PREVALENCE OF ALLO- AND AUTO-ANTIBODIES IN TRANSFUSED SICKLE CELL DISEASE AND CANCER PATIENTS AT KENYATTA NATIONAL HOSPITAL.

CAROLINE EUNICE MANGARE

MASTER OF MEDICAL LABORATORY SCIENCE

JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY.

2016
Prevalence of allo- and auto-antibodies in transfused sickle cell disease and cancer patients at Kenyatta National Hospital.

Caroline Eunice Mangare

A thesis submitted in partial fulfillment for the degree of Master of Medical Laboratory Science in Hematology and Blood Transfusion in the Jomo Kenyatta University of Agriculture and Technology

2016
DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Signature………………………….. Date……………………………………

Caroline Eunice Mangare

This thesis has been submitted for examination with our approval as the university supervisors.

Signature………………………….. Date……………………………………

Dr. Amos Mbugua

JKUAT, Kenya

Signature………………………….. Date……………………………………

Dr. Peter M. Maturi

UON, Kenya

Signature………………………….. Date……………………………………

Dr. Jamilla Rajab

UON, Kenya
DEDICATION

This work is dedicated to my husband Nathan Muya Kimani for his unalteringly encouraging in everything I do.

I also dedicate this work to my mother Rose Makimaro who has always supported me in all that I do, my siblings Neema Mtalian, Sera Kimaro and Eric Kimaro for their continuous assistance and finally to the countless people who are suffering from various forms of cancer and haemoglobinopathies.
ACKNOWLEDGEMENTS

First and foremost, I would like to express my heartfelt thanks to Prof Rainer Blasczyk and Dr. Hans Gert-Heuft of Medizinische Hochschule Hannover, Institute of Transfusion Medicine, for their immense support, guidance and endless efforts to make sure I complete my studies comfortably in Germany. I greatly appreciate their meticulous guidance, patience, encouragement, leadership, moral support and the conducive environment that they created for me to complete my study smoothly and on time. I also extend my thanks my supervisors here, Dr. Amos Mbugua, Dr. Jamilla Rajab and Dr. Peter Maturi for their contributions. I would also like to thank Dr. Jimmy Kihara, Ann Wangui and Elizabeth Luvai for their continued support and advice throughout this journey.

I wish to convey my sincere thanks and acknowledgement to DAAD and Research and Programs department at Kenyatta National Hospital for the research grant, without them this study would not have been possible.

