introduction of molecular techniques such as DNA sequencing techniques and polymerase chain reaction into the field of transfusion medicine. These techniques aid in molecular characterisation of the blood group antigens. The first successful application of molecular techniques in blood transfusion was when the MN gene,

GYPA was cloned in 1986 (Siebert and Fukuda, 1987). In 1990 and 1992, the genes for ABO (Yamamoto *et al.*, 1990) and Rh (Le van Kim *et al.*, 1992) were also cloned and subsequently genes for the other blood group systems.

Application of molecular techniques has overcome the limitations of the traditional hemagglutination method in the following ways:

- It has provided a better understanding of the molecular basis of the blood group antigens and a possibility of predicting blood group antigen profile of individuals using DNA testing.
- Molecular genotyping also allows detection of antigen variants, importantly, Rh antigen variants. This invariably contributes to predicting the risk of and the prevention of alloimmunisation.
- It is able to identify rare phenotypes and rare combination of phenotypes in donors to aid pre- transfusion blood selection.

Nonetheless, molecular based methods have the following limitations:

- They are unable to detect directly the presence of antigens on the surface of the RBCs.
- They may detect normal genes that are unexpressed because of gene silencing mutation, thereby falsely identifying the person as antigen positive. A typical example is the RHD pseudogene, a silencing gene, which when present, prevents the expression of the D antigen. Such patients will appear genotypically as Rh D positive putting them at risk of D-alloimmunisation with Rh D positive transfusion.
- Most DNA based techniques are developed to detect SNPS, so, in the case of complex genetic changes such as hybrid alleles which occurs commonly in RH and MNS, false positive and negative results may be obtained.
- Presently, there are many alleles making it too cumbersome for current molecular methods to practicably address (Denomme, Johnson and Pietz, 2011).

The application of molecular genotyping techniques in blood group antigen determination has proven to be useful, however, whether it should replace serological

phenotyping in the future is a question that requires further research to answer. Some authors believe molecular genotyping is a valuable supplement to the conventional serological antigen typing and not a substitute (Anstee, 2009; Wilkinson *et al.*, 2012). Others also think that in chronically transfused patients, molecular typing is superior to serological typing and have recommended a change in practice to using molecular genotyping as the primary method of extended antigen typing (Costa *et al.*, 2013; Casas *et al.*, 2015; Ye *et al.*, 2016).

2.2 Blood transfusion

2.2.1 What is blood transfusion

Blood transfusion is the process of transferring blood or blood product from one person (donor) to the circulatory system of another person (recipient), both of the same species. Blood transfusion is often required as a lifesaving intervention for replacement of blood cells or blood products lost through severe haemorrhage and surgery or to boost the blood count of an anaemic patient. The need for blood transfusion is driven by major causes of anaemia such as obstetric haemorrhage, malaria, accidents, malnutrition and severe dietary iron deficiency, and parasitic infections. Some groups of people have medical conditions that put them at risk of developing life-threatening anaemia and require intermittent transfusions to maintain adequate levels of haemoglobin, improve the quality of life and growth. Prominent in this group are individuals with SCD, thalassemia, cancer and renal problems.

Blood may be transfused as whole blood, that is, donated blood with all its components intact, or as one of the blood products. Blood products are components of the blood that are prepared following blood donation. Major blood components include: packed RBC, plasma, platelet and cryoprecipitate (Hoffbrand, Moss and Pettit, 2011). Whole blood transfusion is rarely used in many developed countries, because, patients infrequently require all components of a unit of whole blood, therefore, only the component required by the patient for a specific condition or disease is transfused (Erhabor and Adias, 2011). This allows more than one patient to benefit from a unit of donated blood, thereby, maximizing its potential benefit. On the other hand, whole blood transfusion is still widely used in developing countries, where resources for processing whole blood into components are limited and in the

United States military, when specific components required in life threatening emergencies are not readily available (Spinella, 2008).

2.2.2 Types of blood transfusion.

Blood transfusion may be categorized into two types depending on the source of the blood for transfusion: allogenic and autologous transfusion. Allogenic transfusion is when someone's (donor) blood is transfused to a patient (recipient); the donor is different from the recipient. The donor may be related or unrelated to the recipient. It is the most common type of blood transfusion globally. Autologous transfusion refers to when one's own blood is transfused back into oneself (the donor is same as recipient). This is the safest form of blood transfusion in terms of transmission of viral infections; however, it is only practical when preparing for an elective procedure where blood loss may be anticipated. In most other situations, patients are unable to donate their own blood because of the acute nature of the need for the transfusion. Even though autologous transfusion is safe in terms of viral infection, it is prone to bacterial contamination and clerical errors (Hoffbrand, Moss and Pettit, 2011).

Blood transfusion may be given as simple transfusion, in which case the recipient's blood is topped up with donor (figure 2.2), or exchange transfusion, where part of recipient's blood is removed and replaced with donor unit (figure 2.3). Simple or exchange transfusions given either chronically or episodically are discussed further under transfusion in patients with SCD as these types of transfusions are common in patients with SCD.

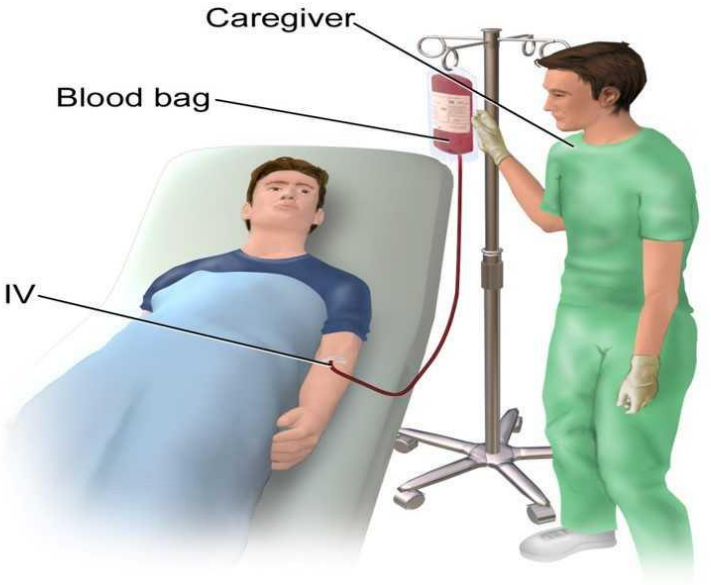


Figure 2. 2: Illustration of simple blood transfusion.

Copied from: https://simple.wikipedia.org/wiki/Blood_transfusion#/media/File:Blausen_0087_Blood_Transfusion.png

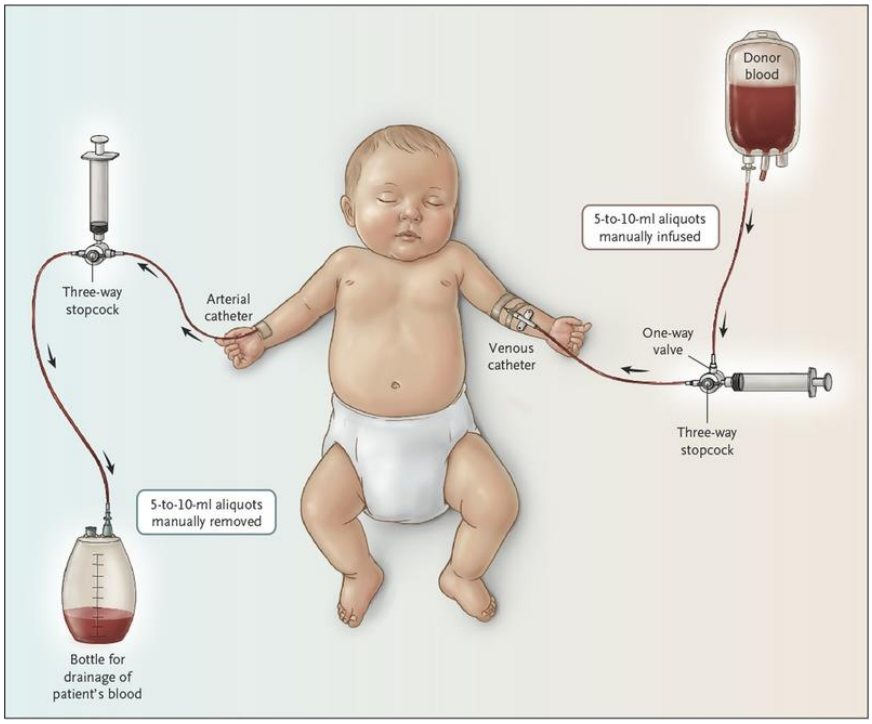


Figure 2. 3: Illustration of exchange blood transfusion.

Copied from https://simple.wikipedia.org/wiki/Blood_transfusion#/media/File:Blausen_0087_Blood_Transfusion.png

2.2.3 Pre-transfusion compatibility screening

Pre-transfusion screening refers to the series of checks and laboratory tests performed to ensure there is compatibility between a transfusion recipient's blood and the intended blood product to be transfused. It begins with proper completion of the blood requisition form and proper patient identification for pre-transfusion blood sample collection, through laboratory testing of both patient (recipient) blood sample and donor blood product. The process ends when a compatible blood product is identified for the patient (figure 2.4). The overall goal of pre-transfusion testing is to provide patients with blood products that are safe and have acceptable survival *in vivo* (Shulman *et al.*, 2001; Lieb and Aldridge, 2005). Properly performed pre-transfusion testing will ensure that the right patient is issued the correct blood component. It will verify ABO compatibility and detect clinically significant unexpected antibodies.

The details of the processes are shown in figure 2.4 and are discussed in detail below.

2.2.3.1 Pre- transfusion patient identification, sample collection and labelling

In making a blood request, all information on the requisition form must be completely filled to aid proper identification of the patient. During the sample collection, the phlebotomist is required to ask the patient to mention his/her first and last name and date of birth. This is done to ensure that blood is collected from the right patient and to reduce errors that may arise from patients wearing the wrong wrist band (Shulman, Nelson and Nakayama, 1990). Collected samples are labelled in the presence of the patient with two unique identifiers; full name and the hospital number or medical record number or national health insurance number. The date of sample collection must be indicated on the sample tube (Murphy *et al.*, 2007) as well as a means of identifying the phlebotomist (JPAC, 2013; CSTM, 2017)

2.2.3.2 Pre-transfusion sample requirements

The pre-transfusion sample may be anticoagulated in EDTA or clotted in a non-additive tube from which serum is obtained. The sample should not be more than 3 days old for patients who in the previous three months had received transfusion or had been pregnant.

2.2.3.3 Pre-transfusion laboratory tests

Pre-transfusion laboratory tests in HIC include ABO Rh typing, antibody screening and crossmatching. Crossmatching may be performed as a serological crossmatch and computerised or electronic crossmatch

2.2.3.3.1 Serological crossmatch

For serological crossmatching, patient serum is mixed with donor cells. There are two types of serological crossmatch; immediate spin crossmatch and complete (indirect antiglobulin) crossmatch. For immediate spin crossmatch, patient serum is mixed with donor cells at room temperature to look for agglutination. Immediate spin crossmatch detects ABO incompatibility. Complete crossmatch is an extended form of the immediate crossmatch; patient serum is mixed with donor cells at room temperature to look for agglutination. If no agglutination is seen, the mixture is incubated at 37^{o}C, if still no agglutination is seen, there is the addition of anti-human globulin. The addition of the anti-human globulin is the final step, after which the outcome, whether agglutination seen, or no agglutination seen, is reported. This extended testing is to be as sure as possible that patient's serum is compatible with donor's red cells. Complete crossmatch detects incompatibility due to immune IgG antibodies.

2.2.3.3.2 Electronic crossmatching

Electronic crossmatching does not physically test recipients's serum against donor cells but uses a computerised program to select the appropriate blood product for the recipient. This method of crossmatching is used in settings where there is a validated computerised algorithm that allows release of only compatible units and for patients with negative antibody screening. For patients who have had ABO incompatible stem cell transplant or solid organ transplant less than 3 months to the intended transfusion, electronic issue is not appropriate (Hoffbrand *et al.*, 2016).

In LMIC, pre- transfusion laboratory tests are ABO grouping, Rh D typing, and crossmatching. Crossmatching is mostly immediate spin crossmatch. Very few facilities would occasionally perform complete crossmatching. RBC antibody screening and identification tests do not form part of the routine pre-transfusion screening method in most LMIC (Natukunda *et al.*, 2010; Meda *et al.*, 2014;

Adewoyin, 2016). This is mainly because of the supposition that, in Africa, donors and recipient antigens are homogenous, so antibody formation is less likely to occur.

2.2.3.4 Emergency blood release

In some situations, the time frame within which the transfusion is required may be so short that initiating and completing the pre-transfusion tests is not feasible. In such instances, blood products may be released for transfusion without matching. If the ABO and Rh D grouping have been completed, group specific uncrossmatched units may be given. However, if the blood group is unknown, group O negative unmatched blood may be released, and patient's blood obtained as soon as clinical circumstances allow, so that, ABO Rh D screening could be performed to issue group specific crossmatched blood.

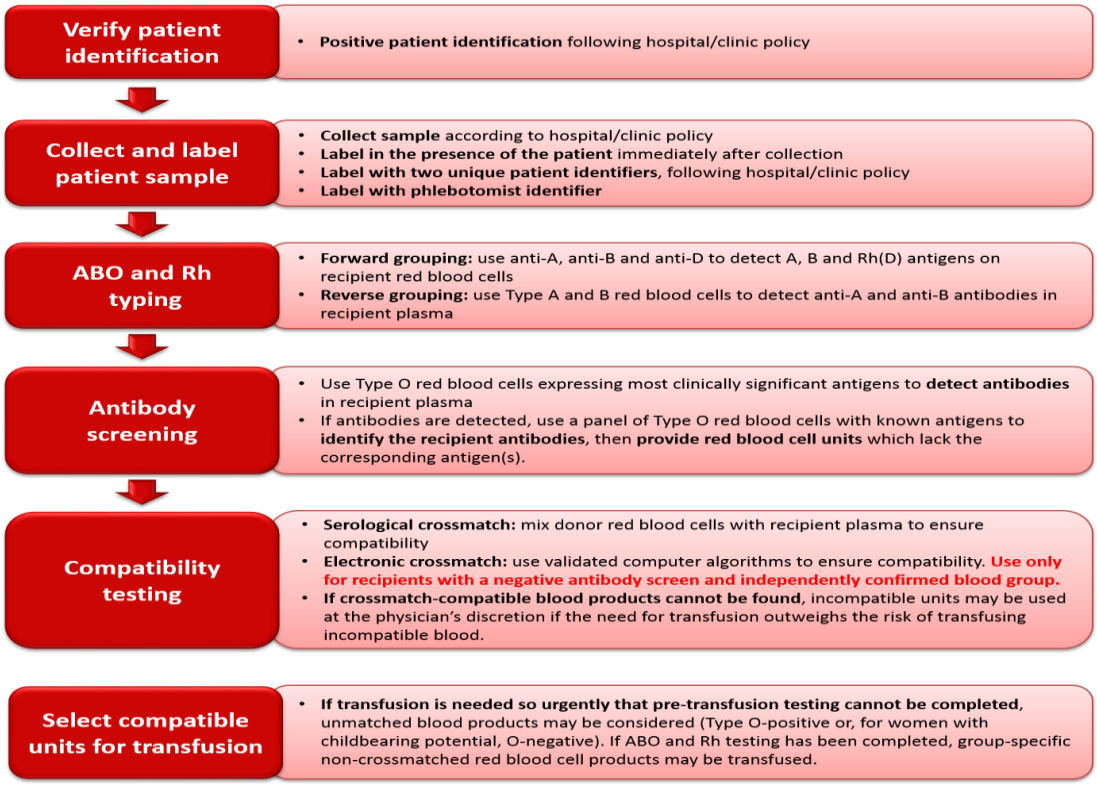


Figure 2. 4: Summary of pre-transfusion compatibility testing copied from Canadian Blood Service Professional Education.

Available at_https://professionaleducation.blood.ca/en/transfusion/guide-clinique/pre-transfusion-testing

2.2.4 Risks of blood transfusion

Even though blood transfusion can save life, it is not without risk. The risks associated with blood transfusion may be infectious or non-infections.

2.2.4.1 Infectious risks of blood transfusion

One of the main risks associated with blood transfusion is the transmission of infectious agents. The infectious agents may be viruses, parasites and bacteria. The frequency of these infectious agents varies from one geographical area to the other depending on the endemicity of specific blood-borne infections within the population.

Viruses that are transmissible by blood transfusion include; West Nile virus, dengue virus, human immunodeficiency virus 1 and 2 (HIV 1 and HIV 2), hepatitis virus (HBV and HCV), human T-cell leukaemia virus 1 and 2 (HTLV 1and HTLV 2) cytomegalovirus (CMV), Epstein Barr Virus (EBV), and parvovirus B19. A few of these viruses show early symptomatic infections after transmission (i.e. parvovirus B19, West Nile virus and dengue virus) while some exhibit cell associated latency (i.e. in a resting state without producing more viruses or showing any noticeable symptoms) and only become active and symptomatic after a long time (eg. HTLV 1 and 2, HIV 1 and 2, CMV).

Bacterial contamination of blood can occur at various points during donation and the transfusion process. Sources of bacteria transmitted through transfusion are in two groups. Endogenous (its source is from the donor) and exogenous (results from external contamination). Examples of endogenous bacteria are *Treponema pallidum, Yersinia enterolitica* and *Salmonella species.* Exogenous bacteria include *Streptococcus species, Pseudomonas species and Serratia species* (Hoffbrand and Moss, 2011). Most transfusion transmitted bacteria are of exogenous sources, however, a few endogenous bacteria species may occasionally be transmitted from donor to recipient.

Some parasites that have been implicated in transfusion associated transmissions are Plasmodium *species*., T*rypanosoma cruzi*, *Babesia microti*, Leishmania species, and *Toxoplasma gondii*. Diseases associated with the infection of these parasites are malaria, Chagas, babesiosis, leishmaniasis and toxoplasmosis respectively. The incidence of infection from these parasites is lower than bacteria and viruses,

however, if transmitted, they can pose considerable risk of illness particularly in immunocompromised recipients.

2.2.4.2 Non- infectious risks of blood transfusion
Non-infectious risks can be immunological or non-immunological.

2.2.4.2.1 Non infectious immunological risks
Immunological risks are immune mediated and can be sub categorized into haematological and non-haematological

Haematologic immunological risk affects the recipeint's blood cells. This occurs when a patient is transfused with an incompatible blood product (blood product containing antigens they lack) and the patient's immune system mounts an immune response, resulting in destruction of the incompatible blood product and sometimes the patient's own blood cells, a situation referred to as transfusion reaction. Transfusion reaction is the most common form of transfusion associated risk in the western countries (Stainsby *et al.*, 2006). Transfusion reactions may occur with all transfused blood cells (RBC, white blood cells (WBC) and platelets). The response against incompatible RBC, WBC and platelet transfusions results in haemolytic transfusion reaction, febrile non-haemolytic transfusion reaction and post transfusion purpura, respectively.

Non-haematologic immunological risks do not affect blood cells but some organs or other body parts such as the lung, skin and eyes. There are two types: transfusion related acute lung injury (TRALI) and allergic reactions. Both are discussed in the following paragraph.

TRALI occurs following passive transfer of human leucocyte antigens (HLA) or granulocyte antibodies from donor to recipient. The risk of TRALI occurrence is 7 to 8-fold increase in plasma rich components such as fresh frozen plasma, platelets and whole blood than packed red cells (Bolton-Maggs and Cohen, 2013). TRALI typically occurs within 6 hours of a transfusion, sometimes during the transfusion. Clinically, TRALI presents with symptoms and signs of fever, hypotension, dyspnea, pulmonary oedema and cyanosis (Latham, 2016).

Allergic reactions are common where transfused products contain immunoglobulin E (IgE) and the recipient has pre-formed anti IgE antibody. Like TRALI, allergic

reactions are common with plasma containing products (Stainsby *et al.*, 2006). Symptoms of allergic reaction include hives and itching. A heightened form of the allergic reaction, anaphylactic reaction, may also occur in transfusions. In most cases the etiology is not known; however, the development of anti immunoglobulin A (IgA) antibodies in Ig A deficient patients, following transfusion with donor plasma containing IgA, has been suggested as the trigger for anaphylaxis. Its incidence is reported to be 1-3 in 10,000 population per year (Soar *et al.*, 2008). Anaphylactic reactions can be severe resulting in death. However, severe anaphylactic reactions are rare (1 in 2.5 million people per year). Symptoms of anaphylactic reaction occur within minutes to hours of exposure to the implicating allergen and include hives, itching, shortness of breath, diarrhea, abdominal pains and unconsciousness (Luo, 2017).

2.2.4.2.2 Non-infectious non-immunological risk

The main non-infectious non-immunological risk of blood transfusion is iron overload. Iron overload is common in patients who receive long term transfusions such as patients with SCD, thalassemia and myelodysplastic syndromes. This is because of the body's limited mechanisms to excrete iron. A unit of transfused packed RBC contains approximately 200-250 mg of iron; since there is no active mechanism to excrete iron, repeated RBC transfusion introduces excess iron which progressively accumulates in tissues. Continuous deposition in the liver, endocrine organs and the heart result in organ damage (Hoffbrand, Moss and Pettit, 2011).

2.3 Alloimmunisation

2.3.1 RBC alloimmunisation

Alloimmunisation is one of the most important complications of blood transfusion besides iron over load and transfusion transmitted infections. It is classified as a non-infectious immunological risk of transfusion. Alloimmunisation is defined as the production of antibodies following exposure to foreign antigens in allogenic blood. Apart from blood transfusion, alloimmunisation could also arise from pregnancy (Moise, 2005) and transplantation (Cummins *et al.*, 1995). Alloantibodies may be formed to RBC antigens in the case of RBC alloimmunisation, Human leucocyte

antigens (HLA) and human platelet antigens (HPA) in leucocyte alloimmunisation and platelet alloimmunisation respectively. For the purposes of this literature, RBC alloimmunisation due to blood transfusion is the focus.

RBC alloimmunisation occurs when an immunocompetent patient (recipient) mounts an immune response to donor antigens following packed RBCs or whole blood transfusion. This is due to genetic variation between the donor and recipient. RBC alloimmunisation is common in people who receive multiple transfusions. The rate has been reported to range from 1-10% in all transfusion recipients (Schonewille, Haak and van Zijl, 1999; Alves *et al.*, 2012) but higher (up to 76%) in chronically transfused patients, like patients with SCD (Olujohungbe *et al.*, 2001; Aygun *et al.*, 2002; Murao and Viana, 2005; Lasalle-Williams *et al.*, 2011; Sins *et al.*, 2016) than other transfused patient populations. Common antibodies implicated in alloimmunisation are directed to antigens in the Rh, Kell, Kidd, MNS and Duffy blood group systems with antibodies to Rh and Kell reported as the most prevalent in most studies.

The risk of alloimmunisation is influenced by the number and frequency of transfusions, antigen disparity between the donor and recipient, the recipient's age and sex, genetic factors and the recipient's inflammatory status at the time of transfusion and underlying disease (Murao and Viana, 2005; Desai *et al.*, 2015; Fasano *et al.*, 2015; Karafin *et al.*, 2015; Sins *et al.*, 2016; Oliveira *et al.*, 2017; Sippert *et al.*, 2017).

The risk of alloimmunisation has been found to increase with increasing number of RBC transfusions, however, in multi-transfused patients, most antibodies are formed following a single allogenic transfusion (Fluit, Kunst and Drenthe-Schonk, 1990; Zalpuri *et al.*, 2012).

2.3.2 Consequences of RBC alloimmunisation

Alloimmunisation can pose a challenge to finding compatible blood for transfusion thereby delaying or preventing transfusion therapy. This could affect the wellbeing of patients who require repeated transfusions. It puts chronically transfused patients at increased risk of developing auto antibodies (Young *et al.*, 2004; Singhal *et al.*, 2017) and a four to five times higher risk of forming additional alloantibodies (Schonewille, de Vries and Brand, 2009). A recent report by Singhal *et al.* (2017),

showed that the intensity of RBC transfusions in patients with myelodysplastic syndrome (MDS) was higher in alloimmunised MDS patients than non alloimmunised MDS patients. Alloimmunisation can also result in both acute and delayed haemolytic transfusion reactions.

A problem of major concern with alloimmunisation is the development of haemolytic transfusion reactions. In the United States of America (USA), non-ABO antibody induced haemolytic transfusion reactions are the third most common cause of transfusion-related mortality (US-FDA, 2013). Haemolytic transfusion reactions may be acute, occurring concurrently or within 24 hours of transfusion, or delayed. Delayed haemolytic transfusion reaction (DHTR) occurs typically five to seven days post transfusion, during which time patients might have been discharged from the hospitals where they received the transfusion, and therefore go unnoticed. In extreme cases, the reaction may occur as early as three days or as late as 2 weeks to three weeks (Issitt, 1985; Klein and Anstee, 2005). Most transfusion reactions resulting from alloimmunisation are delayed (DHTR) (Noizat-Pirenne, 2012; Noizat-Pirenne, 2013). DHTR results from the transfusion of RBC units carrying antigens to which the patients have formed an antibody after a previous transfusion or pregnancy and that has become undetectable in the current pre-transfusion blood sample (i.e. antibody evanescence) (Tormey and Stack, 2009). Transfusion with an incompatible unit can induce a booster immune response, with high titer antibody developing within a few days following the transfusion. DHTR can present as a serious and potentially life-threatening condition, resulting in destruction of transfused cells or even death of patients (Stainsby *et al.*, 2006). Antibodies that have been frequently implicated in DHTR belong to the Rh, Kidd, Duffy, and Kell blood systems (Hoffbrand *et al.*, 2016).

Infrequently, both donor and recipient's own red cells are destroyed. This condition is termed as hyperhaemolysis syndrome

Hyperhaemolysis syndrome has been reported in transfused patients with SCD (Win, 2009), thalassemia (Mechery *et al.*, 2012), myelofibrosis (Treleaven and Win, 2004) and anemia of chronic disease (Darabi and Dzik, 2005). The etiology of hyperhaemolysis syndrome is not clear, however mechanisms such as "bystander haemolysis" (King *et al.*, 1997; Win, 2009), destruction of RBC by macrophage

activation (McGlennan and Grundy, 2005) and concomitant illness or transfusion associated suppression of erythropoiesis (Petz *et al.*, 1997) have been proposed.

Hyperhaemolysis syndrome occurs in two forms; acute and delayed (Win, 2009). The acute form of hyperhaemolysis syndrome typically occurs within 7 days of transfusion. Immunological tests often show no new antibody and negative direct antiglobulin test (DAT). The delayed form, on the other hand, usually occurs beyond 7 days of transfusion, with new/additional antibodies detected and positive DAT (Aygun *et al.*, 2002; Talano *et al.*, 2003). For patients with preformed antibodies, hyperhaemolysis syndrome may still occur even if antigen-matched crossmatched compatible transfusion is given. Prompt diagnosis and provision of intervention is necessary so as to avoid unnecessary transfusion which may exacerbate the condition and lead to death (Friedman, Kim and Manno, 1993).

2.3.3 Pathophysiology of alloimmunisation

The mechanism of alloimmunisation is a complex process that occurs in two ways; direct and indirect pathways (Figure 2.5). However, they are not necessarily mutually exclusive. The direct pathway involves interplay of both donor and recipient's cells. It begins when donor antigen presenting cells (APC), which include monocytes, macrophages and dendritic cells, endocytose donor antigens, process and present them bound to class II major histocompatibility complex (class II MHC) to the recipient T cells. Recipient CD4+T cells bearing specific T cell receptor (TCR) against the antigen/MHC complex recognise the complex and becomes activated with the help of other co-stimulatory factors, from either the donor or recipient. The TH_2 subset of the activated $CD4^+$ T helper cells secrete interleukins (IL-4, IL-5, IL-6, and IL-10) which stimulate antibody producing B cells to make antibodies against the precise antigen. The initial immune response mounted is termed as primary immune response and the T cells become memory T cells. Upon subsequent immune challenge, the memory T cells would neither need class II MHC to recognise the antigen nor require co-stimulatory factors to become activated.

In the indirect pathway recipient APC present donor antigens to self T helper cells. It is akin to normal immune response.

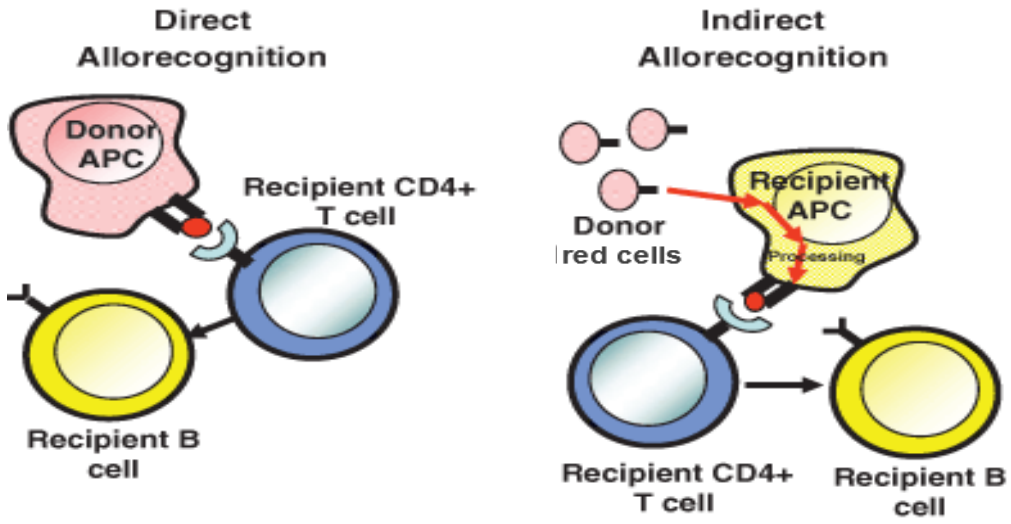


Figure 2. 5: Schematic diagram of the mechanisms for direct (left) and indirect (right) allorecognition of foreign antigens leading to the production of alloantibodies.

Adapted from (Pavenski, Freedman and Semple, 2012).

2.3.4 Types of RBC antibodies

RBC antibodies are immunoglobulin proteins that are secreted by B lymphocytes after specific antigenic stimulation. Depending on the nature of antigenic exposure, two types of RBC antibodies can be made; naturally occurring antibodies or immune antibodies.

Naturally occurring antibodies are antibodies produced following exposure to environmental agents that possess structures analogous to RBC antigens. Sensitisation through pregnancy or blood transfusion is not required. They are usually immunoglobulin M (IgM), a few are immunoglobulin G (IgG), reacting at room temperature or below. These antibodies are not present at birth. They are produced a few months (3-6 months) after birth probably due to interactions with environmental agents such as bacteria, dust and pollen grains which carry antigens similar to red cell antigens. Examples of naturally occurring antibodies include: anti-A, anti-B, anti-M, antibodies in the Lewis (anti-Le^a anti- Le^b) and P (anti P) blood group system. Except for blood group ABO, anti A and anti B antibodies which are clinically significant (can cause haemolytic transfusion reaction), naturally occurring antibodies antibodies are mostly clinically insignificant.

Alternatively, immune antibodies are produced as a result of immune challenge; through transfusion or pregnancy. They are mostly IgG reacting at 37^{o}C, however, IgM and IgA types also exist. Immune antibodies are clinically significant because they can cause haemolytic transfusion reactions and haemolytic disease of the newborn. Examples of immune antibodies include but not limited to antibodies to the Rh, Kell and Duffy blood group antigens.

2.4 Sickle cell disease

2.4.1 Haemoglobin molecule

Haemoglobin is a tetramer of globin chains folded around four haem groups contained in the matured RBC (Figure 2.6). The globin chain consists of two alpha and two non-alpha chains and makes up about 90% of the haemoglobin molecule. The haem portion makes up approximately 10%. Haemoglobin plays the role of oxygen transport to cells and tissues in the body and facilitate the return transport of carbon dioxide to be excreted (Mehta and Hoffbrand, 2009).

The normal adult RBC contains three types of haemoglobin; haemoglobin A (Hb A) which has two alpha globin chains and two beta globin chains (α_2 β_2,), haemoglobin F (Hb F) which has two alpha chains and two gamma chains ($\alpha_2\gamma_2$) and haemoglobin A_2 (Hb A_2) which has two alpha chains and two delta chains ($\alpha_2\delta_2$) (Hoffbrand, Moss and Pettit, 2011).

Quantitative and qualitative mutations occur in the globin genes that alter the formation of the normal haemoglobin types. Quantitative mutations result in the quantitative reduction in the output of the gene. These result in the thalassemias characterised by reduced or absent globin chain synthesis. Qualitative mutations mainly result from single amino acid substitution in the globin protein that alters the amino acid sequence of the protein produced. The qualitative mutations give rise to haemoglobin variants such as Hb C, Hb E and Hb S. Infrequently, the qualitative mutations may be associated with multiple amino acid substitutions, deletions, antitermination mutations and altered posttranslational processing (Hoffbrand *et al.*, 2016). Hb S causes the SCD. SCD will be discussed in the following sections.

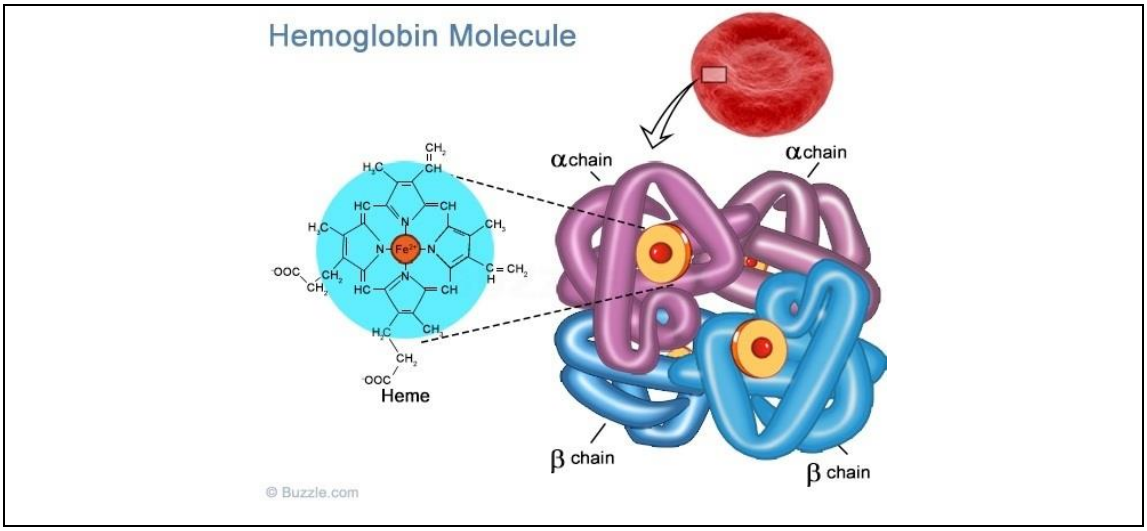


Figure 2. 6: Structure of the haemoglobin molecule

Copied from https://allyouneedisbiology.wordpress.com/2019/03/17/color-blood/

2.4.2 Overview of sickle cell disease (SCD)

SCD was first described in 1910 by Dr. James Herrick in Chicago (Herrick, 2001). SCD is a genetic disorder caused by single mutation in the beta globin gene of haemoglobin. Individuals affected inherit one or two alleles with single nucleotide change (G**A**G to G**T**G) in the beta globin gene coding sequence, thereby forming an abnormal haemoglobin S (Hb S) instead of the normal haemoglobin A (Hb A) (Ingram, 1957). The abnormal haemoglobin S gives rise to sickled RBC (Figure 2.7).

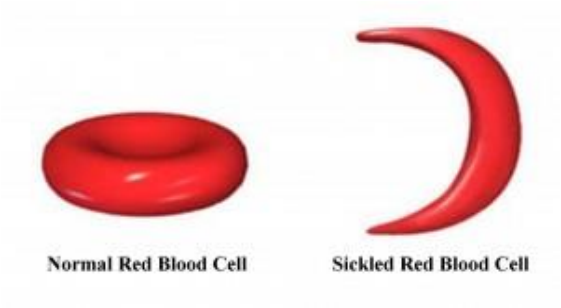


Figure 2. 7: The shape of sickled RBC in comparison with a normal RBC

Copied from http://www.idph.state.il.us/HealthWellness/fs/hemoglobin_sc.htm

The A to T nucleotide change that gives rise to sickle haemoglobin is a qualitative mutation that substitutes valine for glutamic acid at position 6 of the beta globin

chain. The inheritance of a single gene coding for the synthesis of Hb S from one parent and one coding for the normal Hb A from the other parent results in the sickle cell trait (SCT). SCD occurs when there is a homozygous inheritance of the Hb S, (Hb SS) or compound heterozygous inheritance with other abnormal haemoglobin variants (C, D, E) or the thalassaemia. The common haemoglobin genotypes that give rise to the SCD disease are Hb SS, Hb SC, Hb Sβ^+ thalassaemia and Hb Sβ^0 thalassaemia. Other rare genotypes include Hb SE and Hb SD.

The most common and severe form of the SCD is the homozygous inheritance of the Hb S (Hb SS). This form of sickle cell disease is known as sickle cell anaemia (SCA).

2.4.3 Epidemiology of sickle cell disease

The sickle cell gene is common among people from SSA, India, Saudi Arabia, and Mediterranean countries. SCD was initially classified as an inherited disorder of the tropics and subtropics, but due to population migration from regions of high Hb S to areas with low Hb S frequency, it is now a global condition affecting millions of people worldwide (Hoffbrand *et al.*, 2016). It is predominant in areas of the world where malaria is or was endemic, as the sickle cell gene offers protection against severe malaria (Aidoo *et al.*, 2002; Williams *et al.*, 2005).

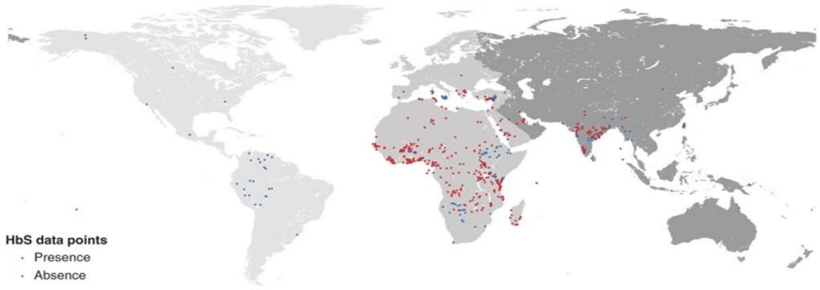


Figure 2. 8: Map showing the global distribution of SCD as of 2010
Red dots represent the presence of the HbS gene and the blue dots represent its absence. The Americas are shown in light grey, Africa, including the western part of Saudi Arabia, and Europe in medium grey and Asia in dark grey.
Copied from Piel *et al.* (2010). Global distribution of the sickle cell gene and geographical confirmation of the malaria hypothesis, Nature communications, 1, p. 104.

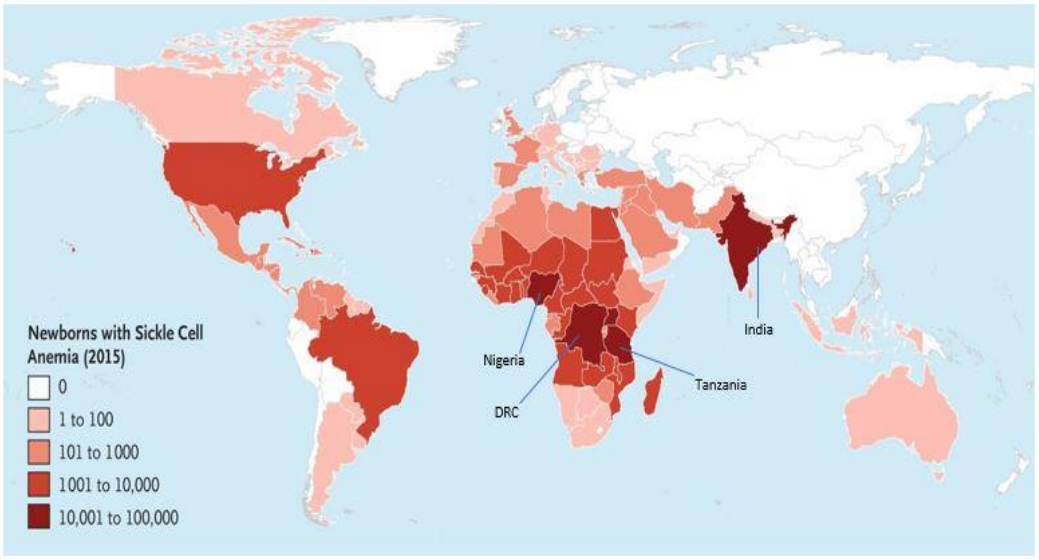


Figure 2. 9: Map showing the global births of of infants with SCD in 2015

Adapted from Piel, Steinberg and Rees (2017). Sickle cell disease, N Engl J Med, 376(16), pp. 1561-1573.

Globally, it is estimated that 550,000 neonates are born with the SCT annually and approximately 400,000 and 300,000 with SCD and SCA respectively (Piel *et al.*, 2013b). More than 75% of these births are currently in SSA (Figure 2.8) and has been predicted to rise to 88% by the year 2050 (Piel *et al.*, 2013a). Areas of Africa, that lie between latitude 15^0 North and latitude 20^0 south have the highest prevalence of the SCT, ranging from 10%-40% (Agasa *et al.*, 2010). More than 50% of the global sickle cell population is in three developing countries; Nigeria, Democratic Republic of Congo and India. Up to 30% and 3% of their population have the SCT and SCD respectively. Tanzania ranks as the fourth highest country with the SCD births globally (Figure 2.9) (Piel *et al.*, 2013a). SCT and SCD in other SSA countries such as Ghana, Cameroon and Gabon also range from 20% to 30% and up to 2% respectively (Ama *et al.*, 2012; Delicat-Loembet *et al.*, 2014). Less than 10% of the global SCD births occur in HIC (Tshilolo *et al.*, 2008; Odame, 2014; Kadima *et al.*, 2015).

Projections by Piel and his group indicated that the global population of patients with SCD is expected to increase by 2050 (Piel *et al.*, 2013a). This is mainly due to the gain in life expectancy in patients with SCD in HIC and the reduced childhood mortality in LMIC (achieved with the millennium development goal 4 which reduced

death in children under five years from 12.7 million in 1990 to approximately 6 million in 2015 (Millenium Development Goal, 2015)), allowing more children to survive to adulthood (Weatherall, 2010; Kyu *et al.*, 2016).

SCD, particularly, in West Africa is a major public health burden and is classified as a non communicable disease. In Ghana, SCD is included as one of the diseases covered under the non communicable disease control programme which is aimed at prolonging the quality of life of individuals and the population through reducing the incidence and morbidity of non communicable diseases and averting the complications and disability caused by non communicable diseases(Bosu, 2012).

2.4.4 Global distribution of the common sickle genotypes

Common haemoglobin genotypes of SCD are Hb SS, Hb SC, Hb Sβ^+ and Hb Sβ^0. The United Kingdom (UK) and the Americas, have a similar distribution of the common sickle genotypes with the highest proportion being the Hb SS genotype (67%-74%), followed in decreasing order by genotypes Hb SC, Hb Sβ^+ and Hb Sβ^0 (Minniti *et al.*, 2009; Sachdev *et al.*, 2011; Leite *et al.*, 2012). However, in other geographical areas, a different distribution pattern has been observed. Three studies in Senegal and Nigeria found very high proportions of Hb SS (87.6%-95.7%) compared to what was reported in the UK and United States (US) followed by Hb SC (3.6%-12.4%). The frequency of the Hb Sβ thalassemia was less than 1% (Diagne *et al.*, 2003; Aliyu *et al.*, 2008; Lagunju, Sodeinde and Telfer, 2012). Two studies in Burkina Faso reports that Hb SC (up to 90%) was the most prevalent genotype followed by Hb SS (up to 20%) (Simpore *et al.*, 2002b; 2002a). The high proportion of Hb SC in Burkina Faso may be attributed to the high prevalence of Hb C variant in West Africa as reported by Modiano et al., (2008). Newborn screening of 2053 babies in Tanzania found no Hb SC genotype (Rwezaula *et al.*, 2015). Further disparities have been observed in Greece and India where the most prevailing genotype was the Hb Sβ then the Hb SS (Voskaridou *et al.*, 2012). In a cohort from India, the highest proportion was Hb SS but Hb Sβ thalassemia accounted for almost one-third of the SCD population (Munshi *et al.*, 2009).

2.4.5 SCD mortality in HIC and LMIC

In the 1960s, Dacie described SCD as a childhood disease with few affected individuals living beyond their 10th birthday (Dacie, 1960). In the early 1970s, SCD median survival was estimated as 14.3 years. Mortality pattern based on autopsies showed that 20% of affected children died before 3 years, about 30% before 5years and 50% between age 5 and 30 years (Diggs, 1997).

Advocacy in HIC in the 1970's on the need to support SCD research, drew the attention of funders to the then neglected disease and this has helped improve SCD survival.

Currently, mortality in SCD varies markedly across the globe. Patients living in HIC have a reduced death rate and tend to survive to adulthood compared to their counterparts in developing countries. In the US, France and UK, life expectancy has increased by about 15 years over the last two decades (Elmariah *et al.*, 2014). In the US, about 94% of children survive to adulthood (Quinn, Rogers and Buchanan, 2004), whilst the UK has an almost 100% survival to adulthood (Telfer *et al.*, 2007). The median life expectancy for male and female patients with SCD in the US is up to 58 years (Elmariah *et al.*, 2014). Median survival in Jamaica stood at 53 and 58.5 years for men and women around 2001 (Wierenga *et al.*, 2001). In the UK, median age of survival was 42 for males and 48 for females in 1994 (Platt *et al.*, 1994) but was reported to have increased to 67 years in both sexes in 2016 (Gardner *et al.*, 2016).

These successes achieved in HIC may be attributed to the introduction of the national Newborn Screening programme; permitting early diagnosis, continued follow up and comprehensive management of patients diagnosed at birth or in early life (Modell and Darlison, 2008; Aygun and Odame, 2012).

Such significant improvements in mortality has not occurred in the SCD counterparts in SSA. Accurate statistics on mortality in SSA are scarce, but, it has been reported that approximately 50-90% of children with SCD die before their 5th birthday (Weatherall *et al.*, 2006; Grosse *et al.*, 2011). More than 6% of deaths in children below 5 years are attributed to SCD (Modell and Darlison, 2008). A report by Makani *et al.* (2011) from Tanzania, reaffirms this; they found that the highest mortality occurred in children less than 5 years old. Median survival in the cohort they studied was 33 years. Some qualitative reports have also stated that, the majority

or almost all children with SCA in Africa die in childhood (Ebrahim *et al.*, 2010; McAuley *et al.*, 2010).

The high rate of mortality observed in SSA compared to HIC is multifactorial. These include the lack of comprehensive SCD management programmes in SSA and the poor access to quality health care. The Newborn Screening programme, a programme that was launched in 1998, in the urban hospitals of some SSA countries, including Ghana, Burkina Faso, Benin, Congo and Nigeria, to screen for SCD at birth or early fetal life and provide appropriate prophylactic treatment, was successfully implemented (Kafando *et al.*, 2005; Ohene-Frempong *et al.*, 2008; Rahimy *et al.*, 2009), and yielded good results. Kuznik et al., (2016) in their cost effective analysis of the newborn screening and prophylactic intervention/programme across 47 SSA countries reported that, the estimated mean life expectancy of unscreened newborns with SCD living in rural and urban areas in SSA were 1.7 years and 6.7 years respectively and ranged from 24.2 years to 32.4 years in children who were identified by screening and treated (Kuznik *et al.*, 2016). Despite this success, the extension of the programme beyond the initial hospitals in the various countries has remained a challenge. The main challenge has been the lack of adequate funding; the pilot programmes were externally funded, therefore, upon completion of the pilot projects and withdrawal of funds, the participating countries lacked the adequate resources to sustain the screening programme. Also, extension of the screening programme to other district and rural areas requires dedicated implementation strategies designed to address the local needs of SSA which is currently unavailable (Kafando *et al.*, 2005; Tshilolo *et al.*, 2008).

2.5 Blood transfusions in patients with SCD: comparison of HIC and LMIC

RBC transfusion is vital in the management of patients with SCD (Miller *et al.*, 2013; Hoffbrand *et al.*, 2016). Although administration of hydroxyurea has improved patients' outcome, blood transfusion therapy remains an important treatment over the last decade and has shown to be associated with reduced morbidity and mortality. Most prominent is the prevention of stroke in children (Adams *et al.*, 1998; Wang *et al.*, 2005; Lee *et al.*, 2006). Transfusion of RBC increases blood oxygen carrying capacity and microvascular perfusion as well as

decreasing the number of circulating sickle cells to help alleviate painful crisis or complications (Davies and Roberts-Harewood, 1997; Wahl and Quirolo, 2009). Current transfusion practices (simple or exchange transfusions) are either episodic or chronic regimens. Episodic transfusions primarily stabilize or reverse acute complications of SCD (Ohene-Frempong, 2001) whilst chronic transfusions are long term (repeated every 3-4 weeks) prophylactic transfusions to prevent recurrent or future complications. The latter is commonly indicated for primary or secondary stroke prevention in children at high risk of stroke.

In HIC, blood transfusion in patients with SCD is an established treatment protocol governed by well documented guidelines (Davis *et al.*, 2017b; 2017a). The majority of patients with SCD are transfused either episodically or chronically. Transfusions may also be exchange and simple transfusions (Telen, 2001; Vishnubhotla, Zielinski and Swerdlow, 2003; Wahl and Quirolo, 2009). Close to 100% of donated blood are from voluntary non-remunerated donors (VNRD) (World Health Organisation, 2020). Donors are mainly Caucasian and racially unmatched to the mostly Black patients (Vichinsky *et al.*, 1990).

The opposite is observed in LMIC where blood transfusion is less frequent. LMIC, especially in Africa, are faced with additional challenges compared to more wealthy countries. There include a higher prevalence of transfusion-transmitted infections, and lack of adequate funds, equipment, reagent supply, training, and expertise. Transfusions are reserved for severe life-threatening anaemia and acute stroke management, mostly one unit of blood at a time (Kanagasabai *et al.*, 2018). Although some patients are transfusion dependent, chronic transfusion regimens are uncommon (Adewoyin, 2016; Weimer *et al.*, 2019). A recent communication with the head of the haematology clinic in one of the tertiary hospitals in Ghana revealed that, in the hospital's SCD clinic which has about 26,000 registered patients, less than 10 patients are on a chronic transfusion regimen. Transfusion practices are mostly simple transfusions; nonetheless, exchange transfusions may be given in some situations (Adewoyin, 2016). Blood donors and patients are mostly of the same Black background.

The main challenges to regular transfusion in patients with SCD in LMIC are blood availability and concerns of transfusion transmitted infections (TTIs) (Makani, Williams and Marsh, 2007). SSA is periodically faced with acute blood shortage and is thus unable to meet the annual blood requirements (Tapko, Toure and Sambo, 2014). In some SSA countries there is a lack of robust screening of allogenic blood for infection markers and a high reliance on first-time donors and family replacement donors who have a higher prevalence of TTI markers than repeat donors (Fernandes, D'souza and D'souza, 2010; Pallavi *et al.*, 2011) predisposing multi transfused patients to TTIs (Allain *et al.*, 2010). However, it is worth mentioning that trends are changing. Over the last decade, strategies have been developed to improve safety of allogenic blood, by careful selection of blood donors and rigorous screening of donated blood (Bloch, Vermeulen and Murphy, 2012; Weimer *et al.*, 2019). Efforts have been made to increase voluntary non remunerated donors (VNRD) and to retain them in the donor pool (Asamoah-Akuoko *et al.*, 2020). These measures are aimed at ensuring safety and an adequate supply of allogenic blood for transfusion (World Health Organisation, 2009). In addition, there have been increased blood donation campaigns in Africa to encourage potential donors and this resulted in an almost 50% increment in blood donations in the African region, between 2008 (2.95 million donations) and 2013 (4.34 million donations) (World Health Organisation, 2017).

2.6 Alloimmunisation in SCD

2.6.1 SCD and alloimmunisation

Multiple intermittent or chronic transfusions raise the concern of RBC alloimmunisation. RBC alloimmunisation is a major complication in transfused patients with SCD and presents significant challenges in their management. Depending on the age, RBC exposure and the extent of RBC antigen matching, alloimmunisation in patients with SCD has been reported to range from 7-47% (Aygun *et al.*, 2002; Sakhalkar *et al.*, 2005) and was up to 76% before the start of RBC antigen matching (Lasalle-Williams *et al.*, 2011).

2.6.2 Risks and genetic associations with alloimmunisation

In HIC, the development of alloantibodies is largely attributed to disparity in RBC antigens between the predominantly Black SCD population and the donor

population, which are mostly Caucasians (Vichinsky *et al.*, 1990; Gader, Al Ghumlas and Al-Momen, 2008; Shaz *et al.*, 2008). However, some patients, classified as non-responders, do not develop alloantibodies despite repeated RBC transfusions from donors with dissimilar antigen profiles (Higgins and Sloan, 2008). The human leucocyte antigen (HLA) type has been reported to play a role in alloantibody formation (Alarif *et al.*, 1986; Reisner *et al.*, 1987); however, the association observed by Reisner was weak. The HLA association with alloimmunisation was further supported by Hoppe *et al.* (2009) who found that, about 34% of alloimmunised SCD patients expressed the HLA-DRB1*1503 allele when compared with non-alloimmunised patients. HLA-DRB1*0901 allele was absent in all alloimmunised patients they studied but present in non-alloimmunised patients suggesting an increased risk of alloimmunisation in patients with SCD who expressed the HLA-DRB1*1503 allele (OR 2.02, p=0.039) whilst HLA-DRB1*0901 appeared to offer some form of protection to developing RBC antibodies (OR 0.13, p=0.008). Further, other studies have also reported a positive association between the HLA DRB1*15 phenotype (Verduin *et al.*, 2016), HLA DRB1*13, DRB1*03 DQB1*06, DQB1*02 and multiple alloantibody formation in responders (Maluskova *et al.*, 2017).

The patients' inflammatory status and genetic polymorphism have been reported to contribute to alloantibody development. A high rate of alloimmunisation was observed in patients with SCD who displayed increased levels of inflammation compared to those who had no inflammation. Patients with high inflammatory levels were defined as those with acute chest syndrome, acute stroke, acute febrile illness in the absence of another SCD co-morbidity, splenic sequestration, aplastic crisis, priapism, vaso-occlusive crisis with and without leukocytosis and elective surgery (Fasano *et al.*, 2015). On the other hand, Desai *et al.* (2015), reported that, patients with vaso-occlusive complications did not have increased alloimmunisation. Desai and colleagues rather observed a positive correlation between alloimmunisation and the age of RBC's transfused. This correlation between alloimmunisation and age of RBC transfused had been previously reported in mouse model by Hendrickson *et al.* (2010) but was disagreed by Yazer and Triulzi (2010) and Zalpuri *et al.* (2013) Zohreh et al., (2013) found a strong association between alloimmunisation and two SNP's (rs708564 and rs2237863) in the CD81 gene. The polymorphism -318C/T of

the CTLA-4 gene have also been reported to be associated with alloimmunisation (Oliveira *et al.*, 2017). A study among Brazillian patients with SCD also found that patients with polymorphisms in the *TNFA*, *IL1B,* and *HLA-DRB1* gene were at higher risk of alloimmunisation than patients who did not have the polymorphisms (Sippert *et al.*, 2017).

Other reported risk factors for alloimmunisation are recipients' sex, age of first transfusion and exposure to episodic transfusions. Murao and Viana (2005) reported that females have higher risk of alloimmunisation than males. Sins *et al.* (2016), identified that patients with SCD whose first transfusions occurred at the age of 5 years and above were at higher risk of developing RBC alloantibodies than patients with SCD whose first transfusions were below age 5 years. Sins and colleagues further observed that patients with SCD exposed to episodic transfusions were at 3-fold higher risk of forming RBC antibodies than those enrolled in a chronic transfusion programme.

2.6.3 Consequences of alloimmunisation in patients with SCD

Alloimmunisation may pose challenges to finding compatible blood for transfusion thereby delaying transfusion therapy or even preventing it. It increases the risk of delayed haemolytic transfusion reaction (DHTR) in about 4-11% of patients with SCD (Talano *et al.*, 2003; Chadebech *et al.*, 2009). In patients with SCD, DHTR tends to mimic sickle cell crises (Garratty, 1997; Scheunemann and Ataga, 2010; de Montalembert *et al.*, 2011), the main treatment of which is transfusion. Thus, if DHTR is undetected and patients are transfused again, this may further exacerbate the haemolytic episode and complications (Friedman, Kim and Manno, 1993).