I would like to express my gratitude to the staff of at Kenyatta National Hospital Blood transfusion Unit, Heamato-oncology clinic and also the staff at the Institute of Transfusion Medicine for their kind support during sample collection and analysis respectively for their great assistance in acquiring samples for my study. Above all, I thank the Almighty God, for giving me the inner strength and ability to accomplish this study.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CONTENT</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DECLARATION</td>
<td>ii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>iii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF APPENDICES</td>
<td>x</td>
</tr>
<tr>
<td>ABBREVIATIONS</td>
<td>xi</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>xiii</td>
</tr>
<tr>
<td>CHAPTER ONE</td>
<td>1</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Background to the study</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Problem Statement</td>
<td>3</td>
</tr>
<tr>
<td>1.3 Justification</td>
<td>4</td>
</tr>
<tr>
<td>1.4 Study Objectives:</td>
<td>5</td>
</tr>
<tr>
<td>1.4.1 General objective</td>
<td>5</td>
</tr>
<tr>
<td>1.4.2 Specific objectives</td>
<td>5</td>
</tr>
<tr>
<td>1.5 Research Question</td>
<td>5</td>
</tr>
<tr>
<td>1.6 Study Limitations</td>
<td>5</td>
</tr>
<tr>
<td>CHAPTER TWO</td>
<td>7</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>7</td>
</tr>
<tr>
<td>2.1 Introduction</td>
<td>7</td>
</tr>
<tr>
<td>2.2 RBC Alloimmunization</td>
<td>8</td>
</tr>
<tr>
<td>2.3 RBC alloimmunization pathobiology</td>
<td>8</td>
</tr>
<tr>
<td>2.5 Factors that influence occurrence of alloimmunization</td>
<td>10</td>
</tr>
<tr>
<td>2.6 Clinical Significance of Most Common Blood Group Alloantibodies</td>
<td>12</td>
</tr>
<tr>
<td>2.7 Consequences of Red Blood Cell Antibodies</td>
<td>12</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>2.8 Consequences of Red Blood Cell alloimmunization</td>
<td>13</td>
</tr>
<tr>
<td>2.8.1 Alloimmunization and delayed hemolytic transfusion reactions</td>
<td>13</td>
</tr>
<tr>
<td>2.8.2 Acute hemolytic transfusion reactions</td>
<td>15</td>
</tr>
<tr>
<td>2.9 Prevention of RBC alloimmunization</td>
<td>16</td>
</tr>
<tr>
<td>2.10 Sickle cell disease</td>
<td>17</td>
</tr>
<tr>
<td>2.11 Transfusion in Sickle Cell Disease</td>
<td>17</td>
</tr>
<tr>
<td>2.11.1 Transfusion management of Sickle cell disease complications</td>
<td>18</td>
</tr>
<tr>
<td>2.12 Transfusion management in cancer patients</td>
<td>20</td>
</tr>
<tr>
<td>2.12.1 Indication for transfusion in cancer patients</td>
<td>22</td>
</tr>
<tr>
<td>2.12.2 Alloimmunization in cancer patients</td>
<td>22</td>
</tr>
<tr>
<td>2.13 Complications of RBC alloimmunization in Sickle cell disease and</td>
<td>22</td>
</tr>
<tr>
<td>2.14 Frequency of RBC Antibodies in Multi-Transfused Patients</td>
<td>23</td>
</tr>
<tr>
<td>2.15 Types of blood products that should be used</td>
<td>24</td>
</tr>
<tr>
<td><strong>CHAPTER THREE</strong></td>
<td>26</td>
</tr>
<tr>
<td><strong>MATERIALS AND METHODS</strong></td>
<td>26</td>
</tr>
<tr>
<td>3.1 Study site</td>
<td>26</td>
</tr>
<tr>
<td>3.2 Study design</td>
<td>27</td>
</tr>
<tr>
<td>3.3 Sampling design</td>
<td>27</td>
</tr>
<tr>
<td>3.4 Study population</td>
<td>27</td>
</tr>
<tr>
<td>3.4.1 Inclusion criteria:</td>
<td>27</td>
</tr>
<tr>
<td>3.4.2 Exclusion criteria:</td>
<td>27</td>
</tr>
<tr>
<td>3.5 Sample size</td>
<td>28</td>
</tr>
<tr>
<td>3.6 Consenting process</td>
<td>29</td>
</tr>
<tr>
<td>3.7 Laboratory procedures</td>
<td>29</td>
</tr>
<tr>
<td>3.7.1 Serologic Testing</td>
<td>30</td>
</tr>
<tr>
<td>3.8 Flow of laboratory analysis</td>
<td>32</td>
</tr>
<tr>
<td>3.9 Quality assurance</td>
<td>33</td>
</tr>
<tr>
<td>3.9.1 Pre-analytical quality assurance</td>
<td>33</td>
</tr>
<tr>
<td>3.9.2 Analytical step</td>
<td>33</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 2.1: Clinically significant antibodies.........................................................................................12
Table 4.1: Characteristics of transfused sickle cell disease and cancer patients at
Kenyatta National Hospital, Nairobi, Kenya, 2014 ........................................................38
Table 4.2: Serological results of transfused sickle cell disease and cancer patients at
Kenyatta National Hospital, Nairobi, Kenya, 2014. .......................................................40
Table 4.3: Characteristics of immunised and non-immunised transfused sickle cell
and cancer patients at Kenyatta National Hospital, 2014 ..........................42
Table 4.4: Rhesus and Kell phenotypes amongst 51 healthy Kenyan blood donors at
Kenyatta National Hospital, 2014..............................................................43
LIST OF FIGURES

**Figure 3.1**: Flow of laboratory analysis.................................................................32

**Figure 4.1**: Transfused sickle cell disease patients by age, sex and seropositivity at
Kenyatta National Hospital, Nairobi, Kenya, 2014.................................36

**Figure 4.2**: Cancer patients by age, sex and seropositivity at Kenyatta National
Hospital, Nairobi, Kenya, 2014.................................................................37
LIST OF APPENDICES

Appendix I: Consent form ................................................................................................................. 62
Appendix II: Statement of Consent: .............................................................................................. 65
Appendix III: Questionnaire: .......................................................................................................... 66
Appendix IV: Laboratory procedure ............................................................................................... 67
Appendix V: Declaration letter ........................................................................................................ 70
Appendix VI: Antigram ....................................................................................................................... 71
Appendix VII: Ethical review committee approval ........................................................................... 73
Appendix VIII: Shipping approval ..................................................................................................... 74
Appendix IX: Shipping guideline ........................................................................................................ 75
Appendix X: Publication ..................................................................................................................... 79
ABBREVIATIONS

AIDS Acquired Immunodeficiency Diseases
AIHA Auto-immune haemolytic anaemia
APC Antigen presenting cells
AZT Azidothymidine
BGAs Blood group antigens
CPDA citrate phosphate dextrose adenine
CMV Cytomegalovirus
CTLA Cytotoxic T lymphocyte-associated molecule
DAT Direct antiglobulin test
DHTR Delayed hemolytic transfusion reactions
DSTR Delayed serological transfusion reactions
EDTA Ethylene diamine tetra-acetic acid
HDN Hemolytic disease of the new born
HLA Human leukocyte antigen
HM hematological malignancies
HTR Haemolytic transfusion reaction
IAT Indirect antiglobulin test
IgM Immunoglobulin M
**IgG**  Immunoglobulin G

**KNH**  Kenyatta National Hospital

**MHC**  Major histocompatibility complex

**RBC**  Red Blood Cell

**TCR**  T-cell receptor

**TR**  Transfusion reactions

**SCD**  Sickle cell disease

**SM**  Solid malignancies
ABSTRACT

Multiple transfusions are required for certain populations such as patients with sickle cell disease (SCD), haematological malignancies (HM) and solid (SM) malignancy. However, the development of auto and alloantibody has not been considered and their prevalence remains unknown. We determined the prevalence and specificities of RBC alloantibodies and autoantibodies in patient groups (SCD, HM and SM) with recurrent transfusion demands at Kenyatta National Hospital, Nairobi, Kenya. Between February and August 2014, 300 whole blood samples from SCD, HM and SM patients were collected and screened for allo- and autoantibodies. Amongst the 228 patients with viable samples (SCD, n = 137; HM, n = 48; SM, n = 43), the median transfusion frequency was two to three events per group, 38 (16.7%) were RBC immunized and 32 (14.0%) had a positive direct antiglobulin test. Specific alloantibodies were identified in six patients (2.6%). Four of these six were SCD patients (2.9%) who had specific RBC alloantibodies (anti-C\textsuperscript{w}, anti-M, anti-Co\textsuperscript{b}, anti-S); amongst HM patients one had anti-K and one had anti-Le\textsuperscript{a}. RBC autoantibody prevalence was 3.1% (7/228). Amongst the healthy blood donors, the Ror, ccD.ee and R2r, ccD.Ee phenotypes accounted for 82% of the Rhesus phenotypes and all were Kell negative. The numbers of transfusions and the rates of RBC allo- and autoantibodies are low. The most important RBC allo- and autoantibody-inducing blood group antigens are relatively homogeneously distributed in this population.