Alloimmunised patients with SCD are also at increased risk of developing additional antibodies (Schonewille *et al.*, 2006) and autoantibodies (Garratty, 2004; Young *et al.*, 2004). Pregnancies in alloimmunised women with SCD may also be complicated by haemolytic disease of the foetus/new born (HDFN). A study in pregnant women with SCD receiving prophylactic transfusions showed that alloimmunisation complicated 5.3% of the pregnancies, leading to severe anaemia and eventual intra uterine death of the foetus within 24 hours (Ngô *et al.*, 2010). Howard et al., (1995) reported two cases of DHTR among 6 (out of 49) pregnant patients with SCD who

became alloimmunised following RBC transfusion during pregnancy, a situation that can endanger both mother and foetus.

3.0 CHAPTER 3: RED BLOOD CELL ALLOIMMUNISATION IN PATIENTS WITH SICKLE CELL DISEASE IN SUB-SAHARAN AFRICA: A SYSTEMATIC REVIEW AND META ANALYSIS

This chapter is already published in Transfusion Medicine Reviews. Full article available at doi: 10.1016/j.tmrv.2019.06.003.

3.1 Brief introduction of chapter

Blood transfusion therapy remains an important part of sickle cell disease (SCD) management; to treat or prevent SCD related complications (Wang *et al.*, 2005; Lee *et al.*, 2006; Wahl and Quirolo, 2009). Red blood cell (RBC) alloimmunisation against foreign RBC antigens in transfused blood remains a major complication in these patients and presents significant challenges in their management.

Most studies reporting on RBC alloimmunisation 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 Sub-Saharan Africa (SSA), where antibody screening is not routinely performed, data on the prevalence of RBC alloimmunisation in patients with SCD are very scarce.

In this chapter, we performed a systematic review of the literature and a meta-analysis to determine the published prevalence of RBC alloimmunisation in SCD patients in SSA. Patients with SCD were selected in this study because they represent the group of transfused patients with the highest risk of alloimmunisation, (up to 76%) due to their lifelong conditions predisposing them to the likelihood of more alloantigen exposure and high level of inflammation (Olujohungbe *et al.*, 2001; Aygun *et al.*, 2002; Lasalle-Williams *et al.*, 2011; Fasano *et al.*, 2015) compared to 1-10% in other transfused populations (Schonewille, Haak and van Zijl, 1999; Alves *et al.*, 2012).

3.2 Methodology

3.2.1 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 alloimmunisation and alloantibody specificities.

3.2.2 Search strategy and study selection:

The review was conducted following the PRISMA flow diagram (Figure 3.1) 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 people.

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, alloimmunisation, 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. Finally,

references of selected articles were searched for any other articles relevant to the studies.

3.2.3 Data extraction

Data extraction was conducted independently by two people 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 3.1).

3.2.4 Quality assessment

The quality of each included study was independently assessed by two people and 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 (Hlela *et al.*, 2009). 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) (Table 3.2).

3.2.5 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) (Schwarzer, 2015). 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 (Fleiss, 1993). 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% (Higgins and Thompson, 2002). 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 odds (Hunter *et al.*, 2014). 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.

3.3 Results

3.3.1 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 (methodology not clearly explained, antibodies were tested at 4^{o}C and antibody specificities were reported in only 18 out of 32 patients with antibodies) of one study (Akre *et al.*, 2008), 15 studies were included in the review, thirteen were in the English and two in the French language (Figure 3.1) (Kuliya-Gwarzo, Akanmu and Dutse, 2005; Batina Agasa *et al.*, 2010; Natukunda *et al.*, 2010; Abbas *et al.*, 2013; Diarra *et al.*, 2013; Meda *et al.*, 2014; Eldour *et al.*, 2015; Kangiwa *et al.*, 2015; Mangare *et al.*, 2015; Ugwu *et al.*, 2015; Adewoyin, 2016; Boma Muteb *et al.*, 2017; Sekongo *et al.*, 2017; Siransy *et al.*, 2018; Boateng *et al.*, 2019a).

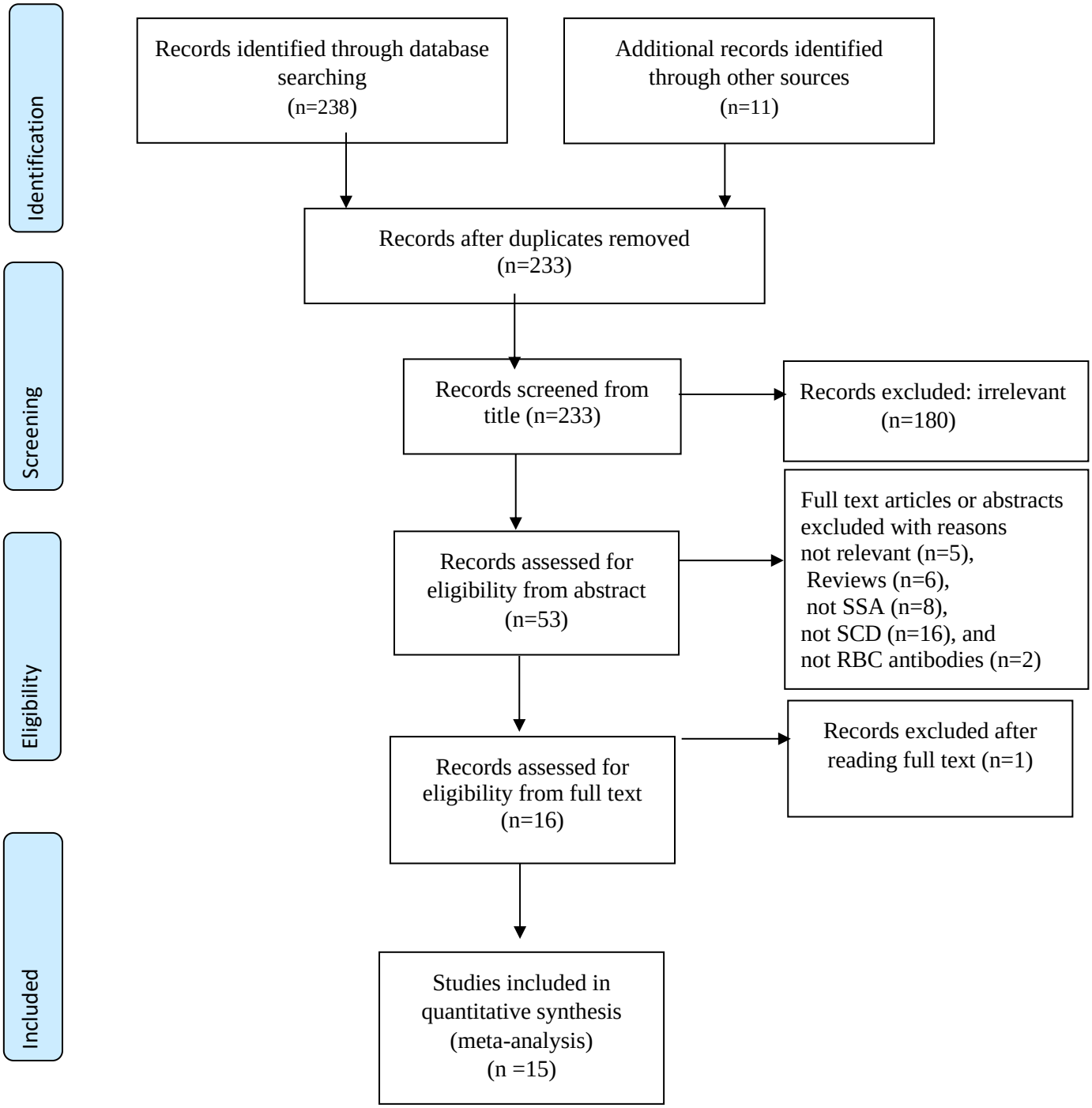


Figure 3. 1**:** Summary of data extraction history

3.3.2 General studies descriptions

The 15 studies were published between 2005 and 2019. Except for the retrospective study by Boma Muteb and collegues, all others had a cross-sectional design, with regard to determination of alloimmunisation frequency (Table 3.1), and all were of moderate quality (i.e. score 4-6) (Table 3.2). The studies were conducted in nine SSA countries. Between two and four studies were conducted in each of four countries. These ten studies were conducted in eight medical centers from different parts of the four countries. However, two studies from Nigeria (Ugwu *et al.*, 2015; Adewoyin, 2016) and the two studies from Cote d'Ivoire (Sekongo *et al.*, 2017; Siransy *et al.*, 2018) were from the same medical centers. Although the study periods differed by about four years, patient overlap cannot be excluded.

The sample size per study ranged from 27 to 428 transfused patients. Eight studies included only individuals with the SS genotype (Batina Agasa *et al.*, 2010; Natukunda *et al.*, 2010; Abbas *et al.*, 2013; Meda *et al.*, 2014; Eldour *et al.*, 2015; Ugwu *et al.*, 2015; Adewoyin, 2016; Boma Muteb *et al.*, 2017), five had a mixture of the SCD genotypes (i.e. SS, SC, Sβthal) (Diarra *et al.*, 2013; Adewoyin, 2016; Sekongo *et al.*, 2017; Siransy *et al.*, 2018; Boateng *et al.*, 2019a) and the remaining two (Kangiwa *et al.*, 2015; Mangare *et al.*, 2015) did not report on the SCD genotype of participants. While 13 studies included children and adults, Abbas *et al* ., studied only children and Ugwu, only adults. Overall participants ages ranged from one to fifty-five years. Nine studies used the column gel technique (CGA) (Natukunda *et al.*, 2010; Diarra *et al.*, 2013; Kangiwa *et al.*, 2015; Mangare *et al.*, 2015; Ugwu *et al.*, 2015; Boma Muteb *et al.*, 2017; Sekongo *et al.*, 2017; Siransy *et al.*, 2018; Boateng *et al.*, 2019a) antibody testing, three, the tube technique (TT) (Abbas *et al.*, 2013; Meda *et al.*, 2014; Adewoyin, 2016), one used both TT and CGA (Kuliya-Gwarzo, Akanmu and Dutse, 2005) and two studies did not report the antibody testing technique (Eldour *et al.*, 2015; Boma Muteb *et al.*, 2017) . All screening and identification reagents were procured from international sources except for the studies performed by Sekongo *et al*., and Kaliyu *et al*.,, which utilized screening and/or identification cells produced at their centre.

3.3.3 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 (Diarra *et al.*, 2013; Eldour *et al.*, 2015; Ugwu *et al.*, 2015; Boma Muteb *et al.*, 2017). 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 (Mangare *et al.*, 2015; Adewoyin, 2016; Boma Muteb *et al.*, 2017; Siransy *et al.*, 2018)

Table 3. 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)	Country (region)	N of Patients/ Transfused patients	Male to femal	Patient age (SD; range)	RBCs transfused (SD; range)	Antibody test method	N of Patients with allo-antibodies	N of allo-antibodies: antibody specificities
Batina Agasa (2010)	Congo (Central)	144 / 127	1.26†	Mean 15.5 (11.1; 4-63)	Mean 5.3 (6.6; 1-40)	CGA-LISS	13 (10.2; 5.6-17)	17: 3D, 3C, 2E, 9UI
Boma Muteb (2017)	Congo (Central)	39 / 39	0.77	Mean 8.6 (6.4;2-27)	82% >2 units (NR; NR)	NR	1 (2.6; 0.1-15.1)	1: 1K
Mangare (2015)	Kenya (East)	137 / 137	0.99	Mean 8 (NR; 1-36)	Mean 2.4 (NR; 1-8)	CGA-NaCl/LISS	4 (2.9; 0.9-7.8)	4: 1C^{w}, 1S, 1M, 1Co^{b}
Abbas (2013)	Sudan (East)	100 / 100	1.33	Median 2-8 (NR; 0.5-17)	Mean 6.7; Median 5 (4.5; 2-20)	TT NaCl/LISS	4 (4.0; 1.3-11)	4: 2K, 1C, 1E
Eldour (2015)	Sudan (East)	210 / 210	1.44	Median 2-5 (NR; 2-20)	≥2 units (NR; NR)	NR	9 (4.3; 2.1-8.3)	10: 5K, 2E, 1c, 1Kidd‡, 1Le^{b}
Meda (2014)	Tanzania (East)	365 / 365	0.81	Median 16 (NR; 0.5-49)	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, 3E, 3C^{w}, 3Lu^{a}, 2C, 2Le^{b}, 2S, 2s, 2Jk^{b}, 2P, 1M, 1N, 1Jk^{a}, 1PAN, 1UI
Natukunda (2010)	Uganda (East)	428 / 428	1.04	Median 12 (NR; 2-44)	Median 3 (NR; 2-100)	CGA-LISS	26 (6.1; 4.1-8.9)	32: 10E, 7D, 4S, 2C, 2Jk^{a}, 2PAN, 1K, 1Fy^{a}, 1Le^{a}, 1C^{w}, 1M
Sekongo (2017)	Cote d'Ivoire	42 / 42	1.00	Mean 24.5 (NR; 4-68)	Mean 9, Median 6-10 (NR; 1-22)	CGA-LISS	12 (28.6; 16-45)	14: 6E, 4C, 1D, 1e, 1S, 1UI
Siransy (2018)	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)	Ghana (West)	154 / 154	1.30	Median 9 (NR; 1-50)	Median 2-4 (NR; 1->10)	CGA-LISS	10 (6.5; 3.3-11.9)	13: 3D, 3M, 2E, 2C, 1e, 1C^{w}, 1UI
Diarra (2013)	Mali (West)	133 / 90	0.73†	Mean 21† (NR; 1-62)	NR	CGA (E-NR)	4 (4.4; 1.4-12)	4: 2C, 1D, 1c
Kuliya-Gwarzo (2005)	Nigeria (West)	135 / 68	1.52	Mean 14 (6.5; NR)	Median 1-5 (NR; 1-10)	TT-Alb and CGA	6 (8.8; 3.6-18.9)	11: 2D, 2E, 1Kp^{b}, 1Js^{b}, 1Wr^{a}, 1M^{g}, 1Vw, 1Di^{a}, 1Go^{a}
Kangiwa (2015)	Nigeria (West)	120 / 80	0.78	Median 21† (NR; 1-50)	Mean 3 (NR; 2-25)	CGA-LISS and enzymes	15 (18.8; 11-29)	13: 2E, 2c, 2e, 1D, 1Fy^{a}, 1Fy^{b}, 1k, 1Kp^{b}, 1Js^{b}, 1Lu^{a}
Ugwu (2015)	Nigeria (West)	86 / 86	1.10	Mean 26 (7.4; 18-48)	≥2 units (NR; 2-NR)	CGA-LISS	8 (9.3; 4.4-18)	11: 3E, 2C, 1D, 1e, 1k, 1Kp^{a}, 1Js^{b}, 1Lu^{b}
Adewoyin (2016)	Nigeria (West)	55 / 41	1.39†	Mean 23† (12; 2-51)	Mean 4.5; Median 2 (7.6; 1-55)	TT (E-NR)	4 (9.8; 3.2-24)	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 3. 2: Quality assessment of eligible studies

Author (year)	External validity				Internal validity					Total sum	Grade
	a	b	c	sum	d	e	f	g	sum		
Abasss et al, 2013	yes	yes	yes	3	yes	yes	no	yes	3	6	B
Adewoyin, 2016	yes	no	yes	2	yes	yes	no	yes	2	4	B
Batina Agasa et al., 2010	yes	no	no	1	yes	yes	no	yes	3	4	B
Diarra et al., 2013	yes	no	yes	2	yes	yes	no	yes	3	5	B
Eldour et al., 2015	yes	no	yes	2	yes	yes	no	yes	3	5	B
Kaliyu et al., 2005	yes	no	yes	2	yes	yes	no	yes	3	5	B
Kangiwa et al., 2015	yes	no	yes	2	yes	yes	no	yes	3	5	B
Mangare et al., 2015	yes	no	yes	2	yes	yes	no	yes	3	5	B
Meda et al., 2014	yes	yes	yes	3	yes	yes	no	yes	3	6	B
Natukunda et al., 2010	yes	no	yes	2	yes	yes	no	yes	3	5	B
Sekongo et al., 2017	yes	yes	yes	3	yes	yes	no	yes	3	6	B
Siransy et al., 2018	yes	no	yes	2	yes	yes	no	yes	3	5	B
Ugwu., 2015	yes	yes	yes	3	yes	yes	no	yes	3	6	B
Boateng et al., 2019	yes	no	yes	2	yes	yes	no	yes	3	5	B
Boma Muteb et al., 2017	yes	no	yes	2	yes	yes	no	yes	3	5	B

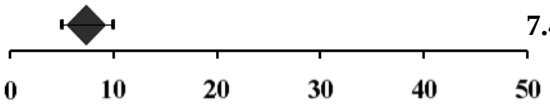
External validity: - a: definition of disease or outcome (alloimmunisation) b: Description of the selection criteria. Was there a clear description of the sampling method used? c: Description of the study population: Are important characteristics (2 or more such as age, ethnicity, gender, socioeconomic status, donor status, etc…) of the population specified?
Internal validity: - d: Type of study: Is it an applicable study design for the question asked? e: Reference standard: Were the screening test results confirmed? f: Reported prevalence: Are gender-specific and age-specific prevalence reported? g: Reproducibility: Evidence for minimization of instrument bias
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).

3.3.4 Prevalence of RBC alloimmunisation

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 (Kangiwa *et al.*, 2015; Sekongo *et al.*, 2017; Siransy *et al.*, 2018) reported high prevalences of RBC alloimmunisation (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).

Table 3. 3 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

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				



3.3.5 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 ($I^2 = 70\%$, $p = 0.01$) (Table 3.4). The leave out sensitivity analysis indicated that no single study influenced the overall outcome nor heterogeneity (Table 3.5).

Table 3. 4: Proportion estimates of RBC alloimmunisation in transfused patients with sickle cell disease by region

			Heterogeneity			**Random effects model**		**Meta-regression**	
		Number of studies; patients	***I*² (%)**	**τ²**	**p-value**	**Proportion (95% CI)**	**% Weight**	**β**	**P-value**
All studies		15; 1994	72	0.005	<0.01	7.4 (5.1-10.0)	100		
Region									0.0048
	Central	2; 166	57	0.0055	0.13	6.8 (1.4-15.2)	12.0		
	West	8; 588	70	0.0082	<0.01	11.4 (6.8-16.9)	46.6	0.0633	0.3125
	East	5; 1240	0	0	0.60	4.6 (3.4-5.8)	41.3	-0.0680	0.2768

Table 3. 5: Leave-one-out sensitivity analysis of 15 studies included in the review

Study	Prevalence (95% CI)	I^2 (95% CI)
All	**7.40 (5.13-10.01)**	**71.7 (52.3; 83.2)**
(-) Siransy (2018)	7.08 (4.89-9.61)	71.1 (50.4; 83.2)
(-) Boma Muteb (2018)	7.70 (5.32-10.45)	73.3 (54.5; 84.3)
(-) Adewoyin (2016)	7.32 (4.99-10.01)	73.2 (54.4; 84.3)
(-) Sekongo (2017)	6.50 (4.65-8.61)	60.4 (29.0; 78.0)
(-) Kaliyu (2005)	7.34 (4.98-10.08)	73.3 (54.6; 84.3)
(-) Kangiwa (2015)	6.63 (4.63-8.94)	64.9 (38.0; 80.1)
(-) Ugwu (2015)	7.30 (4.93-10.04)	73.0 (54.1; 84.2)
(-) Diarra (2013)	7.68 (5.24-10.50)	73.4 (54.8; 84.4)
(-) Abbas (2015)	7.73 (5.28-10.55)	73.2 (54.3; 84.2)
(-) Batina (2010)	7.20 (4.86-9.93)	72.2 (52.4; 83.7)
(-) Mangare (2015)	7.86 (5.43-10.66)	71.6 (51.2; 83.4)
(-) Boateng (2019)	7.55 (5.07-10.43)	73.7 (55.3; 84.5)
(-) Eldour (2015)	7.79 (5.29-10.68)	72.6 (53.1; 83.9)
(-) Meda (2014)	7.58 (5.43-10.01)	59.8 (27.6; 77.6)
(-) Natukunda (2010)	7.69 (5.06-10.76)	73.6 (55.1; 84.5)

3.3.6 Publication bias

Visually, the funnel plot looked symmetric (Figure 3.2) and the p value for Egger's linear regression test was 0.86, indicating no evidence of publication bias.

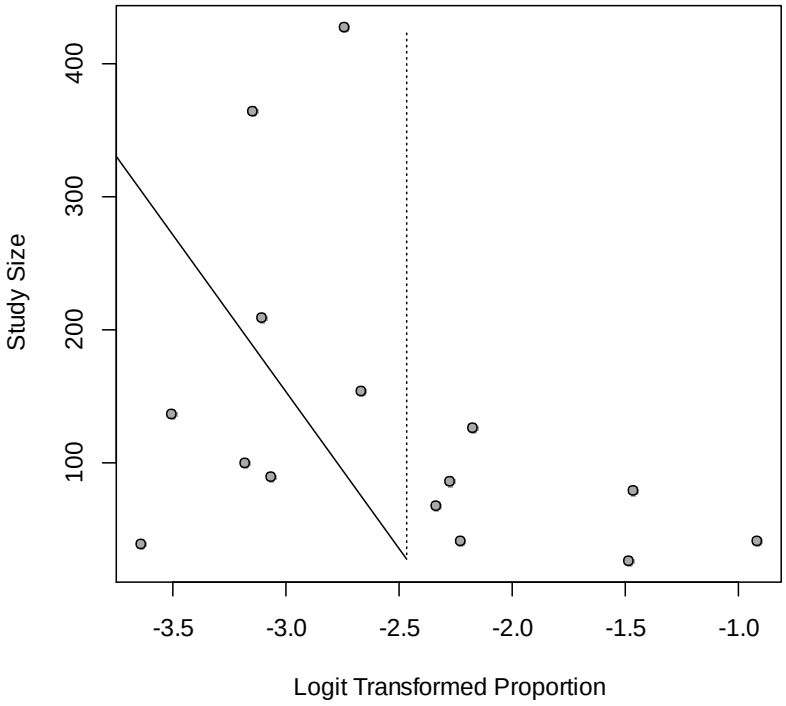


Figure 3. 2**:** Funnel plot of sample size against log odds

3.3.7 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 (Reid, Lomas-Francis and Olsson, 2012) represented 20% and 9% of all specificities, respectively (Table 3.3).

Table 3. 6**:** 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.

3.4 Discussion

This systematic review identified and analyzed 15 cross-sectional studies on RBC alloimmunisation in transfused patients with SCD from nine different SSA countries. The pooled prevalence of RBC alloimmunisation 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 alloimmunisation 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 alloimmunisation in the transfused SCD population in SSA.

3.4.1 Prevalence of RBC alloimmunisation

In a recent review, performed in the general transfused patients in SSA, of which 31% were patients with SCD, the pooled prevalence of alloimmunisation was 6.7% (95% CI: 5.7-7.8) which is comparable to our prevalence of 7.4% (Ngoma *et al.*, 2016). In well-resourced countries, the prevalence of alloimmunisation 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 (Olujohungbe *et al.*, 2001; Fasano *et al.*, 2015). 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 (Fasano *et al.*, 2019). There have been proposals to apply this policy for patients with SCD in SSA, (Kuliya-Gwarzo, Akanmu and Dutse, 2005; Abbas *et al.*, 2013; Ugwu *et al.*, 2015; Adewoyin, 2016; Sekongo *et al.*, 2017). 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.

3.4.2 RBC alloantibody specificity

The observation that anti-E, -D, -C and -K represented almost 50% of antibody specificities in our review is comparable to alloimmunisation rates reported among the general multi transfused patients in SSA (Ngoma *et al.,* 2016) and in non-African countries, despite the frequent Rh and K antigen matched transfusions in the latter (da Cunha Gomes *et al.,* 2018).

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.,* (2010), found that most of their Ugandan patients with anti D had *RHD* variants. *RHD* variants have been reported to be common in individuals of African descent (Noizat-Pirenne and Tournamille, 2011). 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 (Chou *et al.,* 2013), 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 (2012) found the K antigen in 5.6% of 500 random samples from a Sudanese population (East Africa) whilst, studies in Nigeria and Cote d'Ivoire, which are both in West Africa, found lower frequencies, ranging from zero to 2% (Kulkarni *et al.,* 1985; Ugboma and Nwauche, 2009; Siransy Bogui *et al.,* 2014; Osaro *et al.,* 2015; Adewoyin *et al.,* 2018).

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 (Reid, Lomas-Francis and Olsson, 2012). 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 haemolytic transfusion reactions (Larson *et al.*, 1996; Daniels, 2013). 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 (Mattos, 2011). 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 and sustainable means of RBC antibody testing in patients with SCD and other multi-transfused patients.

3.5 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 not have reached, or may have fallen below, detectable levels (Stack and Tormey, 2016). Most of the studies had incomplete descriptions of sampling methods so it was not possible to tell whether studies involved a bias, 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 alloimmunisation.

3.6 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) crossmatch prior to blood transfusion, however, I recognise that unavailability of funds and the need for additional equipment, reagent supply and human expertise may be limiting factors.

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 alloimmunisation risk such as sex, age of patients, number of transfusion events before alloimmunisation 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 haemolytic transfusion reactions (Barro *et al.*, 2018; Weimer *et al.*, 2019). This would help quantify the extent of the clinical consequences of alloimmunisation in patients with SCD.

3.7 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 at a lower frequency compared to those in well-resourced countries, where transfusions are given more frequently. It provides a baseline against which to compare the effect of any intervention to reduce the alloimmunsaition risk.

CHAPTER 4: PREVALENCE, SPECIFICITIES AND RISK FACTORS FOR RBC ANTIBODIES AGAINST EUROPEAN AND AFRICAN ANTIGENS AND ADVERSE TRANSFUSION EVENTS IN MULTI-TRANSFUSED PATIENTS WITH SCD IN GHANA (ANTIBODY STUDY)

Part of this chapter is already published as a letter to the editor in Haematologica. Letter available at doi:10.3324/haematol.2021.278451

4.1 Brief introduction of chapter

Red blood cell (RBC) antibody testing in Ghana and many sub-Saharan African (SSA) countries involve the use of test cells that are predominantly from donors of Caucasian descent that lack antigens that are more prevalent or exclusively present in Africans. This may miss antibodies to the antigens that are predominantly present in Africans.

Two systematic reviews, together including 20 studies and 3683 transfused patients, showed that the prevalence of RBC alloimmunisation in SSA varied between 2.6% and 29%. In the 255 alloimmunised patients, antibodies against common Rh antigens and against low and high frequency antigens accounted for almost 50%, 10% and 5% of all 331 antibody specificities, respectively (Ngoma *et al.*, 2016; Boateng *et al.*, 2019b). However, all these studies used standard test cells of European (Caucasian) origin to determine the prevalence of RBC antibodies and only one study reported on the presence of an anti-Go^{a}, of which the cognate antigen is exclusively found in Africans (Kuliya-Gwarzo, Akanmu and Dutse, 2005). The presence of antibodies against antigens that are almost exclusively present in Africans has not been tested systematically in an African setting, where both patients and donors are Africans. So far, only one study using African red cell antigens has been performed. That study was conducted in France and found that 5.2% of 211 patients with SCD, who had received a mean of 144 RH and K matched transfusions, which were in an estimated 11.5% from Afro-Caribbean donors, had formed antibodies against selected antigens (MNS6, RH10, RH20, RH23, RH30 and KEL6) of Afro-Caribbean descent (Floch *et al.*, 2018).

Compared to studies on transfusion transmissible infections, there is a paucity of studies on non-infectious adverse transfusion events in patients in SSA. The rates of (mainly acute) adverse transfusion reactions in non-selected transfusion recipients in SSA varies between 0.002% and 11.5% per transfused unit in retrospective studies (Ahmed, Kyari and Ibrahim, 2002; Natukunda, Schonewille and Sibinga, 2010; Ughasoro *et al.*, 2013; Mafirakureva *et al.*, 2014; Meza *et al.*, 2014) and between 8.7% and almost 60% in prospective studies

(Mbanya, Binam and Kaptue, 2001; Arewa, Akinola and Salawu, 2009; Gwaram and Abdullahi, 2013; Waiswa *et al.*, 2014; Owusu-Ofori, Owusu-Ofori and Bates, 2017; Sawadogo *et al.*, 2018). Only two cross-sectional studies reported on acute transfusion reactions in patients with SCD in SSA, which occurred in 5.8% and 23.8% of patients (Kangiwa *et al.*, 2015; Ugwu *et al.*, 2015).

In this chapter, we determined the prevalence, and specificities for RBC antibodies against antigens that are more frequent in Europeans and Africans and adverse transfusion events in multi-transfused patients with SCD in Ghana.

4.2 Methodology

4.2.1 Study site

The study was conducted at the sickle cell clinics of KBTH and KATH.

4.2.2 Study population

The study population comprised of patients with SCD. The inclusion and exclusion criteria are described below:

Inclusion criteria

All consenting patients with SCD of any SCD genotype (Hb SS, Hb SC, Hb SD, Hb $S\beta^+$ thalassaemia and Hb $S\beta^0$ thalassaemia) and ethnicity who:

- were two years and above. This age was selected to relieve the very young children the difficulty of blood sampling.
- had at least two RBC transfusions with the last transfusion not less than two weeks before recruitment. This criterion allowed the selection of patients that were likely to become alloimmunised after an appropriate time of exposure to RBC antigens

Exclusion criteria

The study excluded non-transfused patients with SCD and those who had had only one RBC transfusion.

4.2.3 Recruitment strategy

Every consecutive patient with SCD who attended one of the two clinics (that is KBTH and KATH sickle cell clinics) (23rd July to 17th December 2018) and met the inclusion criteria was invited to participate.

Prospective participants were approached when they arrived at the clinic and were in a queue waiting to be seen by the clinician. The principal investigator (myself) explained the study to

them in the local language or English according to their preference using the information outlined in the participant information leaflet (appendix II). They were given time to think this through, discuss with relevant family and friends and consider their participation. After seeing the clinician, they were reapproached for their decision. If they agreed to participate, written informed consent/assent (appendix I) was taken.

Data Collection

Participants' basic demographic characteristics, SCD genotype (determined by the hospital using the high-performance liquid chromatography method), number of previous transfusions and transfusion facilities, age at first transfusion, and history of pregnancy in females were retrieved from patients' hospital files. Patients or children's caretakers provided us with the above information, if missing from the hospital file, using a standard questionnaire (appendix III). Information on patients' previous adverse reactions to transfusion were mostly not complete or not documented in the patients' files held at the clinic. This was because patients might have reported to different hospitals when they experienced the reaction after their discharge or since the hospital uses only paper based documentation system, patients' old files in which some of the reactions were documented were missing or unretrievable. Patients or their caretakers were therefore asked if they recalled adverse events during or after transfusions, i.e. adverse reactions to transfusions (defined as any undesirable and unintended reaction which occurred during or after a blood transfusion and may be related to the administration of the blood) and other events (such as blood too warm or too cold, blood not flowing well, etc), if transfusions were stopped abruptly and if patients had to return to hospital within two weeks after transfusion. Patients were asked if they had experienced one or more of the following, during, within or beyond 24 hours after transfusion: fever, chills, breathlessness, lower back pain, dark urine, rolling tongue, skin eruption, rashes, chest pain, abdominal pain, itching, swelling and reddening of eyes, vomiting and joint pains. Signs suggestive of haemolytic reactions were dark urine, and abdominal/lower back pain. Classification of signs and symptoms into the types of transfusion reactions was based on the Serious Hazards of Transfusion classification, a standardized classification system for adverse events of transfusions. All data were recorded on the patient questionnaire (appendix III) . Information recorded on the questionnaire was entered into an Excel spreadsheet. This was double checked with what was recorded on the questionnaire weekly to ensure data were

complete and correct. Patients with incomplete data were contacted to fill in the missing information.

4.2.4 Blood sample collection

A trained phlebotomist collected 10ml venous blood sample from consenting patients into ethylenediaminetetraacetic acid (EDTA) tubes using a butterfly needle.

4.2.5 Blood samples processing

Blood samples from the patients with SCD were separated into plasma, buffy coat and packed red cells. Briefly, whole blood samples were centrifuged at 4000rpm for 20 minutes, after which plasma was aliquoted into two separate 2ml cryotubes, followed by buffy coat, also into two separate 2ml cryotubes. The remaining red cells were prepared into red cell droplets in liquid nitrogen. The protocol for preparation of the droplet red cells is attached as an appendix (appendix VI).

4.2.6 Samples storage and transport

All cryotubes containing plasma, buffy coat and droplet RBCs were frozen at -80^{o}C either in the serology lab of KATH (Kumasi centre) or in the haematology lab of KBTH (Accra centre). The frozen cryotubes were subsequently shipped on dry ice to Sanquin, Netherlands, for laboratory testing.

4.2.7 Laboratory testing:

Laboratory tests performed under the antibody study were:

1. RBC antibody screening and identification tests on all patients' plasma, using standard Caucassian RBC reagent panel and selected known African red cell antigens,
2. Direct antiglobulin test (DAT) on patients in whom pan reactive results were obtained during RBC antibody testing.
3. Genomic DNA extraction from buffy coat of patients who made antibody D,
4. Rh D genotyping using the Multiplex Ligation-dependent Probe Amplification (MLPA) assay was performed for patients who made antibody to the D antigen. DNA sequencing was performed if the MLPA results were inconclusive. This is because the D antigen is routinely matched prior to transfusions in Ghana, so patients were not expected to make antibody to the D antigen. Genotyping of the D gene helped to explain why those patients made the antibody to the D antigen.

All laboratory tests for the antibody study were performed in Sanquin. I performed the antibody screening and identification testing, assisted by Peter Ligthart, and part of the genomic DNA extraction (the remaining was done by a robot).The MLPA assay was carried out by Ahmad Javadi and I and the DNA sequencing was done by Aicha Soussan and I.

The details of the laboratory tests for the antibody study are discussed in the following sections

4.2.7.1 RBC antibody testing using standard Caucassian red blood cell reagent panel

Antibody screening and identification tests were performed at the diagnostic department of the Experimental Immunohaematology lab in Sanquin, using the column gel agglutination technique. Antibody detection involved antibody screening, using a three-cell screen panel (S I, II and III, Biorad) in a low ionic strength solution (LISS) indirect anti-globulin test (Biorad Gel system, Biorad, Switzerland). A screen was positive if agglutination of reagent screening cells occurred after the manufacturer's recommended incubation period. For a positive screening test, antibody identification was completed using selected cells of 6-8 cells in a panel in a low ionic strength indirect (LISS) anti-globulin test (Diamed Gel system, Dia-Med-ID, Switzerland). Reactivity was documented with standard agglutination grading. (i.e 0 to 4+). The degree of reactivity in the gel was graded according to the following scale:

0, all RBCs at the bottom of the gel;

± or 0.5 or weak, very small amount of RBC agglutinates just above the pellet on the bottom
1+, small amount of RBC agglutinates in the lower half of the gel but most RBCs pelleted at the bottom of the gel;
2+, RBC agglutinates dispersed throughout the gel column,
3+, agglutinated RBC in the upper half of the gel column
4+, solid band of RBC on the top of the gel column
Double population or mixed field reactions, mix of agglutinates at the top of the column (4+/3+ like) with RBCs at the bottom (like a negative reaction). No RBC agglutinates through the column.

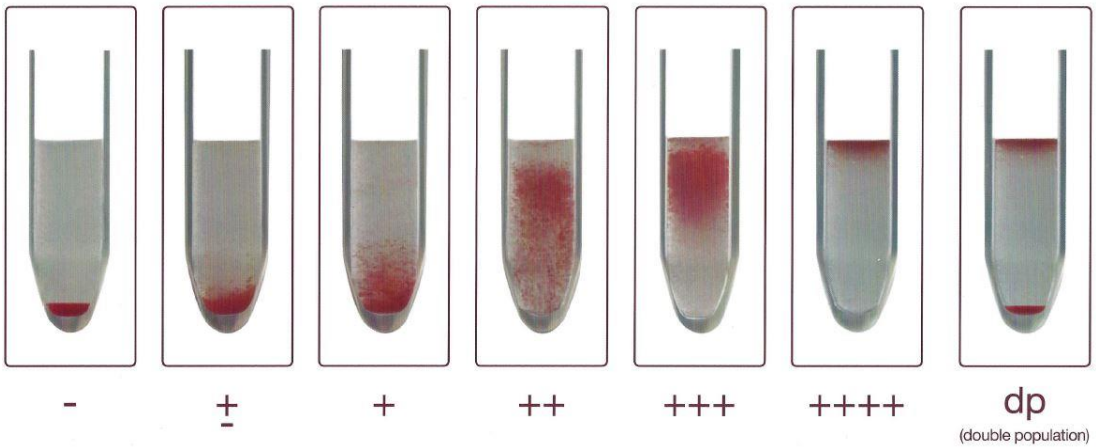


Figure 4. 1: The grading scale of the column gel tecnique for RBC agglutination test.

Gel columns from left to right, showing increasing degrees (0 to 4+/double population) of agglutination. 0 meant no agglutination and 1+ to 4+ meant agglutination present and ± required repition of test to confirm if positive or negative.

4.2.7.2 RBC antibody using selected African red cell antigens

Patients' plasma samples were also tested, using the same column gel technique, against eight selected cells with RBC antigens which are very rare in Caucasians and more frequent in Africans (i.e. MNS6 (He), MNS25 (Dantu), RH10 (V), RH20 (VS), RH30 (Go^a), RH32, RH43 (Crawford) and KEL6 (Js^a)). 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 the plasma with two other RBCs expressing and RBCs not expressing the target antigens. This test sought to find out if there were any antibodies in the patients' plasma directed to Black African polymorphic antigens which could have been missed with the standard Caucassian panel. Briefly, 50 ul of the 8 washed donor cells (prepared in 0.8% suspension in LISS) was pipetted into 8 different column wells. 25 ul of patient plasma was added to each of the wells and incubated for 15 minutes followed by 10 minutes centrifugation. The gel cards were read, and reactivity was documented with standard agglutination grading. A test was positive if agglutination of reaction mixture occurred after the recommended manufacturer's incubation period

4.2.7.3 Direct Antiglobulin Test (DAT)

DAT was performed using the colume gel agglutination technique. Briefly patient red cells (prepared in 0.8% suspension in LISS) was pipetted into three different columns with the first two columns impregnated with IgG and complement component 3d (C3d) respectively. The third column was a control. A test was positive if agglutination of reaction mixture occurred after centrifugation. Reactivity was documented with standard agglutination grading (that is 0 to 4+) as detailed earlier (refer figure 5.1).

4.2.7.4 DNA preparation

Genomic DNA was extracted either manually or with the use of an automated system

Manual extraction: Genomic DNA was extracted from buffy coat and from droplet red cells (for patients whom buffy coat was not available) by manual spin column separation (QIAamp, Qiagen, Valencia, CA, USA), according to the manufacturer's instructions and eluted into 200µl of elution buffer AE. The DNA concentrations were measured in a spectrophotometer at 260nm and the purity was determined by 260/280nm.

Automated extraction: Genomic DNA was extracted from the buffy coat and from droplet red cells (for patients for whom buffy coat was not available) using the automated Chemagen DNA extraction equipment, Perkin Elmer Chemagen XL (PerkinElmer, Waltham, MA, USA). The protocol uses magnetic beads technology to separate DNA from other materials present in whole blood. To briefly describe the principle, DNA become attached to magnetic beads which thereafter become attached to a metal rod magnetized by an electromagnet. The magnetized rod transfers the DNA bound to the beads through processes of washing and purification. The pure DNA is finally eluted in 200ul of elution buffer.

4.2.7.5 Multiple Ligase-dependent Probe Amplification (MLPA) assay

MLPA reaction was performed according to the manufacturer's protocol (MRC Holland, The Netherlands), as previously described by Haer-Wigman et al., (2013), on a thermocycler, (Veriti, Applied Biosystems, Nieuwerkerk aan de Ijssel, The Netherlands), using three probes p401, p402 and p403. In summary, 5µl of 50 ng DNA was denatured. 1.5µl each of probe mix and SALSA MLPA dilution buffer were added. After 16 to 20 hours of hybridisation of the probe combination to the genomic DNA at 60^{o}C, 1µl of SALSA ligase 65, 3µl SALSA ligase buffer A and 3µl SALSA ligase buffer B were added and incubated at 54^{o}C for 15 minutes. Polymerase chain reaction (PCR) was then performed on the complete ligation sample by adding 2µl of universal primers and 0.5µl of SALSA polymerase. The PCR conditions were as follows: 34 cycles of 30 seconds at 95^{o}C, 30 seconds at 60^{o}C and 1 minute at 72^{o}C; followed by 20 minutes at 72^{o}C. A mixture of 0.7µl of MLPA sample, 8.7µl of formamide (Hi-Di, Applied Biosystems), and 0.3 µl size standard (GeneScan 500-Liz, Applied Biosystems) was analysed on a genetic analyser (Model 3730, Applied Biosystems). Each blood-MLPA pool had an artificial control that consisted of a mixture of human cell lines and plasmid DNA, containing targets for probe combinations that were not covered in the cell line. The control samples contained an established number of the blood group alleles in the tested DNA samples. Each control sample was prepared to be used in parallel with the tested samples using the same

protocol. Raw data of the genetic analyser were imported to excel for interpretation. Data were analysed using Genemarker software version 1.85 (Softgenetics).

Figure 4. 2: A picture of the principal investigator performing laboratory investigations in Sanquin Experimental Immunohaematology lab

4.2.7.6 DNA sequencing

DNA sequencing was performed following manufacturer's protocol. The primers for sequencing were synthesized by Eurogentec v. In some cases, all exons and intron boundaries of RHD and RHCE were sequenced. The polymerase chain reaction (PCR) was performed on a Veriti thermocycler in a total volume of 20 µL, containing 50-150 ng DNA, 10 µL of 2x GeneAmp Fast PCR Master Mix (Applied Biosystems) and 0.5 µM each of forward and reverse primers. PCR conditions were: 10 seconds at 95 ºC, 35 cycles of 10 seconds at 95 ºC and a specific annealing/elongation temperature and time for each primer set (ranged from 62 to 70 ºC), followed by 1 minute at 72ºC. PCR products were purified using ExoSAP-IT (GE Healthcare), according to manufacturer's protocol. The sequence reaction was performed on a Veriti thermocycler (Applied Biosystems) in a total volume of 20 µL, containing 1 µL of purified PCR product, 2 µL 2.5x BigDye Terminator v1.1 Cycle (Applied Biosystems), 1 µl 5x BigDye Terminator Buffer (Applied Biosystems) and 0.5 µM forward or reverse primer. Sequence conditions were one minute at 96 ºC, 35 cycles of 10 seconds at 96 ºC, 3 seconds at 50 ºC and 75 seconds at 60 ºC. Sequence products were analyzed on a 3730 Genetic Analyzer (Applied Biosystems).

4.2.8 Sample size calculation

Sample size to determine the prevalence of alloimmunisation was calculated based on the formula, $\mathbf{Z^2xP(1\text{-}P)/d^2}$ for cross sectional studies:

Z is the standard normal variate: at 5% type 1 error (95% confidence interval), Z is 1.96. P is the estimated proportion of alloimmunisation in the population. Based on our previous work on alloimmunisation in Ghanaian multi transfused patients, a prevalence of 9.4% was observed (Boateng et al., 2014). P was therefore 0.094, d is allowable margin of error and this was set at 5% (so 0.05).

Therefore, sample size to determine the prevalence of alloimmunisation (+/- 5%) was calculated as 1.96^2x0.09(1-0.09)/0.05^2= 131patients. This meant that the minimum number of patients required was 131. However, assuming 25% of participants would have insufficient blood samples for the complete analyses, 15% drop out and 10% average sample analysis failures would occur, the sample size calculation was adjusted to (131/(1 - 0.25))/(1 - 0.15)/1-0.1) = 228. This was rounded up to 230.

4.3 Statistical analysis:

Descriptive results for continuous variables were presented as medians (range), and categorical variables were presented as frequencies (percentages). Univariate logistic regression was used to calculate odds ratio (OR) and 95% confidence intervals to determine the association of patients' characteristics with the presence of antibodies and adverse transfusion reactions. The presence of RBC antibodies and adverse transfusion reactions were used as dependent variables. The following variables were included in the analyses; sex, age at enrollment (categorized as <10, 10-19, 20-29, 30-39 and ≥40 years), age at first transfusion (categorized as ≤1, 2-5, 6-9 and ≥10 years), number of transfused units (categorized as 2, 3-5, 6-10 and >10 units), number of years since first transfusion and enrolment in the current study (categorized as <2, 2-5, 6-10 and >10 years), number of years since last transfusion and enrolment in the current study (categorized as <1, 1-2, 3-5, 6-10 and >10 years) and pregnancy with number of pregnancies (categorized as 0, 1 and >1). Because many antibodies are evanescent over time, number of years since first and last transfusion were included in the analyses.

Some studies have suggested that transfusion therapy which starts at a young age (less than one up to five years) results in a lower immunization risk (Verduzco and Nathan, 2009; Sins *et al.*,

2016). Therefore, a sensitivity analysis was performed with dichotomized ages at first transfusion using different cut-offs (≤ or > 1, 2, 3, 4, and 5 years).

Adjusted odds ratio (aOR) was calculated in the final multivariate logistic regression model, including sex and variables with p-values <0.2 in univariate analyses. A 95% confidence interval not overlapping the null value 1.00 for OR was regarded statistically significant.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc, Chicago, IL, USA).

4.4 Results

4.4.1 Patient characteristics

A total of 226 (KBTH n=105 and KATH n=121) multi-transfused patients (126 women and 100 men) of 26 ethnicities (59% Akan, 13% Ga-Adangbe, 12% Ewe, and 16% others) were recruited. The SCD genotypes as obtained from patients' hospital folders were 88% Hb SS, 11% Hb SC, one patient Hb SD and one Hb Sβ^0-thalassemia.

The median age at enrolment was 17.0 years (range 2.0-66.0 years), with female patients being older than male patients (median 21.0 years (range 2.0-66.0 years) and 15.0 years (range 2.0-54.0 years), respectively, p<0.001). Women more often had the Hb SC genotype compared to men (17% and 3%, respectively, p=0.005) (Table 4.1). Patients with the Hb SC genotype were older at enrolment than patients with the Hb SS genotype (31.0 years, range: 8.0-65.0 years and 16.0 years, range: 2.0-66.0), respectively p=0.02) (Table 4.2)

Of the 86 women of childbearing age (i.e. ≥ 16.0 years) and older, 46 had a history of pregnancy (median 1; range, 1-8).

Table 4. 1: Characteristics of 226 multi-transfused Ghanaian patients with sickle cell disease

Characteristic	All patients	Women (n=126)	Men (n=100)	p-value*
SCD genotype: Hb SS, n (%)	200 (88)	104 (83)	96 (96)	**0.005**
Hb SC + Other, n (%)	24 + 2 (12)	21 + 1 (17)	3 + 1 (4)	
Age at enrolment (years)	17 (2-66)	21 (2-66)	15 (2-54)	**<0.001**
Age at first transfusion (years)†	5 (0.1-61)	7 (0.1-61)	3 (0.1-31)	**<0.001**

Number of transfused units	3 (2-40)	3 (2-40)	3 (2-30)	0.67
Number of transfusion episodes	3 (1-21)	3 (1-21)	3 (1-19)	0.90
Single unit transfusion episodes (%)	74	72	76	0.16
Patients with a single transfusion episode, n (%)	34 (15)	23 (18)	11 (11)	0.13
Number of years since first transfusion†‡	8.6 (0-55.5)	7.8 (0-55.5)	9.7 (0-34.8)	0.13
Number of years since last transfusion‡	2.0 (0-55.5)	2.2 (0-55.5)	1.8 (0-31.8)	0.63
Transfused in multiple hospitals, n (%)§	58 (30)	23 (22)	35 (39)	**0.01**
RBC antibodies present, n (%)	36 (15.9)	17 (13.5)	19 (19.0)	0.26

Data presented as median (range), unless stated otherwise; *p-value comparing women and men; †Data available from 174 patients (84 women and 90 men); ‡Number of years between transfusion and enrolment in the current study; §Analyzed for the 192 patients (103 women and 89 men) with multiple transfusion episodes.

Table 4. 2: Characteristics of multi-transfused Ghanaian patients with sickle cell disease, stratified by SCD (Hb SS and Hb SC) genotype

Characteristic	Hb SS (n=200)	Hb SC (n=24)	p-value*
Women, n (%)	103 (52)	21 (88)	**0.003**
Age at enrolment (years)	16 (2-66)	31 (8-65)	**0.02**
Age at first transfusion (years)†	4 (0.1-61)	7 (1-33)	**0.02**
Number of transfused units	3 (2-40)	2 (2-10)	0.11
Number of transfusion episodes	3 (1-21)	2 (1-3)	**0.001**
Single unit transfusion episodes (%)	75	55	**0.007**
Patients with a single transfusion episode, n (%)	23 (11)	11 (46)	**0.001**
Number of years since first transfusion†‡	8.6 (0-55.5)	8.7 (2-25)	0.77
Number of years since last transfusion‡	1.8 (0-55.5)	4.7 (0-35)	0.11
Transfused in multiple hospitals, n (%)§	55 (31)	3 (23)	0.56
RBC antibodies present, n (%)	31 (15.5)	5 (19.2)	0.63

Data presented as median (range), unless stated otherwise; *p-value comparing Hb SS and Hb SC; †Data available from 172 patients (160 Hb SS and 12 Hb SC); ‡Number of years between transfusion and enrolment in the current study; §Calculated for the 191 patients (178 Hb SS and 13 Hb SC) with multiple transfusion episodes.

4.4.2 Transfusion characteristics

The patients had received a median of three (range 2-40; total 1090) whole blood transfusions in a median of three (range 1-21) transfusion episodes. Transfusion episode, for the purpose of this study, was defined as all the RBC components being transfused to a patient in 24 hours. The majority of transfusions (74%) were single unit transfusion episodes. A total of 34 patients (15%) were transfused only once with a median of two units (range 2-6 units). Transfusions had been administered in 60 different hospitals in Ghana. The 192 patients with multiple transfusion episodes (median 3, range 2-21) had been transfused in one hospital in 70% and in multiple (2-3) hospitals in 30%.

The age at first transfusion was known for 174 (77.0%) patients (90 men and 84 women). First transfusions were given at a median age of 5.0 years (range 0.1-61.0 years). The period between the first and last transfusions was median four years (range 0-34 years). The period between the first transfusion and current study was median 8.6 years (range, 0.1-55.5 years) and for the last transfusion, median two years (range 2 weeks-55.5 years).

Some transfusion history characteristics differed between women and men. Women were less likely than men to recall the age at first transfusion (67% and 90%, respectively, $p<0.0001$) were older at first transfusion (7 and 3 years, respectively), the period between first and last transfusion was shorter (2.8 and 5.0 years, respectively) and were less often transfused at multiple hospitals (23% and 39% respectively; Table 4.1). Forty-six of the 86 women of childbearing age and older had a history of pregnancy. Although they had not received more transfusions (median 3; range 2-17) compared to women who had not been pregnant (median 3; range 2-40), their total allo-exposure (transfusions and pregnancies) was higher (median 5; range 3-21; $p=0.001$).

Transfusion characteristics also differed between patients with the Hb SS and the Hb SC genotypes. Patients with the Hb SS genotype were younger at the first transfusion (median 4 and 7 years, respectively), had more transfusion episodes (median 3 and 2, respectively) and more single unit transfusion episodes (75% and 55%, respectively) compared to patients with the Hb SC genotype (Table 4.2).

4.4.3 Red blood cell antibodies

4.4.3.1 Red blood cell antibodies against Eruopean antigens

Antibody screening, using standard test cells from European origin, was positive in 25 patients (11.1%; 11 women and 14 men). Antibody specificities (n=26) were predominantly against Rh antigens (n=19; 73%). Pan-reactive antibodies were present in three patients (Table 4.3).

Anti-D was detected in seven patients (four women and three men). Molecular *RHD* characterization of these patients revealed that three women and two men had only *RHD*-null alleles (three *RHD*01N.01/RHD*01N.01*, and one woman each *RHD*01N.01/RHD*01N.03* and *RHD*08N.01/RHD*08N.01* genotypes). One woman and one man had *RHD* variants (*RHD*03.04/RHD*01N.03* and *RHD*04.01/RHD*04.01* genotypes) associated with D+ serology (Table 4.4).

4.4.3.2 Red blood cell antibodies against selected African antigens

The nine patients who had antibodies against D, G and s antigens in the standard test were not tested against African antigens, because African test cells lacking these antigens were not available. Also, the three patients with pan-reactive antibodies were not tested against African cells. Of the remaining 214 patients who did not have alloantibodies in the three-cell screen panel with European RBC, 11 (5.1%), had positive reactions against African antigens. The observed and confirmed specificities were: 3 anti-Dantu (for one, confirmation by a second Dantu+ test cell was not performed, due to lack of Dantu+ cells), 2 anti-He, 2 anti-V/VS, 1 anti-VS, 1 anti-RH32, 1 anti-Go^{a} and 1 antibody probably against a low frequency antigen, but no plasma was available for further investigation (Table 4.3).

The combined overall prevalence of RBC alloimmunisation was 15.9% (36 of 226 patients; 95% CI 11.5-22.5%) with 37 different antibodies. The rate of antibody formation was 3.4 per 100 units of blood and 4.5 per 100 transfusion episodes.

Table 4. 3: Specificities of the 37 RBC antibodies in the 36 alloimmunised multi-transfused Ghanaian patients with sickle cell disease.

Blood group system	Antibody specificity (n)	
	European panel	African panel
Rh	E (10); D (7); G (2)	V/VS (2); VS (1); Goa (1); RH32 (1)
Kell	K (1)	
MNS	s (1)	Dantu (3); He (2)
Lewis	Le^a (1)	
Unidentified	Pan-reactive (3); Non-specified (1)	Non-specified (1)

The non-specified antibodies were probably against low frequency antigens

Table 4. 4: Characteristics and *RHD* genotypes of the seven patients with anti-D

Patient ID	Sex	Age at inclusion	Number of transfusions	Number of pregnancies	RHD genotype	Predicted phenotype
027	male	18	7	n.a.	*RHD*03.04/RHD*01N.03*	D+
087	female	14	3	n.a.	*RHD*04.01/RHD*04.01*	D+
100	male	14	2	n.a.	*RHD*01N.01/RHD*01N.01*	D-
151	female	50	6	4	*RHD*01N.01/RHD*01N.03*	D-
157	female	39	2	1	*RHD*01N.08/RHD*01N.08*	D-
203	male	45	6	n.a.	*RHD*01N.01/RHD*01N.01*	D-
223	female	34	2	1	*RHD*01N.01/RHD*01N.01*	D-

n.a., not applicable

4.4.4 Risk factors for red blood cell antibodies

Number of transfused units

Univariable logistic regression analysis showed that the presence of RBC antibodies in 36 patients was associated with the number of transfused units (OR 1.70; 95% CI, 1.18-2.44). Antibody prevalence increased from 7% in patients who had received two units to 20% after 3-10 units and 32% after more than ten units.

Age at first transfusion

The sensitivity analyses of dichotomized ages at first transfusion with different cut-offs revealed that, although first transfusions after the age of two and three years were both suggestive for greater antibody positivity, this association was strongest when first transfused after the age of three years (OR 2.24; 95% CI 0.93-5.41) (Table 4.5). Antibody prevalence was 10% in patients first transfused ≤3 years of age compared to 21% when first transfused at an older age. This higher prevalence was not attributable to total transfusion load, because patients who received the first transfusion after the age of three years had received less transfusions (median 3, range 2-40) compared to patients first transfused at a younger age (median 4, range 2-30, p=0.02). Because ages when first and last transfusions were received differed between patients, a stratified ad hoc analysis was performed. Antibody prevalence increased from 6% in the 17 patients only transfused ≤3 years of age, to 12% in the 60 patients who were transfused before and after age three and to 21% in the 97 patients who were first transfused after age three (OR 2.00; 95% CI 0.96-4.15; p=0.063).

Period since last transfusion

A longer period between last transfusion and enrolment in the current study was suggestive for lower antibody positivity (OR 0.80; 95% CI 0.61-1.07).

Other factors

Sex, ethnicity, SCD genotype, age at enrolment and the number of years since first transfusion and current study were not associated (Table 4.5).

Because a number of transfusion characteristics differed between women and men, ad hoc sub-analyses stratified by sex were performed. In the 126 women, a higher number of transfused units was suggestive for more RBC antibodies (OR 1.45; 95% CI 0.87-2.40), while the association was stronger in the 100 men (OR 2.01; 95% CI 1.18-3.44). In the 86 women of

childbearing age (i.e. ≥ 16 years) and older, a higher number of pregnancies was positively associated with antibodies (OR 2.42; 95% CI 1.04-5.64) (Table 4.5).

Multivariable logistic regression analysis, including sex, age at first transfusion ≤3 versus >3 years, number of transfused units and number of years between last transfusion and enrolment in the current study as co-variates, revealed that age at first transfusion (aOR 3.28; 95% CI 1.26-8.55) and number of transfused units (aOR 2.00; 95% CI 1.30-3.07) were positively associated with RBC antibody presence. These associations remained when the analysis was repeated after excluding the 17 patients who received all transfusions before the age of three years (aOR 3.20; 95% CI 1.14-8.98 and aOR 1.97; 95% CI 1.26-3.07, respectively). Restricting the analyses to the 200 patients with the Hb SS genotype, did not change the findings ((age at first transfusion (aOR 3.12; 95% CI 1.17-8.34; p=0.023) and number of transfused units (aOR 2.12; 95% CI 1.36-3.30; p=0.001)) (Table 4.6).