A general change in the Kenyatta National Hospital pre-transfusion test regimen is thus not necessary. The current transfusion practice should be reconsidered if transfusion frequencies increase in the future.
CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Due to increased survival, transfusion remains an important modality in management of patients with sickle cell disease (SCD) and cancer who are dependent on transfusion for a longer period during the course of their treatment. SCD affects 20–25 million people worldwide and 50–80% of children with SCD in Africa die before the age of 5 years (Aygun & Odame, 2012). It is reported that annually, 300 000 children are born with SCD globally and approximately 240,000 of these are born in sub-Saharan Africa (Makani et al., 2011). As part of their management, most SCD patients will require frequent transfusions. Cancer on the other hand has become an important public health problem in Kenya with an estimate of annual incidence of about 28,000 cases, with the annual mortality being over 22,000 (National cancer control strategy, 2011-2016).

Transfusion of red blood cells (RBCs) is the cornerstone of management of patients with SCD and cancer (Nakutunda, et al., 2010). Red blood cell (RBC) alloimmunisation and autoimmunisation are important detrimental effects following repeated transfusions with allogeneic blood (Schonewille, et al., 2008). It results from an immune response to foreign antigens due to the genetic factors related to antigenic response as well as differences between blood donors and recipients (Natukunda, et al., 2010). Other factors implicated in RBC alloantibody formation include recipient age and gender, history of pregnancy, recipient clinical diagnosis and treatment, number of events of blood transfusions and number of blood units transfused (Klein, et al., 2005; Bauer, et al., 2007). Transfusion at an early age has been thought to offer some protection against red cell alloimmunization due to the immune tolerance in young children (Sarnaik, et al., 1986).

SCD and cancer patients will require multiple blood transfusions with whom the rate of RBC alloimmunization has been reported to be in the range of 5–30% (Natukunda, et
al., 2010; Schonewille et al., 1999; Stiegler et al., 2001) which could be as wide as 3–76% in patients with hemoglobinopathies (Natukunda, 2010; Singer et al., 2000) such as SCD (Olujohungbe et al., 2001). Antibodies may become rapidly undetectable in a substantial number of patients, which may result in an underestimation of the prevalence of this complication (Leslie et al., 2010). RBC alloimmunisation is a clinically relevant problem encountered by transfusion specialists because a patient with multiple alloantibodies gives the transfusion laboratory staff difficulties in identifying the specificity of the individual antibodies as well as excluding the presence of other clinically significant antibodies (Miller et al., 2013). This results in difficulty obtaining compatible blood. RBC alloimmunisation can cause hemolytic transfusion reactions; can contribute to perinatal morbidity due to hemolytic disease of the newborn as well as life-threatening events occasionally. In addition, delayed hemolytic transfusion reactions can mimic a sickle cell crisis and may be responsible for major morbidity in the SCD patients (Natukunda, 2010). Usually, many antibodies become undetectable with time potentially confounding future transfusions. This places the patient at risk of anamnestic antibody production and delayed hemolytic transfusion reactions. It is evident that hematopoietic stem cell transplantation as well as other preparative regimens is increasingly being applied in SCD (Shenoy, 2013) and in cancer (Copelan, 2006).

The presence of RBC alloantibodies in the recipient against incompatible RBCs in the graft can create technical obstacles hence will contribute to morbidity and mortality (Rubia et al., 2001; Franchini et al., 2004). The development of alloantibodies was recently found to be associated with the development of RBC autoantibodies. Such autoantibodies can lead to shortened life span of the recipients own RBCs and may cause clinical hemolysis (Schonewille, et al., 2006).