Table 4. 5**:** Univariable analyses of variables associated with the presence of RBC antibodies and transfusion reactions in 226 multi-transfused Ghanaian patients with sickle cell disease

	RBC antibodies		Adverse transfusion reaction	
Variables	OR (95% CI)	p-value	OR (95% CI)	p-value
Sex (women is reference)	1.32 (0.65-2.69)	0.45	0.84 (0.47-1.49)	0.54
Ethnicity*	1.03 (0.76-1.40)	0.84	N.A.	
SCD genotype (SS is reference)	1.30 (0.46-3.70)	0.63	0.30 (0.09-1.06)	0.06
Age at enrolment	1.16 (0.87-1.55)	0.31	1.10 (0.87-1.39)	0.43
Age at first transfusion†	1.22 (0.85-1.76)	0.27	N.A.	
Dichotomized				
≤1 versus >1 year	1.41 (0.46-4.43)	0.54	N.A.	
≤2 versus >2 years	2.15 (0.82-5.64)	0.20	N.A.	
≤3 versus >3 years	2.24 (0.93-5.41)	0.07	N.A.	
≤5 versus >5 years	1.32 (0.59-2.96)	0.51	N.A.	
Number of transfused units	1.70 (1.18-2.44)	**0.004**	2.10 (1.53-2.89)	**<0.001**
Number of years since first transfusion†‡	1.19 (0.80-1.79)	0.40	N.A.	
Number of years since last transfusion‡	0.80 (0.61-1.07)	0.13	0.81 (0.65-1.01)	**0.063**
Number of pregnancies in 86 women ≥16 years of age	2.42 (1.04-5.64)	**0.04**	1.10 (0.63-1.91)	0.74
Presence of RBC antibodies	N.A.		0.88 (0.40-1.93)	0.74

OR, Odds Ratio; CI, confidence interval; N.A., not analyzed; *Akan, Ga-Adangbe, Ewe and other; †Data available from 174 patients; ‡Number of years between transfusion and enrolment in the current study.
Age at enrolment categorized as <10, 10-19, 20-29, 30-39 and ≥40 years; Age at first transfusion categorized as ≤1, 2-5, 6-9 and >10; Number of transfused units categorized as 2, 3-5, 6-10 and >10; Number of years since first transfusion categorized as <2, 2-5, 6-10 and >10 years; Number of years since last transfusion categorized as <1, 1-2, 3-5, 6-10 and >10 years; Number of pregnancies categorized as 0, 1 and >1.

Table 4. 6: Univariable analyses of variables associated with the presence of RBC antibodies in the 200 multi-transfused Ghanaian patients with Hb SS sickle cell disease

Variables	OR (95% CI)	p-value
Sex (women is reference)	1.88 (0.74-4.76)	0.18
Age at enrolment	1.17 (0.79-1.74)	0.43
Age at first transfusion*	1.31 (0.83-2.06)	0.25
Dichotomized		
≤1 versus >1 year	1.04 (0.28-3.87)	0.96
≤2 versus >2 years	1.91 (0.59-6.17)	0.28
≤3 versus >3 years	2.18 (0.73-6.50)	0.16
≤5 versus >5 year	1.60 (0.58-4.39)	0.36
Number of transfused units	1.73 (1.11-2.70)	**0.016**
Number of years since first transfusion*†	1.33 (0.78-2.26)	0.29
Number of years since last transfusion†	0.76 (0.51-1.13)	0.17
Number of pregnancies in 65 women ≥16 years	2.19 (0.74-6.46)	0.15

*Data available from 161 patients; †Number of years between transfusion and enrolment in the current study.
Age at enrolment categorized as <10, 10-19, 20-29, 30-39 and ≥40 years; Age at first transfusion categorized as ≤1, 2-5, 6-9 and >10; Number of transfused units categorized as 2, 3-5, 6-10 and >10; Number of years since first transfusion categorized as <2, 2-5, 6-10 and >10 years; Number of years since last transfusion categorized as <1, 1-2, 3-5, 6-10 and >10 years; Number of pregnancies categorized as 0, 1 and >1.
Multivariable logistic regression analysis, including sex, age at first transfusion ≤3 versus >3 years, number of transfused units and number of years since last transfusion and current study as co-variates revealed that number of transfused units (aOR 2.12; 95% CI 1.36-3.30; p=0.001) and age at first transfusion (aOR 3.12; 95% CI 1.17-8.34; p=0.023) were associated with the presence of red cell antibodies. These associations remained after performing the analysis excluding the 17 patients who received all transfusions before the age of three years (aOR 2.10; 95% CI 1.33-3.33; p=0.002 and aOR 3.11; 95% CI 1.08-8.92; p=0.035, respectively).

4.4.5 Adverse transfusion events

One or more adverse transfusion events were recalled by 79 patients or their caretakers (35%; 45 women and 34 men; 39 children and 40 adults). In 27 (11.9%) patients, a transfusion was abruptly stopped, 58 patients reported clinical signs and symptoms of transfusion reactions (these included fever, chills, breathlessless, lower back pain, dark urine, rolling tongue, skin eruption, rashes, chest pain, abdominal pain, itching, swelling and reddening of eyes, vomiting and joint pains), of which chills/fever, urticaria, dark urine and abdominal pain were the most frequent, during or within 24 hours after transfusion (Table 4.7). 24 (10.6%) patients returned to the hospital within two weeks after a transfusion because they had SCD crises or anaemia

or did not see any improvement in their condition. Multiple adverse events were recalled by 27 patients, of which 'abruptly stopped transfusions with transfusion reactions' (n=16) and 'transfusion reactions with returning to hospital within two weeks' (n=12) were the most frequent. In 68 (30%) patients, the patients' self reported clinical signs and symptoms were classified as adverse transfusion reactions based on the revised Serious Hazards of Transfusion classification (Serious Hazards of Transfusion, 2011). Of the 68 patients, 23 patients (12 women and 11 men; 10%) reported clinical signs and symptoms suggestive of haemolytic transfusion reactions. Antibodies (anti-E, anti-He and pan-reactive) were detected in only three of these 23 patients.

Table 4. 7: Summary of the 68 transfusion reactions and 27 transfusions that were not completed in 226 multi-transfused Ghanaian patients with sickle cell disease

		Frequency (%)
Transfusion reactions	Allergic reaction	16 (23.5)
	Febrile non-haemolytic reaction	13 (19.1)
	Possible haemolytic reaction*	23 (33.8)
	Unclassified	16 (23.5)
Transfusions not completed	Transfusion abruptly stopped:	
	Clotted or haemolysed blood	5 (18.5)
	Venous occlusion	6 (22.2)
	Cannot recall	3 (11.1)
	Other†	13 (48.1)

*Clinical signs and symptoms suggestive of haemolytic reactions were dark urine (n=12), abdominal/lower back pain (n=9) and abdominal pain with dark urine (n=2). In seven of these patients, fever/chills accompanied these reaction.
†Other reasons for stopping transfusion as reported by pateints included itching, fainting, over thawed blood, haemoglobin ok, cold blood, swelling, fever, wrong blood group, transfusion reaction and discomfort.

4.4.6 Risk factors for adverse transfusion reactions

Univariable logistic regression analysis showed that a higher number of transfusions was associated with more adverse transfusion reactions (OR 2.10; 95% CI, 1.53-2.89). SCD genotype (OR 0.30; 95% CI 0.09-1.06 for Hb SC), and number of years since last transfusion and enrolment in the current study (OR 0.81; 95% CI 0.62-1.01) were suggestive for less adverse transfusion reactions. Sex, age at enrolment, number of pregnancies and the presence of RBC antibodies were not associated (Table 4.5). With regard to possible haemolytic transfusion reactions the only positive association was with the number of transfused units (OR 2.25; 95% CI 1.45-3.50; $p<0.001$).

Multivariable logistic regression analysis, including SCD genotype, number of transfusions and number of years since last transfusion and enrolment in current study as co-variates, revealed that only the number of transfusions (aOR 2.06; 95% CI 1.50-2.84; $p<0.001$) was positively associated with adverse transfusion reactions.

4.5 Discussion

4.5.1 Red blood cell antibodies

This cross-sectional study demonstrated RBC antibodies in 36 of 226 (15.9%) multi-transfused Ghanaian patients with SCD. Using cells expressing antigens almost exclusive to Africans revealed antibodies in eleven (5.1%) of 214 tested patients, thus in 31% of immunized patients, which were not detected during standard antibody screening using cells of European origin. These results stress the importance of testing against African antigens, when patients are receiving transfusions from donors of African descent.

Multivariable logistic regression analysis revealed that receiving the first transfusion after the age of three years (aOR 3.28, 95% CI 1.26-8.55) and a higher number of transfused units (aOR 2.12, 95% CI 1.36-3.30) were associated with more RBC antibodies.

In my previous study, using standard test RBC, 6.5% of 154 transfused Ghanaian children with SCD possessed RBC antibodies, and antibody prevalence increased with a higher number of transfusions (Boateng *et al.*, 2019a). In that study, about 30% of patients had received only one unit, while in the current study all patients were multiple transfused and 55% of women of childbearing age and older had been pregnant, and the number of pregnancies was associated with antibodies (OR 2.42). Although many other (environmental and age) factors, that are

presumed to play a role in alloimmunisation, may differ between the two studies, this higher RBC allo-exposure likely contributed to the higher antibody prevalence (11.5% using standard test RBC) in the current study. This is further substantiated by the association between the number of transfusions and the presence of antibodies. The rate of antibody formation (3.4/100 units) is in the range of the rates reported (or calculated from available data) in other cross-sectional studies in patients with SCD from SSA (range 0.6-6.3, with a weighted average of 3.4 antibodies per 100 units) (Batina Agasa *et al.*, 2010; Abbas *et al.*, 2013; Meda *et al.*, 2014; Kangiwa *et al.*, 2015; Mangare *et al.*, 2015; Adewoyin, 2016; Sekongo *et al.*, 2017).

The number of years since last transfusion and current antibody testing varied considerably, ranging from two weeks to 55 years. Antibodies in patients with SCD are notorious for their high evanescence rate, which may be up to 60-80% (Harm *et al.*, 2014; Williams *et al.*, 2016). Indeed, in univariable analysis, a decreased frequency of antibodies (OR 0.8, 95% CI 0.65-1.01) was observed with a longer period between the last transfusion and antibody testing, suggestive of antibody evanescence over time. In addition, about 10% of our patients were tested for antibodies within two months of the last transfusion and this may have been too soon to detect most new antibodies (Schonewille *et al.*, 2016). In many well-resourced countries, patients with SCD may be on a chronic transfusion regime and are routinely tested for the presence of RBC antibodies before each transfusion. The one-time test cross-sectional approach used in our study compared to this regular antibody testing likely results in an underestimation of antibody formation.

In line with other studies of patients with SCD and with thalassemia, we observed that receiving the first transfusion at a later age, in our cohort after three years of age, was associated with a more than three times higher risk of developing antibodies compared to patients of younger age at first transfusion (Rosse *et al.*, 1990; Spanos *et al.*, 1990; Vichinsky *et al.*, 2014; Sins *et al.*, 2016). Although not yet completely elucidated, functional limitations of the immune system or exposure to alloantigens in early childhood leading to immune tolerance/ignorance may explain the lower alloimmunisation rates in young children.[45,46] This lower immune response may persist lifelong, as the risk of having antibodies in patients who received all transfusion after the age of three was 3.2 times higher compared to patients who were also transfused before the age of three years.

Only few studies in transfused patients with SCD report on the presence of antibodies against antigens that are predominantly present in Africans (Rosse *et al.*, 1990; Aygun *et al.*, 2002; Castro *et al.*, 2002; Miller *et al.*, 2013; Karafin *et al.*, 2015; Telen *et al.*, 2015; Yee *et*

al., 2017; Campbell-Lee *et al.*, 2018; Floch *et al.*, 2018; Coleman *et al.*, 2019). Unlike our study, these patients were transfused in the US and Europe and thus not exclusively transfused with blood from African donors. The antibody prevalence against African antigens ranged from 0.7% to 13.3% (median 2.0%) in these studies. For the five studies that provided the number of transfusions, the antibody rate per 100 transfused RBC units varied between 0.03 and 0.17 (median 0.10) (Aygun *et al.*, 2002; Castro *et al.*, 2002; Karafin *et al.*, 2015; Yee *et al.*, 2017; Campbell-Lee *et al.*, 2018). The combined African antibody specificities (n=87) in a total of 4264 patients from these studies were 37 V/VS, 31 Js^a, 12 Go^a, 3 He, 3 RH32 and 1 D^w. Floch and colleagues also systematically screened 211 patients with SCD in France with a panel of selected African antigens. Transient antibodies (4 VS, 1V, 1 Js^a, 1 D^w and 1 RH32) were present in four patients (1.9%) before study inclusion and nine antibodies (5 D^w, 2 Go^a and 2 He) were detected in eight (3.8%) patients after systematic screening. Four antibodies (3 D^w and 1 Go^a) were only detectable with enzyme treated test cells, while we only used a LISS indirect antiglobulin test (Floch *et al.*, 2018). Although the frequency of antibodies against African antigens in our patients was within the range of previous studies, the antibody immunization rate per 100 units in our study was much higher, probably caused by the lower risk to encounter African antigens in the US and Europe, where the majority of donors is from Caucasian background. Furthermore, in our study as well as in the other studies the rate of alloimmunisation against African antigens is probably underestimated due to evanescence of the alloantibodies.

Despite routine matching for D antigen in Ghana, seven patients had anti-D. Two patients possessed variant *RHD* genes (*RHD*03.04* and *RHD*04.01*) associated with D+ serology and it has previously been described that carriers of these variant *RHD* genes can make anti-D after exposure to blood containing the D antigen (Wagner *et al.*, 2000; von Zabern *et al.*, 2013). The remaining five patients had only *RHD*-null alleles. In three D- women, anti-D could have been induced by a previous D+ pregnancy. For two D- men, errors in blood group typing or mistakenly transfusing patients with D+ blood are possible causes for the anti-D. Future research should establish the frequency of *RHD* variants in serological D+ patients in Ghana that will put them at risk of developing anti-D, and institute an appropriate transfusion policy.

Regarding antibody specificities, more than 80% of antibodies were against antigens from the RH (65%) and the MNS systems (16%) and within those systems 25% and 83% were African antibodies, respectively. A remarkable finding was that 19% of all antibodies were

against African antigens of low frequency (i.e. Go^a 2%, RH32 1%, He 1% and Dantu 0.5% in Africans), suggesting that these antigens are highly immunogenic in Ghanaians and more immunogenic than the antigens for which we test in the European setting. This makes the use of African test cells highly relevant. In both our studies in transfused Ghanaian patients with SCD, we did not detect any Duffy, Kidd and S antibodies, which are relatively frequent in patients with SCD in Europe (Sins *et al.*, 2016). Also, in the two reviews on alloimmunisation in transfused patients in SSA, these antibodies were together only present in less than 1.0% of patients (Ngoma *et al.*, 2016; Boateng *et al.*, 2019b).

Although we report African antibodies in African patients transfused with African blood, it is quite likely that similar antibodies may be elicited in Caucasian patients receiving blood from African donors. These antibodies will not be detected during pre-transfusion antibody testing using standard European screen cells and if no indirect antiglobulin crossmatch is done (and many countries only perform type and screen). Since blood bank organizations in many Western countries are developing strategies to recruit and retain more African donors, to fulfill the growing need for an adequate supply of antigen-compatible blood, our findings are also relevant in a Western test approach; with more African donors, African antibodies may increasingly be missed.

4.5.2 Adverse transfusion reactions

Adverse transfusion reactions were recalled in 68 (30%) patients and the number of reactions increased with higher numbers of transfusions (aOR 2.06). Mild reactions, such as fever, chills and/or urticaria rash, were reported by the majority of patients. Adverse transfusion reactions are not uncommon after receiving whole blood and are associated with the presence of donor leucocytes, plasma proteins, pro-inflammatory cytokines and biologic response modifiers. Studies from Africa comparing reported adverse transfusion reactions after whole blood and packed RBC transfusions showed that reactions were 2.5-20 times more frequent after whole blood transfusion (Milner and Butcher, 1978; Mafirakureva *et al.*, 2014). Furthermore, in two studies from Nigeria (Gwaram and Abdullahi, 2013; Ibrahim, Garba and Tilde, 2013), previous transfusions were significantly associated with adverse transfusion reactions (ORs 2.0 and 3.8). Ibrahim and colleagues also showed that, in Nigerian women transfused for pregnancy related complications, transfusion reactions were observed 3.6 times more frequently after uncrossmatched compared to (partly) crossmatched transfusions and 1.4 times more frequently in multigravida compared to primigravida (Ibrahim, Garba and Tilde, 2013). In our 86 women

≥16 years of age, the number of pregnancies was not associated with adverse transfusion reactions (i.e. 33% and 36%, respectively; data not shown).

Abdominal and/or back pain and dark urine were reported by 23 (10%) patients and may be signs of life- threatening HTR. Our ability to demonstrate RBC antibodies in only three of these patients could partly be due to antibody titers below detectable levels, considering that the median follow-up period since last transfusion in these 23 patients was 2.2 years (range 0.2-8.3 years), although it is not uncommon for such reactions to occur without demonstrable RBC antibodies (Habibi *et al.*, 2016). Evanescence of antibodies against European antigens is a well-known phenomenon. For antibodies against African antigens, Floch and colleagues showed that none of the eight historical antibodies were detectable at study enrollment, suggesting that African antibodies also have a high evanescence rate. This may put patients at risk for haemolytic transfusion reactions upon receipt of a transfusion with the cognate antigen, as shown by Coleman and colleagues (Coleman *et al.*, 2019). In their study anti-Go^{a}, anti-Js^{a} and anti-He were involved in severe haemolytic transfusion reactions.

Although protocols for the follow-up of patients with transfusion reactions, including antibody screening and tests for haemolysis, are available in most countries in SSA, it is possible that not all hospitals adhere to these, due to lack of knowledge, and logistical and resource constraints (Natukunda, Schonewille and Sibinga, 2010; Basavaraju *et al.*, 2013).

Currently there are no published data from SSA that prospectively followed patients with SCD after blood transfusion to detect acute or delayed HTR. In our cohort 25 patients returned to hospital within two weeks of a transfusion and eight of them recalled no improvement in their haemoglobin. Based on our data it was not possible to distinguish delayed HTR from sickle cell crisis in these cases. It is however established in well-resourced countries that delayed HTR sometimes tend to mimic sickle cell crises as the anemia and haemolysis can precipitate pain and may go unrecognized (de Montalembert *et al.*, 2011; Coleman *et al.*, 2019). It is therefore imperative that recently transfused patients who return to the hospital with pain crises be investigated for possible delayed HTR before complicating the situation with further transfusions (Coleman *et al.*, 2019).

4.6 Conclusion

In conclusion, this is the first study that has systematically screened for antibodies against African antigens in a large cohort of transfused patients with SCD in an African setting, where patients are exclusively transfused with blood from African donors. Our results show that consideration should be given to the incorporation of RBC antibody testing into routine pre-transfusion screening procedures for patients with SCD in Ghana. Preferably, this should be done with reagent RBC also expressing 'African antigens', but tests should at least include an indirect antiglobulin crossmatch with donor RBC. When antibodies are present, these should be filed on patients' records in the hospital and accessible for the patients' life time. Also, since patients may be transfused in multiple hospitals and inter-hospital electronic records are not available, the patients should receive written information about their antibodies to be able to provide other hospitals with this knowledge and to improve transfusion safety and the efficacy of antibody testing. Patients should be monitored closely during and after transfusion (up to 25 days) as they have a risk of transfusion reactions, especially in patients with a history of previous transfusion. Implementing component therapy (preferably leucodepletion) rather than whole blood transfusion will not only result in a more optimal use of the limited blood supply in SSA but may also serve as a potential strategy to reduce adverse transfusion reactions in Ghana.

CHAPTER 5: RED BLOOD CELL ANTIGEN DISTRIBUTION PATTERN AMONG PATIENTS WITH SCD AND BLOOD DONORS OF DIFFERENT ETHNIC GROUPS IN GHANA (ANTIGEN STUDY)

5.1 Brief introduction of chapter

As described above, red blood cell (RBC) alloimmunisation results from RBC antigen disparities between donors and transfusion recipients and the alloimmunisation risk may be enhanced by ethnic variation in the population. The prevalence of alloimmunisation among patients with sickle cell disease (SCD) in sub-Saharan Africa ranges from 2.6% to 28.6% (overall proportion 7.4%) (Boateng *et al.*, 2019b) . In many studies it was hypothesized that the generally low prevalence of alloimmunisation in Africa is due to RBC antigen similarity between donors and recipients. However, there are only sparse data on RBC antigen distribution prevalence and its variability in ethnic groups in Ghana and Africa as a whole, and most are confined to ABO, Rh and Kell system antigens (Armattoe, 1951; Tills *et al.*, 1979; Acquaye, 1980; Tills *et al.*, 1982; Kulkarni *et al.*, 1985; Acquaye, 1990; Acquaye, 1992; Acquaye, 2004; M'Baya *et al.*, 2010; Siransy Bogui *et al.*, 2014; Kretchy *et al.*, 2017; Adewoyin *et al.*, 2018; Boateng *et al.*, 2019a; Doku *et al.*, 2019). Knowledge of the extended (including non-Rh/K antigens) RBC antigen distribution of donors and recipients would help in predicting alloimmunisation risks and may improve transfusion safety.

This chapter determined the distribution of 24 clinically relevant RBC antigens routinely present on commercially available Caucasian antibody screening panels among patients with SCD and/or blood donors in multi-ethnic Ghana.

5.2 Methodology

5.2.1 Study site

The study was conducted at the sickle cell clinics of KBTH and KATH, the Transfusion Medicine Unit of KATH and the SABC of the NBSG.

5.2.2 Study population

The study population for the antigen study were patients with SCD and blood donors. The inclusion and exclusion criteria for the patients with SCD and the blood donors are described below:

Inclusion criteria for patients with SCD
All consenting patients with SCD, of any SCD genotype (Hb SS, Hb SC, Hb SD, Hb $S\beta^+$ thalassaemia and Hb $S\beta^0$ thalassaemia) and ethnicity.

Exclusion criteria for patients with SCD
Patients who were in severe crises or in so much pain that they could not provide responses to the questionnaire.

Inclusion criteria for blood donors
This included all consenting blood donors of any ethnicity who were either VNRD or FRD. These groups were selected because blood for transfusion in Ghana is obtained mainly from these two types of donors. Selecting both VNRD and FRD provided a good representation of the population whose blood is likely to be transfused to the patients with SCD.

Exclusion criteria for blood donors
Potential blood donors who failed the pre-donation screening checks.

5.2.3 Recruitment strategy
Recruitment strategy for patients with sickle cell disease
Patients with SCD who attended clinic between 10th July to 5th September, 2019 and met the inclusion criteria were enrolled through their respective SCD clinics. The detailed recruitment strategy is the same as described for patients with SCD in the antibody study (refer section 4.2.3).

Detailed recruitment strategy for blood donors
For blood donors, consecutive blood donors who presented at the blood donor clinic or the mobile donation clinic, between 10th July to 5th September, 2019, to donate blood and passed the pre-donation screening checks, was invited to participate. Details of recruitment strategy for blood donors are discussed below.

The routine processes by which the Accra Centre, (SABC of the NBSG), selected their donors was different from that used by the Kumasi centre (KATH Transfusion Medicine Unit). This meant that a slightly different approach was adopted in recruiting the blood donors at the two centres.

At the Kumasi donation centre, FRD were recruited through the hospital's blood donor clinics and VNRD were recruited through mobile donation clinics. Mobile donation clinic for the purposes of this study are the type of blood donation where the hospital's blood bank team schedule and visit senior high schools, churches, mosques, work facilities and appeal to eligible students and/or staff to donate blood at their own convenience. The research team

joined the mobile donation team on their trips to carry out the mobile donation exercise. Mobile donors were recruited from Asawasi mosque, Sawaba senior high school and the Royal parade grounds (Kwame Nkrumah University of Science and Technology). At both the hospital and the mobile donation clinics, donors who agreed to donate blood and passed the pre-donation screening test were asked if part of their donated blood could be used for the study, after explaining thoroughly the study to them as spelt out in the participant information leaflet (appendix IV). Consenting donors signed the consent form (appendix I) and filled a questionnaire (appendix V) designed to capture the following information; age, gender and ethnicity,

At the Accra donation centre, both VNRD and FRD were recruited through the hospital's blood donation clinic. This was because, during mobile donations, partial pre-donation checks (haemoglobin, blood pressure, pulse and weight) were carried out at the mobile donation site. Transfusion transmitted infections were screened at a later date at the NBSG laboratory. It was difficult to keep track of when tests were going to be completed and who qualified or disqualified as a healthy donor after the tests were completed at the the NBSG. It was therefoe pragmatic to recruit all participants from the hospital blood donation centre, where both VNRD and FRD visited and pre-donation checks were completed before donation. Donors who visited the centre's donor clinic were recruited in the same way as described for the Kumasi centre above.

5.2.4 Blood sample collection

Blood sample collection for patients with sickle cell

The protocol for blood sample collection for patients with sickle cell disease was the same as described for patients with SCD in the antibody study (refer section 4.2.4).

Blood sample collection for blood donors

For blood donors, 10mls of blood was collected from the one unit of the whole blood (450 ml) they donated. The 10 ml blood was collected immediately after every donation by connecting the needle of the bag to the EDTA tube and tilting the blood bag to fill the EDTA tube. Once the EDTA tube was filled, the blood bag was sealed.

5.2.5 Blood samples processing

Collected blood samples, from blood donors and patients were processed as follows. One ml each of whole blood was aliquoted into two separate 2ml cryotubes. The remaining whole blood was separated into plasma and packed red cells. Briefly, the whole blood samples were centrifuged at 4000rpm for 20 minutes, after which plasma was aliquoted into two separate

2ml cryotubes. The red cells were frozen in 40% glycerol (high glycerol protocol for -80 storage) and stored in two separate 2ml cryotubes. protocol of the preparation of red cells in the 40% glycerol is attached as an appendix (AppendixVII).

5.2.6 Samples storage and transport

All cryotubes containing whole blood, plasma and glycerolised RBCs were frozen at -80^{o}C either in the serology lab of KATH (Kumasi centre) or in the haematology lab of KBTH (Accra centre). The frozen cryotubes were subsequently shipped on dry ice to the Sanquin Immunohaematology lab, for laboratory testing.

5.2.7 Laboratory tests

Laboratory tests for the antigen study involved:

1. Genomic DNA extraction from whole blood of patients and donors
2. RBC genotyping of all patients and donors
3. RBC serology for the major blood group antigens

DNA extraction was carried out at Sanquin, and RBC genotyping was performed in San Diego, United States of America. RBC antigen serological phenotyping of the major blood groups (ABO, MNS, Rh, Kidd, Duffy) was performed in Sanquin. The serological typing of the major blood group antigens was to validate the genotyping results. For the antigen study, I prepared the whole blood samples for the automated DNA extraction on the Chemagen XL. The RBC genotyping was performed by Dr. William Joseph Lane. I was originally scheduled to travel to Sanquin to perform the RBC serology typing, but due to travel restrions imposed due to the COVID-19 pandemic, Peter Ligthart and Aicha Soussan, based at the Sanquin diagnostic laboratory, were nominated to perform the test.

The laboratory tests are described below:

5.2.7.1 Genomic DNA extraction

Genomic DNA was extracted from one ml whole blood using automated Chemagen DNA extraction equipment, Perkin Elmer Chemagen XL (PerkinElmer, Waltham, MA, USA). The protocol is the same as described for automated DNA extraction (refer section 4.2.7.4)

5.2.7.2 RBC genotyping

RBC genotying was performed as previously described by Gleadell et al., (2020). Briefly, the genotyping was carried out using the Axiom genotyping platform at the Applied Biosystems

Microarray Research Services Laboratory, Santa Clara, CA, in accordance with "Axiom Best Practice Workflows" (figure 6.1). 750 ng total DNA at 30 ng/µl from each sample were used.

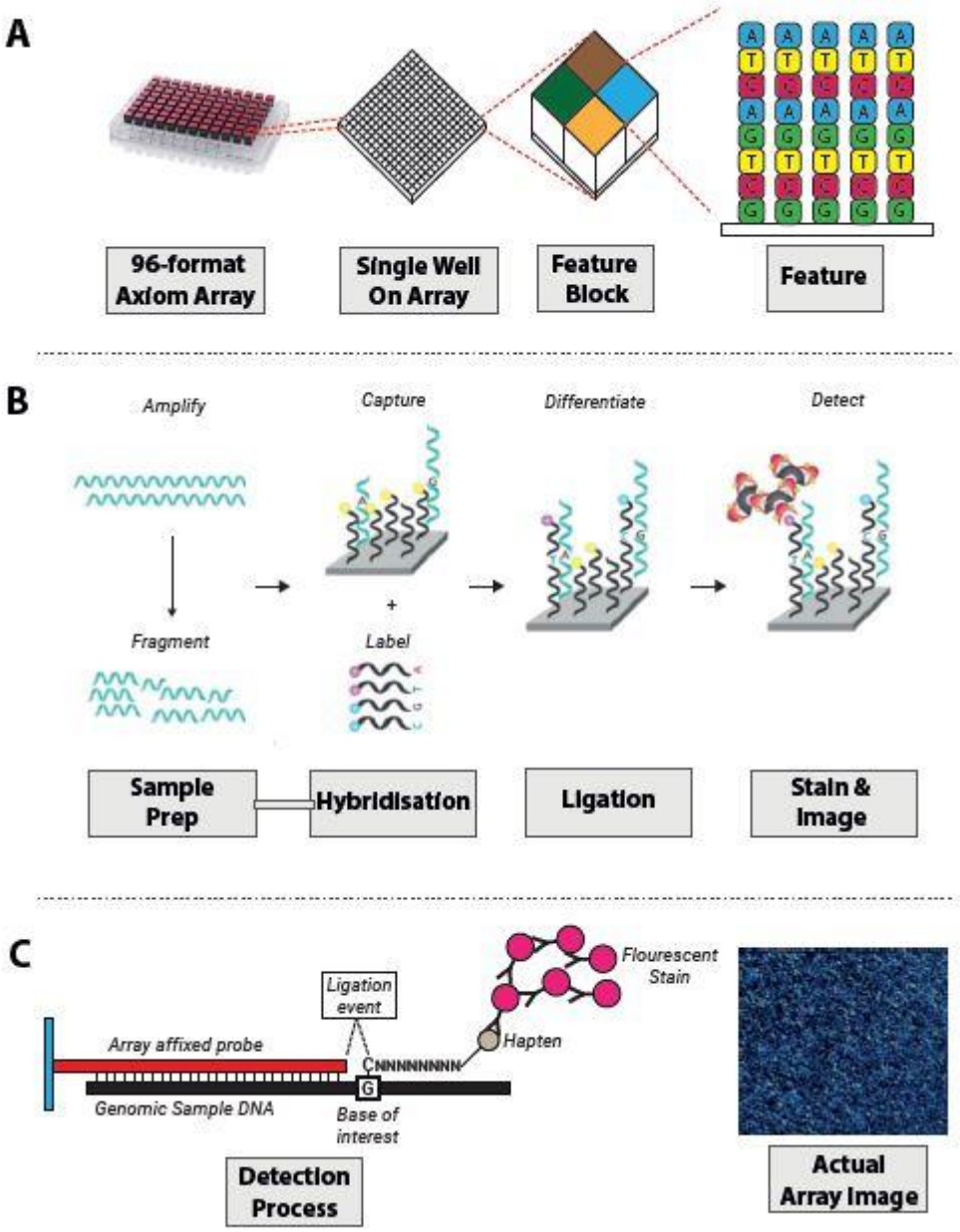


Figure 5. 1: Overview of Axiom array genotyping

(a) A zoom diagram showing the construction of an Axiom array. (b) Axiom genotyping workflow. From left to right; Genomic DNA is amplified then fragmented, fragments are captured and labelled via hybridisation to array probes and detection probes, ligation then covalently bonds the genomic DNA to the array/detection probe complex, finally fluorescent staining is performed followed by excitation and imaging. (c) Cartoon representation of the genomic DNA + array/detection probe complex. An actual image of an array during genotyping is included for reference. Copied from Gleadall N, (2020), thesis submitted to the University of Cambridge.

5.2.7.3 RBC serological phenotyping

RBC serological phenotyping was performed using the tube or microtitre plate method according to the manufacturers' methods. Antigens A, B, D, C, E, c, e, M, N, S,s ,Jka, Jkb were tested with IgM reagents (Sanquin Reagents, Amsterdam, The Netherlands) in direct agglutination. Fya, Fyb antigens were determined with IgG reagents (Sanquin Reagents, Amsterdam, The Netherlands) in indirect anti globulin test with the use of polyclonal anti human IgG (Sanquin Reagents, Amsterdam, The Netherlands). Reactivity was documented with standard agglutination grading (that is 0 to 4+, 0 as no agglutination and 1+ to 4+, showing agglutination in increasing strength of reactivity).

5.2.8 Sample size calculation for patients with SCD and blood donors

Sample size to determine the frequency of blood group antigens was calculated based on the formula, $\mathbf{Z^2xP(1-P)/d^2}$ for cross sectional studies:

Where Z=1.96, d=5% (so 0.05), P=10%

It is estimated that the frequency of the various blood group antigens vary. But our antigens of interest are antigens that are likely to be encountered often in transfused blood. This will usually exclude low prevalence antigens. Low prevalence antigens are defined as antigens present in less than 10% of the population. We therefore chose 10% as the estimated lowest frequency of the blood group antigens and went through the same procedure as described for the antibody study (refer section 4.2.3).

1.96^2x0.1(1-0.1)/0.05^2=138.

With the same assumptions as for the antibody study, 25% with insufficient blood sample to undertake the additional/ complete analyses, 15% drop out and 10% sample analysis failure (138/(1 - 0.25))/(1 - 0.15)/1-0.1) = 240.5. This was rounded up to 250.

5.3 Statistical analysis

The prevalence of the antigens was expressed as percentages when more than ten participants were tested. The prevalence of RBC antigens and blood group phenotypes was compared between donors and patients and between ethnic groups. Chi squared test was performed to look for differences in antigen frequencies between donors and patients. A P-value less than 0.05 was deemed statistically significant.

5.4 Results

5.4.1 Participant demographics

A total of 505 participants were recruited, 171 females and 334 males, 253 donors (182 from KATH and 71 from KBTH) and 252 patients (159 from KATH and 93 from KBTH) with SCD. The median age of the participants was 18 years (range, 2-72 years). The participants belonged to a total of 26 ethnic groups which were grouped into Akan (n=300), Ga (n=88), Ewe (n=46), and other ethnic groups (n=71) (table 5.1). The donors were 159 (62.8%) voluntary non remunerated donors and 94 (37.2%) family replacement donors. Due to funds availability, the number of participants tested per antigen ranged from 117 to 505 (table 5.2).

Table 5. 1: Demographics characteristics of the Ghanaian participants

Characteristic		All participants n=505	Patients with SCD n=252	Blood donors n=253
Age, median years (range)		18 (2-72)	15 (2-72)	21 (16-47)
Gender	Male	334 (66.1)	114 (45.2)	220 (87)
	Female	171 (33.9)	138 (54.8)	33 (13)
Ethnic group	Akan	300 (59.4)	160 (63.5)	140 (55.3)
	Ga	88 (17.4)	37 (14.7)	51 (20.2)
	Ewe	46 (9.1)	28 (11.1)	18 (7.1)
	Other	71 (14.1)	27 (10.7)	44 (17.4)

Data presented as n (%), unless stated otherwise.

5.4.2 RBC antigen frequency

In the total cohort, the prevalence of c, e, k, Kp^b, Js^b, and Lu^b antigens was more than 98%. None of the participants had C^w, Kp^a, Fy^a and Fy^b antigens, K antigen was present in 0.9% and Lu^a antigen in 3.9%. The remaining antigens were present in 12% to 94% (table 5.3). The prevalence of antigens (excluding Duffy and MNSs antigens, which were only tested in donors) was comparable in patients and donors, except for C which was more frequent in donors (17% and 26%, respectively).

When the total population was stratified by ethnicity, comparing Duffy and MNSs antigen prevalence was not performed for Ga and Ewe ethnicities, because less than 10 participants

were typed. The A and E antigens had the lowest and the B and Lu^a antigens the highest prevalence in Ewe participants compared to the other ethnicities (13% and ≥20%; 2.2% and ≥12%; 39% and ≤30%; 6.7% and ≤3.9%, respectively). Akan participants had the lowest prevalence of the B antigen and Ga participants the lowest prevalence of Js^a (23% and ≥28%; 11% and ≥22%, respectively). The prevalence of the other antigens was comparable between ethnicities (table 5.3).

When patients and donors were further stratified by ethnicity, the prevalence of the C antigen was lower in Akan patients compared to Akan donors (14% and 26%) and in Ga patients compared to Ga donors (19% and 29%). The Ga patients had a higher prevalence of Js^a and Jk^b antigens than the Ga donors (17% and 3.8%; 46% and 37%, respectively) whilst the Akan patients had a lower prevalence of the Jk^b compared to the Akan donors (39% and 49%). The prevalence of the other antigens was comparable between patients and donors (table 5.3).

Table 5. 2: Number of Ghanaian patients and donors tested for red blood cell antigens according to ethnicity.

Ethnic groups	Antigens									
	A, B	D, C, E, c, e	C^w	K, k	Kp^a, Kp^b	Js^a, Js^b	Fy^a, Fy^b	Jk^a, Jk^b	M, N, S, s	Lu^a, Lu^b
Total cohort	505	505	440	440	440	440	117	505	117	431
Patients	252	252	225	225	225	225	0	252	0	222
Donors	253	253	215	215	215	215	117	253	117	209
Akan	300	300	289	289	289	289	91	300	91	284
Patients	160	160	149	149	149	149	0	160	0	148
Donors	140	140	140	140	140	140	91	140	91	136
Ga	88	88	55	55	55	55	7	88	7	53
Patients	37	37	29	29	29	29	0	37	0	28
Donors	51	51	26	26	26	26	7	51	7	25
Ewe	46	46	31	31	31	31	4	46	4	30
Patients	28	28	22	22	22	22	0	28	0	21
Donors	18	18	9	9	9	9	4	18	4	9
Other	71	71	65	65	65	65	15	71	15	64
Patients	27	27	25	25	25	25	0	27	0	25
Donors	44	44	40	40	40	40	15	44	15	39

Table 5. 3: Frequency (%) of blood group antigens in Ghanaian patients with SCD and donors according to ethnic group.

Ethnic	Blood group antigens																
groups	A	B	D	C	E	c	e	K	Js^a	Js^b	Jk^a	Jk^b	M	N	S	s	Lu^a	Lu^b
All	20	26	92	22	12	100	99	0.9	22	99	94	43	77	80	27	94	3.9	100
Patients	20	26	90	17	10	100	100	0.4	24	98	93	41	na	na	na	na	4.1	100
Donors	20	26	94	26	13	99	99	1.4	19	99	95	44	77	80	27	94	3.8	100
Akan	20	23	91	20	12	100	99	1.0	23	99	93	43	79	76	29	93	3.9	100
Patients	21	23	89	14	11	100	99	0.7	26	99	93	39	na	na	na	na	3.4	100
Donors	19	22	94	26	14	99	99	1.4	20	99	93	49	79	76	29	93	4.4	100
Ga	23	28	94	25	13	99	100	1.8	11	98	93	41	na	na	na	na	3.8	100
Patients	24	24	95	19	14	100	100	0	17	97	89	46	na	na	na	na	0	100
Donors	22	31	94	29	12	98	100	3.8	3.8	100	96	37	na	na	na	na	8.0	100
Ewe	13	39	89	22	2.2	100	100	0	26	97	94	41	na	na	na	na	6.7	100
Patients	14	36	89	21	3.6	100	100	0	32	96	89	39	na	na	na	na	9.5	100
Donors	11	44	89	22	0	100	100	na	na	na	100	44	na	na	na	na	na	na
Other	20	30	93	27	16	100	99	0	22	98	97	42	73	93	13	93	3.1	98
Patients	15	37	89	30	11	100	100	0	16	100	100	52	na	na	na	na	8.0	96
Donors	23	25	96	25	18	100	98	0	25	98	95	36	73	93	13	93	0	100

The number of patients and donors tested in each ethnic group is shown in table 5.2
na, not applicable because less than 10 participants were tested (see table 5.2).
Percentages greater than 10 were rounded to an integer.
Antigen frequency of 0% (i.e. C^w, Kp^a, Fy^a and Fy^b) and of 100% (i.e. k and Kp^b) in all participants are not presented in the table.

5.4.3 Blood group phenotypes

The prevalence of ABO blood group phenotypes within the total population, stratified as donors and patients and by ethnicity, all followed the O>B>A>AB pattern. The prevalence of the O blood group was lowest in Ewe ethnicity (48%) and highest in Akan ethnicity (61%). None of the Ewe participants had the AB blood group compared to about 3% in the other ethnicities (table 5.4). There were eight Rh phenotypes, and the most common phenotype was ccDee (62%). The ccDEE and CCDee phenotypes were the least common, both 0.4%. The ccddee phenotype prevalence was 8.3% in patients and 4.0% in donors. Ewe participants had the lowest prevalence of ccDEe and the highest prevalence of ccDee and Ccddee phenotypes compared to the other ethnicities (2.1% and ≥9.7%; 72% and ≤63%; 6.5% and ≤2.3%,

respectively). In the Kell blood group system, the frequency of the Js(a+b+) phenotype in the Ga population was low (9%) compared to the other ethnicities (range 20%-23%). The Jk(a-b), M-N- and S-s- phenotypes were found in two participants each (table 5.4).

Table 5. 4: Frequency (%) of ABO and Rh phenotypes in Ghanaian patients with SCD, blood donors and ethnic groups.

Blood group system	Phenotype	All	Patients	Donors	Akan	Ga	Ewe	Other
ABO	O	57	57	57	61	52	48	54
	B	23	23	23	19	25	39	27
	A	17	17	17	17	19	13	17
	AB	3.0	2.8	3.2	3.3	3.4	0	2.8
Rhesus	ccDee	62	65	59	63	60	72	52
	CcDee	18	15	21	16	21	15	25
	ccDEe	9.9	9.1	11	9.7	11	2.1	14
	CcDEe	1.6	0.8	2.4	2.3	1.1	0.0	0.0
	ccDEE	0.4	0.4	0.4	0.3	0.0	0.0	1.4
	CCDee	0.4	0	0.8	0.3	1.1	0	0
	ccddee	6.1	8.3	4.0	0.3	3.4	4.3	5.6
	Ccddee	2.0	1.6	2.4	1.3	2.3	6.5	1.4
Kell	Js(a+b-)	1.4	1.8	0.9	1.0	1.8	3.2	1.5
	Js(a-b+)	78	76	81	77	89	74	79
	Js(a+b+)	20	23	18	22	9.0	23	20
Kidd	Jk(a+b-)	57	58	56	56	59	59	58
	Jk(a-b+)	6.1	6.7	5.5	6.7	6.8	6.5	2.8
	Jk(a+b+)	36	35	38	37	34	35	39
MN	M+N-	19	na	19	22	na	na	6.7
	M+N+	58	na	58	57	na	na	67
	M-N+	21	na	21	19	na	na	27
Ss	SS	4.3	na	4.3	5.5	na	na	0
	ss	71	na	71	70	na	na	80
	Ss	23	na	23	23	na	na	13
Lutheran	Lu(a+b-)	0.2	0.5	0	0	0	0	1.5
	Lu(a-b+)	96	96	96	96	96	93	97
	Lu(a+b+)	3.7	3.6	3.8	3.9	3.8	6.7	1.5

na, not applicable because less than ten participants were tested (see table 5.2).
Percentages greater than 10 were rounded to an integer.
Two Akan patients with SCD had the Jka-b- phenotype, two Akan donors the M-N- phenotype and two donors (one Akan and one other ethnicity) had the S-s- phenotype. Kell and Duffy phenotypes were not presented in the table, because 436 participants had the k+k+ and four participants the K+k+ phenotype and all participants had the Fy(a-b-) phenotype.

5.4.4 Comparison of antigen and phenotype frequencies between different ethnicities in Ghanaian studies

From the eight studies (Armattoe, 1951; Acquaye, 1980; Acquaye, 1990; Acquaye, 1992; Acquaye, 2004; Kretchy et al., 2017; Boateng et al., 2019a; Doku et al., 2019) that were previously published on antigen distribution in Ghanaian ethnic groups and the present study, we compared the frequencies of RBC antigens (table 5.5) and phenotypes (table 5.6). Among the Akans, the prevalence of the antigens was comparable across studies except for A, C, and E antigens which were higher and Jk^a antigen was lower in our Boateng et al 2019 study compared to the present and other studies (34% and 20-26%, 38% and 19-20%, 27% and 12-17% and 78% and 93%). Likewise, the Rh phenotypes CcDee and ccDEe were about one and half times and four times higher respectively (25% and 16% and 10% and 2.2-2.3%), and the ccDee and ccddee phenotypes were lower (44% and 61-63% and 1.9% and 4.9-7.3% respectively), also in our 2019 study compared to the present and other studies.

Among the Ga studies, the prevalence of the antigens and phenotypes were consistent except for antigen Jk^a which was two times less in Acquaye (1990) than in the present study (47% and 93%).

In the Ewe studies, antigen A was up to two times higher in the other studies compared to the present study (20-28% and 13%). Antigen B was higher in the present study (39%) and Armattoe, 1951 (37%) compared to the rest of the studies (25%-29%). Antigens E and K were more prevalent and Jk^a and N were less prevalent in Acquaye 1992 than the present study (21% and 2.2%; 11% and 0%; 69% and 94%; 53% and 72%, respectively). For the phenotypes, the AB and ccddee phenotypes were higher in Armattoe 1951 than the other studies (7.4 and 0-4.1%; 10% and 3.9-4.3%, respectively). The ccDee and Ccddee phenotypes were higher in the present study (72% and 53-54%; 6.5 and 1.5-2.6%, respectively) and ccDEe was higher in Acquaye, 1992, (16% and 2.1-5.1%) compared to other studies.

Table 5. 5: Blood group antigen frequency (%) in Akan, Ga and Ewe ethnicities in studies from Ghana

Ethnic group	Number of participants	First author-year of publication	Antigen A	B	D	C	E	c	e	K	Jk^a	Jk^b	M	N	S	s
Akan	5,439	Acquaye-1980[1]	23	20	98											
	1,533	Acquaye-2004[2]	23	24	94	19	17	100	100	3.3						
	6,503	Kretchy-2017[3]	22	22	94											
	16,896	Doku-2019[4]	26	30	93											
	125	Boateng-2019[5]	34	21	96	38	27	100	98	0	78	50			38	94
		Current study	20	23	91	20	12	100	99	1.0	93	43	79	76	29	93
Ga	230	Acquaye-1990[6]	20	27	96	28	11	99	100	1.3	47	30	94	79	15	97
	2,579	Kretchy-2017[3]	19	21	91											
	10,637	Doku-2019[4]	26	31	94											
		Current study	23	28	94	25	13	99	100	1.8	93	41				
Ewe	853	Armattoe-1951[7]	28	37	90	31	8.6	97	100				86	72		
	328	Acquaye-1980[1]	20	28	95											
	586	Acquaye-1992[8]	22	26	93	26	21	100	100	11	69	38	78	53	26	98
	1,431	Kretchy-2017[3]	23	25	93											
	9,554	Doku-2019[4]	22	29	94											
		Current study	13	39	89	22	2.2	100	100	0	94	41				

The studies were performed in four regions in Ghana: Ashanti (Ash), Greater-Accra (G. Acc), Northern (North) and Eastern.

Percentages greater than 10 were rounded to an integer.

MNSs antigen frequencies from the current study were not presented in Ga and Ewe because only 4-8 participants were tested.

[1]Adapted from Acquaye JK. The incidence of the ABO blood group genotype and the Rhesus D negative frequency in some Ghanaian population groups. Ghana Med J. 1980;6:84-87. This study was conducted in Ashanti and Northern Regions.

[2]Adapted from Acquaye JK. ABO, Rhesus and Kell blood groups in the Akans of Ghana. Ghana Med J. 2004;38:68-71. This study was conducted in Greater Accra Region.

[3]Adapted from Kretchy et al. Distribution of ABO blood group/Rhesus factor in the Eastern Region of Ghana, towards effective blood bank inventory. Sch J App Med Sci. 2017;5(3B):821-826. This study was conducted in Eastern Region.

[4]Adapted from Doku et al. Frequency of ABO/Rhesus (D) blood groupings and ethnic distribution in the Greater-Accra region of Ghana, towards effective blood bank inventory. Int J Immunogenet. 2019;46:67-73. This study was conducted in Greater Accra Region.

[5]Adapted from Boateng et al. Red blood cell alloimmunisation and minor red blood cell antigen phenotypes in transfused Ghanaian patients with sickle cell disease. Transfusion 2019;59:2016-22. This study was conducted in Ashanti Region. Antigen frequencies in these 125 Akan were calculated from the raw patient data.

[6]Adapted from Acquaye JK. Red cell antigens in the Ga ethnic group of Ghana. Ghana Med J. 1990;24:177-182. This study was conducted in Greater Accra Region.

[7]Adapted from Armattoe REG. ABO and RH blood types among the Ewes of West Africa. Am J Phys Anthropol. 1951;9:371-374. This study was conducted in Ashanti Region.

[8]Adapted from Acquaye JK. Red cell antigens in the Ewes of Ghana. Ghana Med J. 1992;26:438-447. This study was conducted in Greater Accra Region.

Table 5. 6: Blood group phenotype frequency (%) in Akan, Ga and Ewe ethnicities in studies from Ghana

Ethnic group	Number of participants	First author-year of publication	Phenotype											
			O	B	A	AB	D-positive						D-negative	
							ccee	Ccee	ccEe	CcEe	CCee	ccEE	ccee	Ccee
Akan	5,439	Acquaye-1980[1]	59	18	21	1.3								
	1,533	Acquaye-2004[2]	55	22	21	2.1	61	16	14	2.2	0.2	0	4.9	0.8
	6,503	Kretchy-2017[3]	59	19	19	2.7								
	16,896	Doku-2019[4]	49	25	21	5.2								
	125	Boateng-2019[5]	51	15	28	6.5	44	25	15	10	0	1.9	1.9	0.9
		Current study	61	19	17	3.3	63	16	9.7	2.3	0.3	0.3	7.3	1.3
Ga	230	Acquaye-1990[6]	53	25	18	3.7	59	25	9.5	1.3	0.9	0.4	3.0	0.9
	2,579	Kretchy-2017[3]	62	18	17	3.0								
	10,637	Doku-2019[4]	49	25	21	5.2								
		Current study	52	25	19	3.4	60	21	11	1.1	1.1	0	3.4	2.3
Ewe	853	Armattoe-1951[7]	43	29	20	7.4	53	23	5.1	3.6	2.6	0	10	2.6
	328	Acquaye-1980[1]	54	27	19	0.9								
	586	Acquaye-1992[8]	48	26	22	3.4	54	20	16	3.8	0.2	0.2	3.9	1.5
	1,431	Kretchy-2017[3]	56	21	19	4.1								
	9,554	Doku-2019[4]	54	24	17	4.9								
		Current study	48	39	13	0	72	15	2.1	0	0	0	4.3	6.5

Percentages greater than 10 were rounded to an integer.
The studies were performed in four regions in Ghana: Ashanti (Ash), Greater-Accra (G. Acc), Northern (North) and Eastern.
Phenotype percentages for Boateng-2019 were calculated from the raw patient data.
Not presented in the table are: frequency of the CcddEe phenotype, which was 0.9% in Akan in Boateng-2019 and 0.2% in Ewe in Acquaye-1992 and of the ccddEe phenotype, which was 0.3% in Akan in Acquaye-2004 and 1.0% in Ewe in Acquaye-1992. These phenotypes were absent in ethnic groups in the other studies.
[1]Adapted from Acquaye JK. The incidence of the ABO blood group genotype and the Rhesus D negative frequency in some Ghanaian population groups. Ghana Med J. 1980;6:84-87.
[2]Adapted from Acquaye JK. ABO, Rhesus and Kell blood groups in the Akans of Ghana. Ghana Med J. 2004;38:68-71.
[3]Adapted from Kretchy JP et al. Distribution of ABO blood group/Rhesus factor in the Eastern Region of Ghana, towards effective blood bank inventory. Sch J App Med Sci. 2017;5(3B):821-826.
[4]Adapted from Doku GN et al. Frequency of ABO/Rhesus (D) blood groupings and ethnic distribution in the Greater-Accra region of Ghana, towards effective blood bank inventory. Int J Immunogenet. 2019;46:67-73.
[5]Adapted from Boateng LA et al. Red blood cell alloimmunisation and minor red blood cell antigen phenotypes in transfused Ghanaian patients with sickle cell disease. Transfusion. 2019;59:2016-22.
[6]Adapted from Acquaye JK. Red cell antigens in the Ga ethnic group of Ghana. Ghana Med J. 1990;24:177-182.
[7]Adapted from Armattoe REG. ABO and RH blood types among the Ewes of West Africa. Am J Phys Anthropol. 1951;9:371-374.
[8]Adapted from Acquaye JK. Red cell antigens in the Ewes of Ghana. Ghana Med J. 1992;26:438-447.

5.5 Discussion

In this study, the prevalence of 24 RBC antigens and antigen phenotypes was studied in Ghanaian patients with SCD and blood donors of various ethnicities (Akan, Ga, Ewe and Other). Among patients and donors, the antigen and antigen phenotypes frequencies did not differ, except for the C antigen (P= 0.02) and the ccddee phenotype (P=0.04). Among the four ethnic groups, frequency variations were observed for some antigens with the Ewe population showing the highest number of antigen (A, B, E, and Lu^a) and antigen phenotype (ccDEe, ccDee and Ccddee) differences compared to the other ethnic groups. The Ga population also differed from the other ethnic groups in the frequency of the Js^a antigen and the Js(a+b+) phenotype. The small number of participants in most ethnic groups limited statistical analyses of frequency variations within ethnicities.

When antigens and phenotypes frequencies between Akan, Ga and Ewe ethnicities were compared in the present and the eight previously published studies, antigens and phenotypes frequencies varied considerably between studies within ethnicities. Most of the variations were within the Ewe ethnicity (antigens A, B, E, K, Jk^a and N and phenotypes AB, ccDee, ccDEe, ccddee and Ccddee) and then the Akan (antigens A, C, E and Jk^a and phenotypes ccDee, CcDee, CcDEe and ccddee). The Ga ethnicity had comparable frequencies across studies except for antigen Jk^a.

5.5.1 Comparing study findings with other studies in Ghana

Among patients and donors, our findings that the ccddee phenotype was more prevalent in patients than the donors and the C antigen was more frequent in blood donors than in patients are important for transfusion purposes, as these are likely to put the patients at risk for allo anti-C and anti-E formation following unmatched transfusions with units from a donor population with relatively high frequencies of antigens C and antigen E. In Motswaledi, Kasvosve and Oguntibeju (2016), the C antigen was shown to offer protection to HIV infection. Since donors are screened for HIV and those who are negative are selected, this may result in the selection of C positive donors.

Within the four categorized ethnic groups in the present study, we observed that the frequencies of some antigens varied in the Ewe population (A and E were lower) and (B and Lu^a were higher) compared to the other ethnicity groups. A study that genetically characterized 268 unrelated males of five different ethnicities (Akan, Ga, Ewe, Guang, Mole-Dagbon) at the 23 Y chromosome short tandem repeat loci, found that the Ewe males were significantly different

from the other ethnicities (Kofi *et al.*, 2019), which may have contributed to the different antigen frequencies. The Ewe population resides in the Volta Region of Ghana, which is the area where Ghana shares border with Togo. There is free movement between this Region and the Togoland and possible intermarriage and breeding. It is therefore possible that Togo might have contributed to the gene pool in the Ewe resulting in the variations from the other ethnic groups observed.

The pattern of the ABO blood groups frequency distribution in this study (O>B>A>AB) was consistent with some previous studies on ABO conducted in Ghana.(Acquaye, 1990; Acquaye, 1992; Kretchy *et al.*, 2017; Doku *et al.*, 2019) and differed from others also in Ghana (Acquaye, 1980; Boateng *et al.*, 2019a). In the study of Doku et al (2019), even though the pattern observed in the whole population was consistent with what we observed in our population, when the participants were divided based on regions, the frequency for those from the Northern region of Ghana followed a different pattern O>A>B>AB, confirming the possible regional differences in blood group frequencies.

The 100% frequency of the Duffy null phenotype, Fy(a-b-), within the Ghanaian population is not surprising, because the Duffy antigen acts as a receptor for the invasion of the Plasmodium vivax parasite into the human RBC, and the null phenotype offers protection against malaria (Barnwell, Nichols and Rubinstein, 1989) which is endemic in Ghana.