Studies have shown that many patients become alloimmunized and develop immune anti-RBC antibodies early during transfusion therapy usually before the 10th transfusion (Blumberg et al., 1983; Fluit et al., 1990) RBC alloimmunization has been shown to be high 18.4% among those by history receiving at least one transfusion (Klein, 2005). The
antigens most frequently involved belong to the Rh, Kell, Kidd, Duffy, Lewis, and MNS blood group systems (Strother et al., 2013, Salama et al., 2004) that transfusions given to patients who are likely to become transfusion-dependent over a longer period of time should be matched for. Minimizing RBC transfusion and the use of more extensive phenotypic matching of blood antigens other than ABO and D in an attempt to prevent alloimmunization, particularly the Rh and Kell blood group systems has been advocated by several authors (Fluit et al., 1990; Hmida et al., 1994; Campbell et al., 2000) in an attempt to decrease the risk of alloimmunization (Vichinsky et al., 2000). Although this is costly and impractical in many health settings, transfused patients should receive at the minimum, leukocyte-reduced RBC units that are E, C, and Kell-negative (Fluit et al., 1990; Rosse et al., 1990; Hmida, 1994; Campbell et al., 2000; Seeyave et al., 2006).

1.2 Problem Statement

The prevalence of RBC alloimmunization following exposure to allogeneic RBCs in frequently transfused patients with SCD and various malignancies has been reported to be in range of 5–30% in African and other non-African populations (Stiegler et al., 2001; Natukunda et al., 2010; Schonewille et al., 1999). The situation in Kenya is however, currently not known. Pretransfusion testing in Kenya is currently only limited to ABO/D grouping and cross-match, antibody identification is not done routinely. This means that immune anti-RBC antibodies may not be detected at the time of a new transfusion event. Thus, patients do not receive antigen-negative blood for the alloantibodies they may have. There is always a high possibility that the donor might have minor blood groups antigens which may not be present in the recipient blood. This may eventually lead to formation of RBC allo- and autoantibodies (Bilwani et al., 2005). The hazards associated with the generation of these antibodies include: difficulty in obtaining compatible blood, causes delayed transfusion reactions and hemolysis as well as life-threatening events occasionally.
The aim of this study was to determine the prevalence and specificities of RBC allo- and autoantibodies in patients with recurrent transfusion needs at Kenyatta National Hospital.

1.3 Justification

Transfusion of red blood cells (RBCs) remains an important modality in management of patients with SCD and cancer patients who are dependent on transfusion for a longer period during the course of their treatment. SCD affects 20–25 million people worldwide and 50–80% of these children die before the age of 5 years in Africa (Aygun & Odame, 2012). Cancer has become an important public health problem with an estimated annual incidence of about 28,000 cases. The annual mortality stands at over 22,000 (National cancer control strategy, 2011-2016).

Transfusion support remains a cornerstone in the management of SCD and malignancy patients (Nakutunda, et al., 2010). These patients have an increased risk of developing RBC allo- and autoimmunisation, a consequence of regular blood transfusions. This is a clinically relevant problem encountered by transfusion specialists because it results in difficulty obtaining compatible blood. RBC allo and autoantibodies causes delayed transfusion reactions and hemolysis as well as life-threatening events occasionally. Hematopoietic stem cell transplantation is and will increasingly be applied in these patients as curative measure (Mulumba, & Wilson, 2015). However, the presence of RBC allo- and autoantibodies in the recipient against incompatible RBCs in the graft can create technical obstacles hence will contribute to morbidity and mortality.

There was paucity of data on the prevalence of RBC alloimmunization in transfused recipients in Kenya as pre-transfusion antibody screening or identification is not done routinely. Therefore, immune anti-RBC antibodies may not be detected at the time of a new transfusion event. In addition, no studies have been conducted regarding RBC allo- and autoimmunization in chronically transfused patients in Kenya.
This study aimed to fill the knowledge gap about the occurrence of RBC allo- and autoantibodies at Kenyatta National Hospital. This will help in determining whether the current practices need to be improved in managing patients who undergo multiple transfusions in order to reduce blood-transfusion related hazards.

1.4 Study Objectives:

1.4.1 General objective

- To determine the prevalence of RBC alloimmunization and autoimmunization in transfused sickle cell disease and cancer patients at Kenyatta National Hospital.

1.4.2 Specific objectives

1. To screen for and determine the prevalence of RBC alloantibodies in SCD and cancer patients.

2. To detect and determine the prevalence of RBC autoantibodies in SCD and cancer patients.

3. To determine the specificities of alloantibody and autoantibody using an 11 cell panel.

1.5 Research Question

What is the prevalence and specificities of RBC alloantibodies and autoantibodies in transfused in SCD and cancer patients?

1.6 Study Limitations

It has been reported that 25% of alloantibodies become undetectable within a median of 10 months of follow-up, which may lead to the underestimation of the prevalence of antibodies formed (Rosse, et al., 1990; Schonewille, et al., 2000). This can result in a
patient receiving RBCs and consequently experiencing a secondary immune response that may compromise the benefit of the following transfusion (Natukunda, et al., 2012).

Because this was a cross-sectional study, some RBC alloantibodies might have been missed, since they have been reported to disappear with time. (Schonewille, et al., 2000).

Other factors that might also be responsible for the disparity in results include; the fact that the majority of the study patients were children; low mean of transfused units; inability to meet optimal transfusion needs for these patient groups; and the frequency of testing.

Drugs and other underlying conditions such as HIV/AIDs that could have been confounding factors to RBC alloimmunisation were not recoded.

Shipping the samples took a long time hence delayed the study for a while and the quality of the samples could have been compromised.
CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Transfusion of red blood cells (RBCs) remains a cornerstone of treatment of many patients thus, the frequency of RBC alloantibodies that do not belong to the ABO system among these patients is rapidly increasing (Alves et al., 2012). RBCs transfusion is recommended primarily to infuse erythrocytes needed to increase the oxygen-carrying capacity of blood in the anemic patients. Red blood cells (RBCs) are not only vehicles of oxygen but are also carriers of more than 245 other blood group antigens classified into 29 blood group systems. Most blood banks provide ABO and Rh (D) antigens matched blood. Even though blood is ABO and Rh compatible, a recipient of a blood transfusion may still develop an alloantibody to one or more of the hundreds of red blood cell antigens that are present.

The most important blood group system is the ABO system followed by the Rh. Blood group antigens can act as functional molecules but also can evoke autoantibodies and alloantibodies, causing autoimmune hemolytic anemia, hemolytic disease of the newborn and hemolytic transfusion reactions (Nay et al., 2001). RBCs transfusion is also needed for the replacement of the abnormal RBCs that occur in sickle cell patients with normal ones so as to alleviate the symptoms or prevent the complications of the disorder (Rosse et al., 1990). Other purposes of transfusion of other blood components are to maintain appropriate blood volume as well as to provide various coagulation factors. Issuing or administration of homologous blood often depends on hemoglobin or hematocrit levels of the patient. Factors that are involved in transfusion timing include the patient's cardiovascular state, anticipated additional blood loss, age, arterial blood oxygenation, PO₂ in mixed venous blood, cardiac output, and total blood volume (Miller, 2009). Healthy adults often do not require transfusion until hemoglobin (Hb) decreases
to 6 g/dl, but patients with severe underlying conditions such as coronary or chronic lung
diseases need transfusions even when Hb is as high as 10 g/dl (Miller, 2007)

2.2 RBC Alloimmunization

Alloimmunization occurs when a foreign antigen is introduced in an immuno-competent
host of the same species evoking an immune response. Alloimmunization responses to
red blood cell antigens occur when unexpected antibodies called alloantibodies are
developed after exposure to red blood cell antigens which are foreign to the patient (Han
et al., 2006). The immune response to the antigens is usually thymus independent. The
most important antigens for blood transfusion practice are the A- and B-antigens.
Multivalent antigens directly stimulate B cells to synthesize antibodies mostly IgM
antibodies; individuals who lack either the A or B antigen make anti-B- or anti-A
antibodies. Since IgM antibodies are complement-binding, these antibodies can cause
severe intravascular hemolysis after transfusion of incompatible red cells. Many other
blood group antigens reside on membrane proteins consisting of protein antigens that
can stimulate a thymus-dependent immune response, resulting in IgG antibodies that can
cause extravascular clearance of the antigen-positive cells.