Comparing the antigens and phenotype frequencies across ethnicities in the present and the other eight published studies on ethnicities in Ghana, we found that some antigens and phenotype frequencies varied between studies according to ethnicity. The variations were many and did not follow a particular trend making it difficult to draw any strong conclusions.

5.5.2 Comparing study findings with other studies in SSA

Comparing our findings with some other SSA countries, the frequencies of most RBC antigens in our population was comparable to those published for other Sub-Saharan African countries (M'Baya *et al.*, 2010; Jeremiah *et al.*, 2011; Siransy Bogui *et al.*, 2014). The ABO phenotype distribution pattern was similar to what has been reported in Guinea and Madagascar (Loua *et al.*, 2007; Randriamanantany *et al.*, 2012) but differed from the pattern (O>A>B>AB) observed in Cameroon, Ethiopia, Malawi and Tanzania (M'Baya *et al.*, 2010; Ndoula *et al.*, 2014; Tesfaye, Petros and Andargie, 2015). The frequencies of the Rh phenotypes ccDEE (18%) and CcDee (0.4%), in our study were similar to what was reported by Adewoyin *et al.* (2018) (15.2% and 1.7% respectively) among Nigerians from Benin but were inconsistent with

what was observed by Jeremiah and Odumody (2005) among Nigerians from Calabar (1.9% and 4.5% respectively). The differences in frequencies of some of the ABO and Rh phenotypes observed between our population and some other sub-Saharan African countries may be explained by the high genetic variations within the African population (Yu *et al.*, 2002). These differences were however not surprising, because, within the same Ghanaian population variations existed between ethnicities and even between studies within the same ethnic groups. This suggests that data for one African country may not be readily extrapolated for another. For example, blood group frequencies established for Blacks, mainly based on the Black American population, may not be used for Blacks from other origins as this may not reflect the genetic differences observed within the specific population.

5.5.3 Varying Jk^a antigen frequencies between studies in Ghana and SSA

The frequency of the Jk^a antigen varied between 5% and 94% in studies from Ghana (Acquaye, 1990; Acquaye, 1992; Boateng *et al.*, 2019a) and other SSA countries. (M'Baya *et al.*, 2010; Erhabor *et al.*, 2014; Traore *et al.*, 2019). The low frequencies may have been introduced by the type of Jk^a antisera and the technique used. Wu *et al.* (2019), found that serology in donors with weak *JK*A* allele showed discrepant results with different manufacturers of antiserum. Our preliminary results on *JK* genotyping showed that weak *JK*A* alleles are frequent in Ghanaians. Some previous studies with low prevalence of the Jk^a antigen may have used antisera that did not react with all weak *JK*A* and patients and donors may have been incorrectly classified as Jk^a negative.

Many authors have speculated that for patients with SCD (recipients) and blood donors (donors) in Africa, RBC antigens are similar (Natukunda *et al.*, 2010; Meda *et al.*, 2014; Mangare *et al.*, 2015; Adewoyin, 2016). From our observations in this study, this statement holds true for many, but not all RBC antigens.

Overall, the varying observations suggest the need to establish the antigen frequencies for the various antigens in different ethnicities and populations in SSA and to compare the distribution patterns between donors and recipients. This would help determine which antigens differ between ethnicities and between the donors and recipients and further aid in the management of alloimmunisation.

5.6 Conclusion

In conclusion, this is the first study to determine the frequency of many RBC antigens, beyond ABO, Rh and Kell in patients with SCD and blood donors and to compare the frequencies of antigens between the patients and the donors and for different ethnic groups in Ghana. The antigen distribution differed between the patients with SCD and blood donors for the C antigen and the ccddee phenotype. These findings show that the distribution of some antigens differ between patients and donors in Ghana and calls for studies, which include antigens untested in this cohort, to compare the frequencies of antigens between patients and donors of different ethnic backgrounds in Ghana and for other parts of Africa. To improve transfusion management of the patients, antigens distributions that differ between the donors and patients and likely to put the patients at risk for alloimmunisation, should be prioritized in the selection of cells for RBC antibody testing in Ghana.

CHAPTER 6: GENERAL DISCUSSION

6.1 Summary of project findings

Blood transfusions are vital in the management of patients with SCD. Repeated transfusions with blood from allogenic donors which may contain antigens foreign to the patients predispose them to the development of RBC alloantibodies. RBC alloantibodies could induce mild to serious transfusion reactions upon subsequent transfusions with the cognate antigens to which the patients have made antibodies, cause haemolytic disease of the newborn/foetus or even limit the availability of further compatible blood thereby delaying or preventing transfusion therapy. Pre transfusion testing protocols in Ghana and many sub-Saharan African countries tests for ABO, Rh D compatibility and perform crossmatching (mostly immediate spin crossmatch) without screening for the presence of these antibodies. The few studies on RBC alloimmunisation in patients with SCD in SSA have highlighted the need to screen for RBC antibodies during pre-transfusion testing. There have been proposals to include RBC antibody testing into pre-transfusion screening procedures for patients with SCD in SSA (Natukunda *et al.*, 2010; Meda *et al.*, 2014; Adewoyin, 2016). Because many SSA countries do not prepare reagent red cells for antibody testing and are likely to rely on the commercially available cells, the main concerns for introducing the test are:

1. the cost of importing the reagents: the cost of procuring and shipping the reagents from international sources will be high and a major hindrance. Also, screening and identification cells expire within four weeks. Repeated ordering of reagent red cells every three to four weeks is not likely to be sustainable in resource limited areas like Africa.
2. the antigens present on the panel : blood group antigens have shown to be inherently polymorphic and vary between populations (Mattos, 2011). When reagent red cells are procured from international sources where antigens represented are mainly of Caucasian origin, some antibodies exclusively present in Africans are missed.

In this project, I performed a systematic review of the published literature to estimate the burden of alloimmunisation in SSA, investigated post transfusion red blood cell alloimmunisation in patients with SCD in Ghana and explored the possible ways of

optimising transfusion practices in patients with SCD to detect RBC alloimmunisation to clinically relevant RBC antigens.

To determine the estimated burden of alloimmunisation in patients with SCD in SSA, I performed a systematic search of published literature and a meta-analysis including 15 studies with a total of 1994 transfused patients that reported on RBC alloimmunisation in patients with SCD from nine different SSA countries. The prevalence of alloimmunisation ranged from 2.6%-28.6% with an overall pooled proportion of 7.4% (CI 5.1-10.0). Antibodies E, D, C and K accounted for about half of the total antibody specificities. Antibodies to low and high frequency antigens were also common accounting for 29% (20% and 9% to low and high frequency antigens respectively) of the antibody specificities.

Further, in a cross-sectional study of antibody and antigen positivity and adverse transfusion events to explore the possible ways of optimising transfusion practices in patients with SCD to detect RBC alloimmunisation to clinically relevant RBC antigens, I determined the prevalence, specificities, and risk factors for RBC antibodies against European and African antigens and adverse transfusion events in multi-transfused patients with SCD and the distribution of clinically relevant RBC antigens -routinely present on commercially available antibody screening panels- among patients with SCD and blood donors in multi-ethnic Ghana .

In the antibody study, I demonstrated that 36 of 226 patients (16%) had RBC antibodies. In eleven of these patients (31%) antibodies were against African antigens. Receiving the first transfusion after the age of three years (aOR 3.28) and the number of transfusions (aOR 2.00) were associated with alloimmunisation.

Sixty-eight patients (30%) recalled they experienced adverse transfusion reactions of which 23 patients (10%) had symptoms suggestive of haemolytic reactions. Adverse transfusion reactions were positively associated with the number of transfusions (aOR 2.06).

In the antigen study, I studied the prevalence of 24 RBC antigens and antigen phenotypes in Ghanaian patients with SCD and blood donors. The number of samples

tested ranged from 117 to 505. The data was analysed by first grouping the participants as patients with SCD and donors and then into different ethnic groups. The participants, who belonged to 26 different ethnic groups were categorised under four main ethnic groups, Akan, Ga, Ewe and other. Other represented all the ethnic groups other than Akan, Ga and Ewe. Among patients and donors, the antigen and antigen phenotypes frequencies did not differ, except for the C antigen, and the Rh ddccee phenotype. Among the four ethnicity groups, frequency variations were observed for some antigens with the Ewe population showing the highest number of antigens (A, B, E, and Lu^a) and antigen phenotypes (DccEe, Dccee and ddCcee) variations compared to the other ethnicity groups, The Ga population also differed from the other ethnicity groups in the frequency of the Js^a antigen and the Js(a+b+) phenotype.
Alloimmunisation was mainly towards the major Rh and some African Antigens. It is unclear whether these antibodies are the highly immunogenic antigens in the African patients with SCD or if the other antibodies which we did not encounter might have disappeared, considering the cross sectional design of all the studies on this subject in SSA and the high evanescence rate of some of the antibodies (particularly Kidd and Kell antibodies) (Harm *et al.*, 2014) in these patients. Longitudinal studies that test patients at specific time points after transfusions would be helpful to further elucidate this finding. Further studies that prospectively investigate adverse transfusion events are needed to better determine the consequences of alloimmunisation in the patients with SCD.

Also, because it was confirmed that some antibodies to African antigens are missed when the standard Caucassian panel is used, there is the need for research to determine the immunogenicity of these African antigens and in approaches to develop African specific RBC antibody panel that incorporate African RBC antigens. The panel could be used locally for RBC antibody testing. This would allow detection of clinically significant antibodies that may be specific to Black Africans. Moreover, it would provide an affordable and sustainable means of implementing RBC antibody screening tests for SCD and other transfused patients which could be integrated into routine pre-transfusion screening procedures.

Besides the importance of my findings to Africa, they are also 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.

6.2 Strengths of study

6.2.1 Assuring quality of study

6.2.1.1 Data collection

The study participants were randomly selected with no restrictions on age, gender, ethnicity and SCD genotype. Data collection was thorough. Effort was made to collect all information relevant to answering the research questions. This was done by ensuring that patients' information on age, sex, ethnicity, transfusion, pregnancy and medical histories filled in the questionnaire were complete. Participants with incomplete information were followed up with a phone call to fill in the missing information, unless they already indicated they could not recall. Data entered in excel were validated weekly to ensure information entered matched what was entered in the questionnaire.

6.2.1.2 Sample processing

Blood samples were processed and frozen daily, at the end of the same working day they were collected to reduce the possibility of sample number mix-ups associated with processing bulky samples in a shift and to preserve the quality of the samples. Cryotubes were carefully labelled. Both the EDTA tubes and cyrotubes were arranged in order of increasing numbers before the start of the process to ensure accuracy. To ensure the correct samples go into the correct tubes, samples numbers were double checked before transferring samples from the EDTA tubes into the cryotubes. The samples were frozen immediately after processing to preserve their quality.

6.2.2 Laboratory testing

Laboratory testing was comprehensive and was carried out at the state-of-the-art transfusion research laboratory in Amsterdam, Sanquin. This laboratory is enrolled in the external quality assurance services. Laboratory equipment were duly calibrated and reagents, quality controlled, ensuring high level of quality assurance . Genotype test

results were validated with serology. Serology tests were repeated any time results were inconclusive, to ensure accuracy of results.

6.2.3 Supervision

This project was supervised by renowned researchers from the United Kingdom, and the Netherlands. These supervisors have long term research experience in different aspects of transfusion medicine. Bringing together their rich and varying expertise enriched the output of the project.

6.2.4 Research collaborations

Through this project, strong and durable collaborations have been formed with the NHSBT, Cambridge, Sanquin, Amsterdam and the NBSG, Ghana. These collaborations are already providing the platform for future research projects tailored to further optimising transfusion practices and safety in Ghana.

6.3 Challenges

6.3.1 Loss of RBCs and repeat fieldwork

Due to power outage during storage of samples in Ghana and technical problems (extreme storage temperature changes) with samples shipment and subsequent storage at Sanquin, all RBCs that were collected during the June 2018 to January 2019 field work haemolysed. This was realized during the laboratory analysis in March 2019 and the fieldwork had to be repeated in July to September 2019 to enable recollection of the lost blood samples. This impacted negatively on my timelines and funds available for the project.

6.3.2 Impact of COVID-19

The COVID-19 pandemic like its impact globally also significantly delayed my work (for almost one year).

I had to abruptly end the second phase of my laboratory analyses (which involved RBC antibody testing against the selected cells expressing African Antigens and RBC serological typing of the major blood group antigens, to validate the genotype results) in Sanquin in March, 2020. I could not finish the serology typing. This was because the laboratory where I was working prioritized COVID 19 research and ceased all non-COVID related research, and the whole country (Netherlands) announced a national

lockdown. I therefore had to immediately return to the UK. The unfinished work was halted until December 2020 when a member of staff at Sanquin resumed the testing.
Another delay relates to my red blood cell (RBC) genotyping results – the genotyping testing was completed in February 2020, but the data needed to be translated to readable information and this depended on a supercomputer based in Cambridge. The job was sent to the supercomputer in March 2020 but was not executed because all non-COVID related work had been halted as the computer was diverted to executing COVID 19 related research. The remaining aspects of the project work were also dependent on these results so further laboratory testing which was originally scheduled for May 2020 had to be put on hold. The results were finally received in October 2020. This coincided with the second wave of the virus in the UK and some other countries, so international travel was not approved by both LSTM and the Commonwealth Scholarship Commission. I therefore could not travel to the Netherlands for the follow up laboratory tests. This was suspended until December when arrangements were made for Sanquin laboratory personnel to continue the testing on my behalf. The assigned staff performed the serological tests for the samples, however, it was not possible to fully resolve discrepancies between serology and genotype results within my PhD timelines. Finally, I have had to work on my thesis from home since April 2020. This was also the period when the nation had been in a lockdown, schools were closed and visit to other homes banned. Being stuck indoors with two young children impacted negatively on my ability to concentrate on my results analysis and thesis write up.

6.3.3 Coordinating samples and data between multiple partners

I collaborated with two independent institutions, in two different countries, in the laboratory analysis of my blood samples; Sanquin in the Netherlands where I performed all the laboratory work related to the antibody study and part of the laboratory work for the antigen study (DNA extraction) and the National Health Service Blood and Transplant (NHSBT) in Cambrigde, United Kingdom, where the genotyping test was carried out. The NHSBT collaboration extended to Thermofischer in Boston, USA. The Thermofischer lab in Boston ran the genotype test and transferred the data to a supercomputer in Cambridge for interpretation of data. These

collaborations are mutually beneficial since these blood services need more information on alloimmunisation to cater for their ethnic minorities.

All these institutions worked independently and sometimes coordinating, receiving, and communicating information between the three was difficult. It was quite challenging at times to know who the best contact for a particular information would be or to relay particular message. Miscommunication sometimes affected the effective execution of some tasks. Also, differences in partners' interests affected the extent of work (in some cases over-testing and others under-testing) we could carry out on the samples. Coupled with the busy schedule of study partners in these uncertain times, information flow was sometimes challenging leading to delays in the execution and completion of some tests.

6.4 Limitations of study

The project had the following limitations.

6.4.1 Cross sectional design

Sub-optimal timing for antibody testing might have underestimated the number of alloimmunised patients. This is because patients might have been sampled too early when antibodies might not have been formed or too late when the antibodies might have disappeared.

6.4.2 Information documentation at hospitals

Although I endeavoured to collect all information such as participants' demographics and transfusion/pregnancy/medical histories relevant to answering the research questions, information on transfusion history may not have been complete for all patients, due to sub-optimal hospital documentation and because patients' (or guardians') memory may not have been exactly accurate. Also since patients or their guardians had to recall information on adverse reactions to transfusions, there could possibly be recall bias leading to the under- or over- estimation of the frequency of adverse transfusion reactions.

6.4.3 Sample size

For the antigen typing study, the sample size was powered for the total population, but some of the ethnic groups were underrepresented making it difficult to stratify donors and patients with SCD into different ethnic groups and to statisticaly analyse to see if

significant differences existed between donors and patients within and across ethnic groups.

6.4.4 Sample population

In the antigen study, almost 40% of the donors were family replacement donors and the probability that two relatives share the same blood group is significantly higher than for two random individuals (van Sambeeck *et al.*, 2020). The results should therefore be intetpreted with caution and the findings may not be readily extrapolated to situations where the blood donors have a lower possibility of being related.

6.5 Next Steps

6.5.1 Ongoing laboratory work

Laboratory tests are still ongoing to resolve discrepancies between genotype and serology. When this is complete an RBC antibody testing panel which will be specific for Africa will be developed.

6.5.2 Further research

Once the ongoing discrepancies are resolved and the Africa specific panel is prepared, I will carry out a mixed method study to assess the feasibility of incorporating RBC antibody testing into routine pretransfusion testing in Ghana.

I have submitted a proposal for the Wellcome Trust International Training Fellowship to secure additional funds for further prospective investigations. These include:

1. The investigation of antibodies to antigens that are predominantly present in Black Africans.
2. To perform a longitudinal study to characterise the development and immunogenicity of RBC antibodies to African antigens.
3. To investigate thoroughly the adverse reactions associated with blood transfusion.

I further plan to design a study that would determine the frequency of RHD variants in serological D positive patients with SCD at risk for making anti D alloantibody.

6.6 Recommendations/ implications for transfusion practice in patients with SCD in Ghana

The effective transfusion management of patients with SCD is vital to improving the overall wellbeing of the patients. To achieve this requires the collaborative effort of the relevant healthcare team and the National Blood Service. From the findings of this study, I developed some recommendations and have categorised them under the relevant teams. Recommendations that require changes to national systems or introduction of policies were categorised under the National Blood Service. Those that pertained to the day-to-day laboratory testing to improve the blood transfusion process were assigned to the blood banks and finally, recommendations that related to improving the primary care of patients were categorised under clinicians and nurses.

6.6.1 For the National Blood Service in Ghana

- Consideration should be given to the incorporation of RBC antibody testing into routine pre-transfusion screening procedures for patients with SCD. Preferably, this should be done with reagent RBC that also express African antigens.
- The national blood service should institute appropriate measures to transfuse patients who have a variant form of the Rh D antigen (and are therefore at risk for anti-D antibody formation in Ghana) with D negative blood units.

6.6.2 For blood banks

- Blood banks should always perform indirect antiglobulin crossmatch between donor RBC and patient serum before blood is issued for transfusion.
- When antibodies are present, these should be filed on patients' records in the hospital/blood bank and should be accessible for the patients' lifetime.
- Since patients may be transfused in multiple hospitals and because there is no information technology system that links the hospitals, the patients should receive written information about their antibodies to be able to provide other hospitals with this knowledge to improve transfusion safety and the efficacy of antibody testing.
- Blood banks should consider implementing component therapy (preferably leucodepleted) rather than whole blood transfusion. This will result in a more optimal use of the limited blood supply and will improve blood safety by reducing adverse transfusion reactions in SSA. However, I recognise that unavailability of funds and the need for additional resources/cost may be limiting factors.

- There should be a clearly written protocol (standard operating procedure) for the investigation of HTR in the blood bank.

6.6.3 For clinicians and nurses

- Patients with a history of transfusion, especially those who are first transfused after three years, should be monitored closely during and after transfusion as they have a higher risk of transfusion reactions.
- Recently transfused patients with SCD who return to the hospital with pain crises should be investigated for possible delayed HTR before complicating the situation with further transfusions.
- During internship, clinicians and nurses should be educated and trained on how to identify a HTR and what they need to do in a suspected case of HTR.
- There should be a clearly written protocol (standard operating procedure) for the collection and processing of blood for a suspected HTR in the ward.

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Appendices

Appendix I: Consent forms for study participants

1. Consent Form (blood donors/ adult patients with SCD)

CONFIDENTIAL

Study Title: Investigation of post-transfusion red blood cell alloimmunisation in Ghana	
Principal Investigator: Lilian Antwi Boateng	**Study Site: Ghana**

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information about the study, including procedures, risks and benefits, sample transport and handling, to enable the prospective participant to make an informed decision to or not to participate.

DATE: _____ NAME: _____

SIGNATURE: _____

Statement of person giving consent:

I confirm I have read and understood the information sheet dated 15/05/18 (Version 1.2) for the above study or have had it translated into a language I understand. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.

I understand that my participation is voluntary (not compulsory).

I agree to donate a small sample of blood for use in health-related research. I understand that my samples may be transferred between international research institutions.

I give permission for the long-term anonymised storage of my blood samples (including cells and DNA) for health-related research purposes (even in the event of my incapacity or death).
I understand my anonymised sample may be used for further research.
I understand that my samples will be tested as outlined in the information leaflet and this may include the reading of my entire DNA code.
I give permission to the researchers to access my medical notes and other health-related records. I understand that information from my medical notes and other health-related records may be used to provide information about my health status and I give permission for long-term anonymised storage of this and other information about me for health-related research purposes (even in the event of my incapacity or death) (for Patients with SCD only)
I understand that I may freely stop being part of this study at any time without having to explain myself.
I have received a copy of this information leaflet and consent form to keep for myself.
I agree / do NOT agree to the data about me/ my blood sample collected in this study being stored for further use in the future. (please underline)
NAME:_____

DATE: _____ SIGNATURE/THUMB PRINT: _____

Statement of person witnessing consent (Process for Non-Literate Participants):

I _____ (Name of Witness) certify that information given to

_____ (Name of Participant), in the local language, is a true reflection of what l have read from the study Participant Information Leaflet, attached.

WITNESS' SIGNATURE (if participant is non-literate): _____

When complete: 1 copy for participant; 1 copy (original) for research

2. Consent Form (parent of minor SCD patient)

CONFIDENTIAL

Study Title: Investigation of post-transfusion red blood cell alloimmunisation in Ghana	
Principal Investigator: Lilian Antwi Boateng	**Study Site: Ghana**

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information about the study, including that on procedures, risks and benefits, sample transport and handling, to enable the prospective participant to make an informed decision to or not to participate.

DATE: _____ NAME: _____

SIGNATURE: _____

Statement of person giving consent:

I confirm I have read and understood the information sheet dated 15/05/18 (Version 1.2) for the above study or have had it translated into a language I understand. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

I understand that my child's participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want him/her take part in it.

I agree that my child will donate a small sample of blood for use in health-related research. I understand that these samples may be transferred between international research institutions.

I give permission for the long-term anonymised storage of the blood samples (including cells and DNA) for health-related research purposes. I understand the anonymised sample may be used for further research.

I understand that the samples will be tested as outlined in the information leaflet and this may include the reading of my child's entire DNA code.

I give permission to the researchers to access my child's medical notes and other health-related records. I understand that information from my child's medical notes and other health-related records may be used to provide information about my child's health status and I give

permission for long-term anonymised storage of this and other information about me for health-related research purposes (for parents of Patients with SCD only)

I understand that my child can freely stop being part of this study at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.

I agree / do NOT agree to the data about my child collected in this study being stored for further use in the future.

NAME: _____

DATE: _____ SIGNATURE/THUMB PRINT: _____

Statement of person witnessing consent (Process for Non-Literate Participants):

I _____ (Name of Witness) certify that information given to

_____ (Name of Participant), in the local language, is a true reflection of what l have read from the study Participant Information Leaflet, attached.

WITNESS' SIGNATURE (if participant is non-literate): _____

3. Assent Form (minor SCD patient)

CONFIDENTIAL

Study Title: Investigation of post-transfusion red blood cell alloimmunisation in Ghana	
Principal Investigator: Lilian Antwi Boateng	**Study Site: Ghana**

Hello …… (name of Child)

We are doing a study to see if the blood transfusion that you receive in the hospital when you are sick makes you feel better or worse. We are asking you to be take part so that we can learn more about how much the blood transfusion helps you. If we find that the blood transfusion makes you feel worse we can learn why and how this happens. We will then be able to try and prevent this happening to you and your friends in the future.

If you agree to be part of our study, we are going to take a small amount of blood from you (2 teaspoonful) and we will share this with people helping us with our study in other countries, such as the United Kingdom. If you are meant to be having a normal blood test today we will collect the sample for the study at the same time.

If you sign this paper, it means that you have read this and that you want to be in the study. If you don't want to be in the study, don't sign this paper. Being in the study is up to you, and no one will be upset if you don't sign this paper or if you change your mind later.

Your Signature …… (Date)

Your name……

Signature of person taken assent……..

Name of person taking assent……

Appendix II: Participant information leaflet for patients with SCD

"Title of Study: Investigation of post-transfusion red blood cell alloimmunisation in Ghana

Participant Information Leaflet

(For sickle cell disease patients)

My name is Lilian Antwi Boateng. I am a student at the Liverpool School of Tropical Medicine working with Prof. Imelda Bates. We would like to invite you to take part in our research study. Before you decide, we would like you to understand why the research is being done and what it would involve for you. **I will go through the information sheet with you and answer any questions you have.** This should take about 10 minutes. Please ask me if anything isn't clear.

Background

Sickle cell disease (SCD) patients require frequent blood transfusion. Studies have shown that Patients with SCD develop some substances in the blood that can cause them to react badly to blood transfusions or make it difficult for the laboratory to find a suitable blood donor match for them.

Unfortunately, we do not know how often Patients with SCD in Ghana develop these substances. Blood type mismatch between patients and blood donors is what causes the patients to develop these substances. We do not whether the blood type of the Patients with SCD and the blood donors are the same or not.

Purpose of study

We are carrying out this study to determine how often Patients with SCD in Ghana make these substances in their blood following blood transfusion and the nature of the substances. We will also determine the blood types of the patients and the blood donors to see if they are similar or not. Based on our findings, we will use blood from the donors to prepare a solution that will help us test for the substances in the blood before every transfusion in Patients with SCD and other people who often

require blood transfusion. This will help us provide the patients with suitable blood and to prevent future development of the substances.

Why have I been invited?

You have been invited because you have sickle cell disease and have received blood transfusion before. You will be one of 200 participants.

Do I have to take part?

No. It is up to you to decide whether to join the study. We will describe the study and go through this information sheet with you. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive.

What will happen to me if I take part?

You will only need to attend one appointment which should take no more than [15] minutes. In the future, you may be invited to provide a further small sample of your blood, or further samples, if the initial sample you provided was insufficient for certain types of testing.

Below are highlights of what we will do:

- We will take your personal information from your hospital folder and add it to a questionnaire. The information we will take is your age, the number of blood transfusions you have received and when you received them, how often you receive blood transfusions, the reasons why you receive blood, if you have reacted to any blood transfusion before and what was done, the type of sickle cell disease you have.
- You will be asked to help us with information we are unable to get from your hospital folder and to confirm information taken from your hospital folder.
- We will then take 10 mls (equivalent to 2 teaspoons) of blood from one of your arms. If you are having a hospital test on the day of the sample collection, the blood sample for your hospital test and the research sample will be taken at the same time.

What will happen to the samples I give?

Blood samples will be shipped to the laboratories of international collaborators (e.g. Sanquin, Amsterdam, The Netherlands or NHS Blood and Transplant, Cambridge, United Kingdom) for blood group type and protein analysis.

This may include determining the sequence of all or part of your DNA code. We may also isolate and test other components of your blood such as cells, RNA, protein and metabolites. Researchers may seek access to your samples and related data, but your personal details will never be released to researchers without your knowledge. Your samples will be kept in a secure location.

The data we obtain from this will be analysed together with the data from other participants and this will help us to make meaning of the results we will get.

We would also want to seek your consent to store your remaining blood sample for future studies and to use your data in future research similar to this work. All future research will be anonymous and will not be identifiable with you

What will happen to any data produced from the samples I give?

Anonymous data about you may be stored in an electronic archive and made available to researchers. This data may include part of or your entire DNA code, or the results of other tests performed with your samples and other information from the research database that does not identify you personally, e.g. your age in years, your gender.

Researchers will have to make a request to access this data and explain how they will use it, e.g. which research question they are trying to answer. This type of system is referred to as 'managed access'. Genuine researchers will be given access to the data for their research, and they will be reminded of their obligation to keep your data safe by accepting the terms of a data transfer/access agreement.

'Managed access' requests could come from researchers who are working in the public and charitable sector (Universities, Research Institutes) or in commercial companies, either in the UK or overseas.

Researchers share the results of their studies by means of reports or publications, which includes placing information/data on the internet, in press articles, in project leaflets and through other media. Under no circumstances will information that identifies you personally be disclosed in any of these documents.

Compensation

You will not receive any payment in return for your participation in this study

What are the possible disadvantages and risks of taking part?

The only risk associated with taking part in this study is that providing a blood sample may cause slight pain from insertion of the needle and, in rare cases, may be followed by bruising or swelling which resolves after a few days. However, the latter risk is very minimal as blood will be collected by an experienced phlebotomist.

What are the possible benefits of taking part?