The most important and frequently encountered irregular red blood cell alloantibodies in
daily transfusion practice, are directed towards the RH (anti- D, -C, -E, -c and -e), KEL
(anti-K), JK (anti-Jka and -Jkb), FY (anti-Fya and -Fyb), and the MNS (anti-M, -S and -
s) blood group systems. Of these, the D-antigen is the most immunogenic; the ability of
a given antigen to stimulate antibody production in a patient lacking the antigen.
(Schonewille et al., 2006).

2.3 RBC alloimmunization pathobiology

Antigenic differences between donor and recipient RBCs are mainly the requisite for the
initial trigger for RBC alloimmunization (Yazdanbakhsh et al., 2012).
Alloimmunization to red blood cells has been shown to involve a series of steps including:

1. RBC antigen recognition, processing, and presentation of antigen by HLA class II to TCR,
2. activation of CD4 helper T cells,
3. interaction of T and B cells, and
4. Finally B-cell differentiation into plasma cells.

The immune system consists of the innate and adaptive immune response that recognizes a foreign antigen. The main mechanism for alloimmunization involves the presentation of the donor antigen by APCs to the T-cell receptor (TCR) on recipient CD4 T cells. This may involve the direct and indirect pathway of allore cognition. In direct recognition, the donor HLA class II antigens expressed on donor APCs are directly recognized by recipient CD4 T-cells (Fast, 2000). Leucoreduction of blood products, removing donor APCs, has greatly reduced the occurrence of HLA immunization (Fast, 2000). However, mature RBC lack HLA class II antigens therefore direct antigen presentation will not occur.

2.4 Factors linked to alloimmunization

RBC alloimmunization can also occur due to transfusion that results from genetic disparity of RBC antigens between donor and recipients, or can occur due to pregnancy, abortion and miscarriages. Any RBC antigens that the patient does not possess are potentially immunogenic. The recipient's immune system will react depending on genetic or acquired factors that could be patient-related, or due to dose and route of administration and the immunogenicity of the antigen. These factors play a significant role in alloimmunization. Additionally, antibody formation can occur due to an anamnestic response when there is repeated exposure to the same red blood cell antigen. Alloantibodies due to alloimmunization are mostly not formed immediately after initial exposure to foreign red blood cell antigens; it can take weeks or even months. In some
instances, alloantibodies can appear rapidly within 48-72 hours after transfusion due to repeated exposure to the same antigen and reach a maximum 7-10 days later (Brecher, et al., 2002). Sometimes, RBC alloimmunization can occur without prior exposure to foreign RBC antigens; in this case presumably the stimulus is from antigens located outside the RBC membrane, or from other substances such as bacteria. Ingestion or inhalation of certain bacteria, or substances in the environment which are antigenically similar to RBC alloantigens may also result in the formation of “naturally occurring” (usually IgM) antibodies.