We cannot promise that the study will help you directly but the information we get from this study should help to improve transfusion management of individuals with sickle cell disease

What will happen if I don't want to carry on with the study?

You can withdraw from the study at any time, and you do not need provide a reason.

If you withdraw from the study, information collected may still be used. Any stored blood or tissue samples that can still be identified as yours will be destroyed if you wish.

Will my taking part in this study be kept confidential?

All the information about you will be handled in confidence, stored securely in locked cabinets and on password protected computers. Your data will be looked at only by responsible and authorised personnel from the project.

What will happen to the results of the research study?

The results of the study will be presented to the hospital management team in charge of the sickle cell clinic. A lay summary of the research findings will be available in the clinic.

Also, the results will be published and/or presented at conferences. In all these presentations, you will be anonymous.

Study Conduct

The Commonwealth Scholarship Commission is the sponsor of this project and is ultimately responsible for the safe conduct of the study and the well-being of participants. Any unforeseen circumstances will be reported to the sponsor and dealt with appropriately.

Complaints

If you have a concern about any aspect of this study, you should ask to speak to Lilian Antwi Boateng by calling +233200315879, who will do her best to answer your questions. If you remain unhappy and wish to complain formally, you can do this by contacting [Prof. Imelda Bates, at Imelda.Bates@lstmed.ac.uk]. Details can be obtained from [Lilian Antwi Boateng].

Sponsorship and Funding

This research is sponsored by Commonwealth Scholarship Commission and has been reviewed and approved by Liverpool School of Tropical Medicine Research Ethics Committee and the committee on Human Research Publication and Ethics of Kwame Nkrumah University of Science and Technology, Kumasi and Korle Bu Teaching Hospital, Accra.

It is funded by the United Kingdom government.

Contact Details

Lilian Antwi Boateng

+233200315879

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Ethics committee in Ghana

Committee on Human Research Publication and Ethics

School of Medical Sciences, Kwame Nkrumah University of Science and Technology

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Korle Bu Teaching Hospital

Korle Bu - Accra

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Appendix III: Questionnaire for patients

Study title: Investigation of post-transfusion red blood cell alloimmunisation in Ghana

(Data capture sheet for patients with SCD only)

This questionnaire will help us gather your personal information, transfusion and medical history

Study ID number

…….

Date……..

study site

…….

A. Demographic data

1. Name:……
2. Contact No:
3. Date of Birth……...
4. Age…… (years)
5. Sex: a. Male b. Female c. Prefer not to say (please Circle)
6. Ethnicity (please circle) a. Akan b. Ewe c. Ga-Adangbe d. Guan e. Mole-Dagbani

   f. Other, specify

   …….
7. Educational level: a. Pre-primary b. Primary c. JHS c. SHS/Voc/Technical d. post sec e. University
8. Mother's occupation……
9. Father's occupation……..
10. Religion: a. Catholic b. protestant

    c. Charismatic d. Muslim e. traditional f. other

    Specify……

B. Medical information

11. Sickle cell disease status: a. SS b. SC c. S Beta Zero Thal d. S Beta Plus Thal e. other (…….) please specify f. Don't know

12. Do you have any chronic condition?

    No b. Yes (…….) please specify

13. Are you on any medication for a chronic condition?

    a.No b. Yes (…….) please specify

14. Have you been pregnant before: a. Yes b. No c. N/A (please circle)

15. If yes how many times (including still births, miscarriages, abortions etc)…….

16. Have you experienced any of the following before (please tick)

    Still birth ( ) how many……..

    Miscarriage ( ) how many……..

    Abortion ( ) how many……..

C. Transfusion history

17. Age at first transfusion…….. 18. Age at last transfusion……..

19. How many times have you been transfused……..

20. were your blood transfusions given in emergency situations only?

    a. Yes b. No (please circle)

21. Are/were you on a regular scheduled blood transfusion programme?

    a. Yes b. No (please circle)

22. Please indicate date, type, number of units, where and reason for the transfusion

1st transfusion	2nd transfusion
Date	Date
Type of product(s)	Type of product(s)
No of units	No of units
Facility/place	Facility/place
Reason for transfusion	Reason for transfusion

3rd transfusion	4th transfusion
Date	Date
Type of product(s)	Type of product(s)
No of units	No of units
Facility/place	Facility/place
Reason for transfusion	Reason for transfusion

5th transfusion	6th transfusion
Date	Date
Type of product(s)	Type of product(s)

No of units	No of units
Facility/place	Facility/place
Reason for transfusion	Reason for transfusion

7th transfusion	8th transfusion
Date	Date
Type of product(s)	Type of product(s)
No of units	No of units
Facility/place	Facility/place
Reason for transfusion	Reason for transfusion

9th transfusion	10th transfusion
Date	Date
Type of product(s)	Type of product(s)
No of units	No of units
Facility/place	Facility/place
Reason for transfusion	Reason for transfusion

Transfusion reaction

23. Has there been any instance where the hospital had to stop your transfusion abruptly?

a. Yes b. No (please circle)

24. If you answered yes to 23, please state how many times this has happened

……..

25. If you answered yes to 23, please state why each transfusion was stopped (continue at the back of sheet if you need more space)

I.

II.

III.

IV.

26. Were any of the transfusions continued after being stopped?

Yes (please state how many of them) ……

No

27. Had there been an instance where you had to be transfused less than 2 weeks after a previous transfusion a. Yes b. No (please circle)

If you answered yes to question 27 please continue with 3.5.6 and 3.5.7

28. Why were you transfused again?.....

29. How long after the transfusion, were you transfused with the next? ……

30. Have you experienced any of these during or within/after 24 hours after a blood transfusion? (please circle as many as applicable)

Sign of reaction	Time of occurrence		
	During transfusion	Within 24hrs	After 24hrs
Fever	1=Yes 2=No	1=Yes 2=No	1=Yes 2=No

Chills	1=Yes	2=No	1=Yes	2=No	1=Yes	2=No
Dark urine	1=Yes	2=No	1=Yes	2=No	1=Yes	2=No
Dyspnoea/breathlessness	1=Yes	2=No	1=Yes	2=No	1=Yes	2=No
Lower back pain	1=Yes	2=No	1=Yes	2=No	1=Yes	2=No
Abdominal pain	1=Yes	2=No	1=Yes	2=No	1=Yes	2=No
Nausea/ Vomiting	1=Yes	2=No	1=Yes	2=No	1=Yes	2=No
Diarrhoea	1=Yes	2=No	1=Yes	2=No	1=Yes	2=No
Skin eruptions/urticaria	1=Yes	2=No	1=Yes	2=No	1=Yes	2=No
Jaundice	1=Yes	2=No	1=Yes	2=No	1=Yes	2=No
Itchy eyes with reddening and swelling	1=Yes	2=No	1=Yes	2=No	1=Yes	2=No
Edema of lips and tongue	1=Yes	2=No	1=Yes	2=No	1=Yes	2=No
Hypotension	1=Yes	2=No	1=Yes	2=No	1=Yes	2=No
Chest pain	1=Yes	2=No	1=Yes	2=No	1=Yes	2=No
Other1, specify	1=Yes	2=No	1=Yes	2=No	1=Yes	2=No
Other2, specify	1=Yes	2=No	1=Yes	2=No	1=Yes	2=No

Thank you for your time

Appendix IV: Participant information leaflet for blood donors

Study Title: Investigation of post-transfusion red blood cell alloimmunisation in Ghana

Participant Information Leaflet

(For blood donors)

My name is Lilian Antwi Boateng. I am a student at the Liverpool School of Tropical Medicine working with Prof. Imelda Bates. We would like to invite you to take part in our research study. Before you decide, we would like you to understand why the research is being done and what it would involve for you. **I will go through the information sheet with you and answer any questions you have.** This should take about 10 minutes. Please ask me if anything isn't clear.

Background

Sickle cell disease (SCD) patients require frequent blood transfusion. Studies have shown that Patients with SCD develop some substances in the blood that can cause them to react badly to blood transfusions or make it difficult for the laboratory to find a suitable blood donor match for them.

Unfortunately, we do not know how often patients with SCD in Ghana develop these substances. Blood type mismatch between the patients and the donors of the blood is what causes the patients to develop these substances. We do not know whether the blood type of the Patients with SCD and the blood donors are the same or not.

Purpose of study

We are carrying out this study to determine how often patients with SCD in Ghana make the substances in their blood following blood transfusion and the nature of the substances. We will also determine the blood types of the patients and the blood donors to see if they are similar or not. Based on our findings, we will use blood from the donors to prepare a solution that will help us test for the substances in the blood before every transfusion in patients with SCD and also other people who often require blood transfusion. This will help us provide the patients with suitable blood and to prevent future development of the substances.

Why have I been invited?

You have been invited because you are a blood donor and our blood may be transfused to patients with SCD. You will be one of 200 participants.

Do I have to take part?

No. It is up to you to decide whether to join the study. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason. This would not prevent you from donating blood now or any other time.

What will happen to me if I take part?

You will only need to attend one appointment which should take no more than [15] minutes.

Below are highlights of what we will do:

- We will take your personal information from your pre-donation check form and add it to a questionnaire. The information we will take is yourage, whether you have donated before, how many times you have donated and ethnicity of your parents.
- You will be asked to help us with information we are unable to get from your form
- We will then take 10 mls (equivalent to 2 teaspoons) of blood out of your planned donation today.

What will happen to the samples I give?

- Blood samples will be shipped to the laboratories of international collaborators (e.g. Sanquin, Amsterdam, the Netherlands or NHS Blood and Transplant, Cambridge, United Kingdom) for blood group type and protein analysis.
- This may include determining the sequence of all or part of your DNA code. We may also isolate and test other components of your blood such as cells, RNA, protein and metabolites. Researchers may seek access to your samples and related data, but your personal details will never be released to

researchers without your knowledge. Your samples will be kept in a secure location.

- The data we obtain from this will be analysed together with the data from other participants and this will help us to make meaning of the results we will get.
- We would also want to seek your consent to store your remaining blood sample for future studies and to use your data in future research similar to this work. All future research will be anonymous and will not be identifiable with you

What will happen to any data produced from the samples I give?

- Anonymous data about you may be stored in an electronic archive and made available to researchers. This data may include part of or your entire DNA code, or the results of other tests performed with your samples and other information from the research database that does not identify you personally, e.g. your age in years, your gender.
- Researchers will have to make a request to access this data and explain how they will use it, e.g. which research question they are trying to answer. This type of system is referred to as 'managed access'. Genuine researchers will be given access to the data for their research, and they will be reminded of their obligation to keep your data safe by accepting the terms of a data transfer/access agreement.
- 'Managed access' requests could come from researchers who are working in the public and charitable sector (Universities, Research Institutes) or in commercial companies, either in the UK or overseas.
- Researchers share the results of their studies by means of reports or publications, which includes placing information/data on the internet, in press articles, in project leaflets and through other media. Under no circumstances will information that identifies you personally be disclosed in any of these documents.

Compensation

You will not receive any payment in return for your participation in this study

What are the possible disadvantages and risks of taking part?

There is no anticipated risk or disadvantage as we will only take part of the blood you are donating today.

What are the possible benefits of taking part?

We cannot promise that the study will benefit you directly, but the information we get from this study will help to improve transfusion management of individuals with sickle cell disease.

What will happen if I don't want to carry on with the study?

You can withdraw from the study at any time, and you do not need provide a reason.

If you withdraw from the study, information collected may still be used. Any stored blood or tissue samples that can still be identified as yours will be destroyed if you wish.

Will my taking part in this study be kept confidential?

All the information about you will be handled in confidence, stored securely in locked cabinets and on password protected computers. Your data will be looked at only by responsible and authorised personnel from the project.

What will happen to the results of the research study?

The results of the study will be presented to the hospital management team in charge of the sickle cell clinic and the Transfusion Medicine Unit of the hospital. A lay summary of the research findings will be available in the sickle cell and blood donor clinics.

Also, the results will be published and/or presented at conferences. In all these presentations, you will be anonymous.

Study Conduct

The Liverpool School of Tropical Medicine is the sponsor of this project and is ultimately responsible for the safe conduct of the study and the well-being of participants. Any unforeseen circumstances will be reported to the Sponsor and dealt with appropriately.

Complaints

If you have a concern about any aspect of this study, you should ask to speak Lilian Antwi Boateng by calling +233200315879, who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this by contacting [Prof. Imelda Bates, at Imelda.Bates@lstmed.ac.uk]. Details can be obtained from [Lilian Antwi Boateng].

Sponsorship and Funding

This research is sponsored by Commonwealth Scholarship Commission and has been reviewed and been approved by LSTM Research Ethics Committee and the committee on Human Research Publication and Ethics of Kwame Nkrumah University of Science and Technology, Kumasi and Korle Bu Teaching Hospital, Accra.

It is funded by the United Kingdom government

Contact Details

Lilian Antwi Boateng

+233200315879

Email: lilian.boateng@lstmed.ac.uk

Ethics committees in Ghana

Committee on Human Research Publication and Ethics

School of Medical Sciences, Kwame Nkrumah University of Science and Technology

Kumasi, Ghana. Tel: 233 3220 63248 or 233 20 5453785.

Email: chrpe.knust.kath@gmail.com

Korle Bu Teaching Hospital

Korle Bu - Accra

Info@kbth.gov.gh or pr@kbth.gov.gh

Tel: +233 302667759/673034-6

Appendix V: Questionnaire for blood donors

Investigation of post-transfusion red blood cell alloimmunisation in Ghana

(Data capture sheet for Blood donors only)

This questionnaire will help us gather your personal information and donation history. This will complement the results we will obtain after testing your blood

Study ID number …….

Study date…….. study site …….

Demographic data

1. Name:……
2. Contact No:……...
3. Date of Birth……...
4. Age…… (years)
5. Sex: a. Male b. Female c. Prefer not to say (please Circle)
6. Ethnicity (please circle) a. Akan b. Ewe c. Ga-Adangbe d. Guan e. Mole-Dagbani f. Other, specify ……..

Thank you for your time

Appendix VI: Protocol for preparation of red cell droplets in liquid nitrogen

Materials used included: 30% polyvinyl pyrolidone (PVP), 30% bovine serum albumin (BSA) liquid nitrogen (Ln2), liquid nitrogen resistant bucket, pasture pipette, low temperature gloves.

preparation of 30% PVP

Reagent	Quantity Required
PVP (Polyvinyl pyrrolidone) MW 44000	100 g
Purified (Ro) water	333 ml

Measure 333ml of purified water into a glass beaker, cover and heat gently on a heated magnetic stirrer. Do not allow to boil.

Add the PVP in approximately 10g portions to the pre-heated water and mix vigorously until all the PVP has been added and has dissolved (approximately 3-4 hours).

Aliquot the PVP into glass universals at 23ml each and loosely screw the caps on.

Sterilise in the autoclave. Use a liquids cycle of 121°C for 15 min. Tighten caps when removing from autoclave.

Label “30% PVP”, batch number and expiry date.

preparation of 30%BSA/PVP

Reagent	**Quantity Required**
30% BSA (Bovine Serum Albumin) Serologicals NN-6	27ml
30% PVP (Polyvinyl pyrrolidone)	23ml

Measure the required amounts of each reagent into measuring cylinders and add to a 100ml screw cap plastic container, allow to drain. Mix well and label as described.

Label: "**30% BSA/PVP"**, assigned batch number and expiry date.

Store at 2-8°C for up to 1 year.

Process for the cryopreservation of red cells in liquid nitrogen

Place the black bucket, with the polystyrene tray inside it, onto the trolley and push over to the LN_2 fill point.

Add LN_2 to the bucket, ensuring the wells of the inner polystyrene tray are filled with LN_2, until the bucket is half full.

Put the lid on the bucket. Carefully push the trolley back to the freezing bench. Transfer the bucket to the work surface.

Remove the lid from the bucket. Using a disposable plastic Pasteur pipette drop the cells into LN_2 in each compartment of the polystyrene tray sequentially, (this allows the previous drop to freeze by the time the next drop is added to that compartment, so the drops do not stick together).

When all the cells have been frozen carefully lift the polystyrene tray and drain excess LN_2 into the black bucket by holding the polystyrene tray at a 45^o angle with forceps.

Transfer the frozen red cell droplets and small amount of remaining LN_2 in the polystyrene tray into the small metal red cell droplet recovery tray. IMPORTANT: Make sure no red cell droplets are stuck to the polystyrene tray by 'tapping out' the polystyrene tray before it starts to defrost.

Transfer the frozen droplets with forceps and/or a scooping action into the labelled 2ml cryovials and screw a top onto each one.

As they are filled, the cryovials should be stored in the LN_2 remaining in the black bucket, until they are all ready to be placed in the appropriate storage tank.

Ensure no red cell droplets remain in the polystyrene tray or the red cell droplet recovery tray. If either of these have blood on them, wash gently in water and pat dry before being used for the next sample to prevent cross contamination.

Place the Nalgene dewar on the trolley. Carefully pour the LN_2 and the filled cryovials from the black bucket into the Nalgene dewar. Replace the lid of the dewar carefully, ensuring it is in the fully closed position.

Push the trolley to the appropriate LN_2 storage tank.

Turn the lid of the dewar to the pouring position (ensuring the hole is too small for any cryovials to pour out) and pour any residual LN_2 into the LN_2 storage tank. Remove the lid of the dewar. Remove the filled cryovials and place into the allocated storage location.

Return to the work bench and write the location of the cryovials onto the back of the relevant frozen cell record card (as described in 4.3). Enter details (as listed in 2.3) and location of the frozen cells into the LN_2 storage book (as described in 4.5).

Immediately discard any labelled cryovials that were not filled, to prevent them being used for another sample.

If further samples are to be frozen, repeat the procedure from 2.10. IMPORTANT: Complete the freezing and storage of one sample before commencing the next.

Once all samples have been frozen, return to the Red Cell Reference Laboratory. All sample tubes should be annotated with "+ PVP". The sample tubes should then be returned to their relevant storage locations in the fridge.

Appendix VII: Protocol for glycerolisation of RBC (Schmid *et al.*, 2011)

For each sample, 2 vol of glycerol freezing solution (Glycerolyte 57 freezing solution, Fenwal, Lake Zurich, IL) was used for every 1 vol of RBCs.

First, 20% of the total glycerol freezing solution volume was added dropwise to the RBCs and allowed to incubate with mixing for 10 minutes.

Second, the remaining glycerol freezing solution was added dropwise to the RBCs with gentle mixing.

Third, the glycerol-RBC mixture was incubated for 10 minutes with gentle mixing.

Finally, the RBCs were transferred directly to cryovials and placed at -80°C or the cryovials were cooled slowly at 1°C/min by incubating in a controlledrate freezing tray at -80°C (Nalgene Cryo 1°C freezing container, Thermo Fisher Scientific, Pittsburgh, PA).

A quantity of 1.8 mL of RBCs mixed with glycerol was in each cryovial.

Appendix VIII: Protocol for washing thawed RBC (previously frozen with 40% glycerol and stored at -80°C)

To 1 mL thawed RBC suspension (in a round-bottom tube)

- Add 250 µl of 12% NaCl, mix (carefully) and wait for 3 minutes;
- Add 1.25 ml of 0.9% NaCl mix and wait for 3 minutes;
- Add 1.5 ml of 0.9% NaCl, mix and wait for 3 minutes;
- Add 8 ml of 0.9% NaCl, mix and wait for 3 minutes.
- Centrifuge the tube (5 min, 3000 rpm), remove supernatant and resuspend the cells in 0.9% NaCl.

The cells are now clean enough for blood group typing.

Appendix IX: Ethical Approval letters

Mrs Lilan Antwi Boateng
Liverpool School of Tropical Medicine
Liverpool
L3 5QA

LSTM
LIVERPOOL SCHOOL OF TROPICAL MEDICINE
Pembroke Place,
Liverpool, L3 5QA, UK
Tel: +44(0)151 705 3100
Fax: +44(0)151 705 3370
www.lstmed.ac.uk

Friday, 01 June 2018

Dear Mrs Boateng

Research Protocol (18-010) Optimizing transfusion in sickle cell disease (SCD) patients to avert red blood cell alloimmunisation

Thank you for your letter of 31 May 2018 providing the necessary in-country approvals for this project. I can confirm that the protocol now has formal ethical approval from the LSTM Research Ethics Committee.

The approval is for a fixed period of three years and will therefore expire on 31 May 2021. The Committee may suspend or withdraw ethical approval at any time if appropriate.

Approval is conditional upon:

- Continued adherence to all in-country ethical requirements.
- Notification of all amendments to the protocol for approval before implementation.
- Notification of when the project actually starts.
- Provision of an annual update to the Committee.
  Failure to do so could result in suspension of the study without further notice.
- Reporting of new information relevant to patient safety to the Committee
- Provision of Data Monitoring Committee reports (if applicable) to the Committee

Failure to comply with these requirements is a breach of the LSTM Research Code of Conduct and will result in withdrawal of approval and may lead to disciplinary action. The Committee would also like to receive copies of the final report once the study is completed. Please quote your Ethics Reference number with all correspondence.

Yours sincerely

Dr Angela Obasi
Chair
LSTM Research Ethics Committee

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICAL SCIENCES / KOMFO ANOKYE TEACHING HOSPITAL
COMMITTEE ON HUMAN RESEARCH, PUBLICATION AND ETHICS

Ref: CHRPE/AP/194/18

17th April, 2018.

Mrs. Lillian Antwi Boateng
Department of Medical
Laboratory Technology
Faculty of Allied Health Sciences
KNUST-KUMASI.

Dear Madam,

LETTER OF APPROVAL

Protocol Title: ***"Optimizing Blood Transfusion in Sickle Cell Disease Patients to Avert Alloimmunisation."***

Proposed Site: ***Sickle Cell Clinic and Blood Donor Clinic, Komfo Anokye Teaching Hospital, Kumasi & Korle Bu Teaching Hospital, Accra.***

Sponsor: ***Commonwealth Scholarship Commission.***

Your submission to the Committee on Human Research, Publications and Ethics on the above named protocol refers.

The Committee reviewed the following documents:

- A notification letter of 14th March, 2018 from the Komfo Anokye Teaching Hospital (study site) indicating approval for the conduct of the study at the Hospital.
- A Completed CHRPE Application Form.
- Participant Information Leaflet and Consent Form.
- Research Protocol.
- Questionnaire.

The Committee has considered the ethical merit of your submission and approved the protocol. The approval is for a fixed period of one year, beginning 17th April, 2018 to 16th April, 2019 renewable thereafter. The Committee may however, suspend or withdraw ethical approval at any time if your study is found to contravene the approved protocol.

Data gathered for the study should be used for the approved purposes only. Permission should be sought from the Committee if any amendment to the protocol or use, other than submitted, is made of your research data.

The Committee should be notified of the actual start date of the project and would expect a report on your study, annually or at the close of the project, whichever one comes first. It should also be informed of any publication arising from the study.

Yours faithfully,

Osomfo Prof. Sir J. W. Acheampong MD, FWACP
Chairman

Room 7 Block J, School of Medical Sciences, KNUST, University Post Office, Kumasi, Ghana
Phone: +233 3220 63248 Mobile: +233 20 5453785 Email: chrpe.knust.kath@gmail.com / chrpe@knust.edu.gh

NATIONAL BLOOD SERVICE, GHANA

Our Ref: NBTS/RES-76/RDAL-02

Your Ref:.....

Post Office Box KB 78
Korle-Bu, Accra

28th September, 2018

Lilian Antwi Boateng
Liverpool School of Tropical Medicine,
Pembroke Place L3 5QA

Dear Lilian Antwi Boateng,

Re: Research Protocol (NBSGRD/180731/01) "Optimising Blood Transfusion in Sickle Cell Disease Patients to Avert Red Blood Cell Alloimmunisation"

Thank you for your letter seeking approval to conduct the above study at the Southern Area Blood Centre (SABC). You provided the following documents for consideration:

- Project Proposal
- Ethical Clearance by the Korle-Bu Scientific and Technical Committee (KBTH-STC)
- Participant Information & Consent Form
- Completed Registration Form

These documents have been considered and the project has been approved. Approval is conditional upon:

- Continued adherence to NBSG approved operating procedures.
- Adherence to all ethical requirements.
- Provision of notification of when the data collection commences and ends.

You are to collect **10mls** of blood samples and **interview data** from **100** consenting blood donors, who present at the static or mobile donor clinics of the Southern Area Blood Centre as per your proposal by 30th February 2019.

You are required to submit a copy of the final report once the study is completed.

Yours sincerely,

Dr. Lucy Asamoah-Akuoko
Head, Research & Development
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Research Officer, R&D

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And the date of this
Letter should be quoted

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23rd August, 2018

LILIAN ANTWI BOATENG
INTERNATIONAL PUBLIC HEALTH/MEDICAL LABORATORY TECHNOLOGY
LIVERPOOL SCHOOL OF TROPICAL MEDICINE/KNUST

OPTIMIZING BLOOD TRANSFUSION IN SICKLE CELL DISEASE PATIENTS TO AVERT RED BLOOD CELL ALLOIMMUNISATION

KBTH-IRB /00029/2018

Investigator: Lilian Antwi Boateng

The Korle Bu Teaching Hospital Institutional Review Board (KBTH IRB) reviewed and granted approval to the study entitled "Optimizing blood transfusion in sickle cell disease patients to avert red blood cell alloimmunisation"

Please note that the Board requires you to submit a final review report on completion of this study to the KBTH-IRB.

Kindly, note that, any modification/amendment to the approved study protocol without approval from KBTH-IRB renders this certificate invalid.

Please report all serious adverse events related to this study to KBTH-IRB within seven days verbally and fourteen days in writing.

This IRB approval is valid till 30th July, 2019. You are to submit annual report for continuing review.

Sincere regards,

MR OKYERE BOATENG
CHAIR (KBTH-IRB)

Cc: The Chief Executive Officer
Korle Bu Teaching Hospital

Seen
Dan
11/10/18

Appendix X: List of publications/ Abstracts

A. Papers published during PhD in peer reviewed journals

1. **Boateng AL**, Campbell AD, Davenport RD, Osei-Akoto A, Hugan S, Asamoah A, Schonewille H., (2019). Red blood cell alloimmunisation and minor red blood cell antigen phenotypes in transfused Ghanaian patients with sickle cell disease. Transfusion vol 59 Issue 6, pp 2016-2022

2. **Boateng LA**, Ngoma AM., Bates I, Schonewille H (2019). Red blood cell alloimmunisation in transfused patients with sickle cell disease in Sub-Saharan Africa; a Systematic Review and Meta-Analysis'. Transfusion Medicine Reviews, Vol 33, Issue 3, pp. 162-169

3. **Boateng LA,** Schonewille H, Ligthart P, Javadi A, Veldhuisen B, Adomako Y, Osei-Akoto A, Bates I, Schoot EVD, (2021), One third of alloantibodies in patients with sickle cell disease transfused with African blood are missed by the standard red blood cell test panel, Haematologica 106(7).

B. Abstracts submitted for conference presentations during PHD

1. **Boateng LA**, Campbell AD, Davenport RD, Akoto AYO, Schonewille H, Hugan S, (2018), Red blood cell antigens and red cell alloimmunisation in sickle cell disease patients in Ghana. *Proceedings of the 35th International Congress of the International Society of Blood Transfusion, Toronto, Canada. (Abstract accepted for poster presentation).*

2. **Boateng LA**, Schonewille H, Ligthart P, Javadi A, Veldhuisen B, Adomako Y, Osei-Akoto A, Bates I, Schoot EVD, (2019), Red blood cell antibodies in transfused patients with sickle cell disease in Ghana; prevalence, specificities and risk factors. *proceedings of the 29th International Congress of the International Society of Blood Transfusion, Basel, Switzerland. (Abstract accepted for oral presentation)*

3. **Boateng LA**, Schonewille H, Ligthart P, Javadi A, Veldhuisen B, Adomako Y, Osei-Akoto A, Bates I, Schoot EVD, (2020). Red blood cell antibodies and adverse transfusion events in multi-transfused patients with sickle cell disease in Ghana. *Proceedings of the British Society of Haematology 2020 Annual Society Meeting*, Virtual meeting, (*Abstract accepted for poster presentation*)

4. **Boateng LA**, Gleadell N, Lane W, Ligthart P, Javadi A, Soussan A, Twumasi-Oteng AK, Duku J, Asamoah-Akuoko L, Owusu-Ofori S, Schonewille H, Bates I,

Veldhuisen B, Schoot EVD, (2021), Validation of blood group genotyping array on Ghanaian blood samples. *Proceedings of the 31st Regional Congress of the International Society of Blood Transfusion, virtual meeting (Accepted for poster presentation)*

C. Manuscript in preparation

1. Red blood cell antigen distribution pattern in patients with sickle cell disease and blood donors of different ethnic groups in Ghana.