2.5 Factors that influence occurrence of alloimmunization

Bauer et al., suggests that factors for alloimmunization are complex and involve: The RBC antigenic difference between the blood donor and the recipient could occur due to racial differences existing between blood donors and transfusion recipients (Bauer et al., 2007); the recipient’s immunestatus; and the immunomodulatory effect of the allogeneic bloodtransfusions on the recipient’s immune system, patient-related factors such as the nature of the underlying disease, treatment may influence alloimmunization in patients with inherited risks. Recipient's formation of antibodies as mentioned earlier also depends on dose and route of administration and the immunogenicity of the antigen. Some studies claim that women often show a higher rate of alloimmunization which can partially be explained by exposure through pregnancy. However, other studies done by (Redman et al., 1996) refute this and showed no difference in gender regarding alloimmunization rates. The immune response to alloimmunization may also be affected by the patient’s age at the start of transfusion. Transfusion at an early age especially less than 1-3 years old has been thought to offer some protection against red cell alloimmunization because of the immune tolerance in young children (Singer et al., 2000). Consequently, the start of regular blood transfusions at an older age increases the chance of alloimmunisation. The relation between the number of blood units transfused or transfusion events and antibody formation is an important factor for increased alloimmunization in multitransfused patients, including sickle cell disease and cancer.
patients (Lee et al., 2008; Fluit et al., 1990; Michail-Merianou et al., 1990; Wendell et al., 1990). Previous studies have reported a rate of red cell alloimmunization in multitransfused patients to be ranging from 5 to 30% (Spanos et al., 1990). Certain human leukocyte antigen (HLA) play an active role in RBC alloimmunization in autoimmune disorders and diseases, which develop via T cell-mediated immunity (Lee et al., 2000). Patients who have had a splenectomy have been shown to have a higher rate of alloimmunization as compared to patients who have not had a splenectomy (Singer et al., 2000).

Evidence shows that low rate of alloimmunization occurs when there is homogeneity of RBC antigens between the blood providers and recipients (Walker et al., 1989). Immunocompromised patients have been proved to have a lower risk to develop alloantibodies such as those reported in some D negative AIDS patients receiving D positive RBC transfusions and in multiply transfused AIDS patients with AZT-associated anaemia (Calverly et al., 1991) due to immunosuppression in these patients. According to several studies, haematological malignancies such as leukaemia and lymphoproliferative disease have been shown to have a lower risk to RBC alloimmunization in terms of their ability to produce blood group alloantibodies. This can be attributed to lymphocyte dysfunction by concomitant chemotherapy and radiotherapy as well as suppression of the immune response that is characterized by an impaired immunological response and therefore, alloimmunization to RBC antigens following multiple transfusions is less common (Fluit et al., 1990; Han et al., 1981). However, Singer claims that this is not the case in patients with myeloproliferative disorders since antibodies develop more easily probably because of their chronic inflammatory state (Singer et al., 1995). Other unexpected risk factors have also been found, such as diabetes and solid tumors which have considerably an increased risk.
2.6 Clinical Significance of Most Common Blood Group Alloantibodies

Since the discovery of ABO blood group system, it still remains the most important and clinically significant of all blood groups. Blood group antibodies play an important role in transfusion medicine; both in relation to the practice of blood transfusion and in pregnancy. However, not all antibodies are clinically significant. Clinically significant antibodies are capable of causing adverse events following transfusion, ranging from mild to severe, and of causing hemolytic disease of the fetus and newborn (Poole, et al., 2007). Some of the most clinically significant antibodies are outlined on the table below.

Table 2.1: Clinically significant antibodies

<table>
<thead>
<tr>
<th>Usually Clinically Significant</th>
<th>Sometimes Clinically Significant</th>
<th>Insignificant if not reactive at 37°C</th>
<th>Generally Clinically Insignificant</th>
</tr>
</thead>
<tbody>
<tr>
<td>A &amp; B</td>
<td>Colton</td>
<td>A₁</td>
<td>P₁</td>
</tr>
<tr>
<td>Diego</td>
<td>Dombrock</td>
<td>H</td>
<td>Sd⁺</td>
</tr>
<tr>
<td>Duffy</td>
<td>Yt⁺</td>
<td>M,N</td>
<td>Chido, Rodgers</td>
</tr>
<tr>
<td>Kell</td>
<td>Le⁺</td>
<td></td>
<td>Cost</td>
</tr>
<tr>
<td>Kidd</td>
<td>Lutheran</td>
<td></td>
<td>Knops</td>
</tr>
<tr>
<td>Rh</td>
<td></td>
<td></td>
<td>Leᵇ</td>
</tr>
<tr>
<td>S,s,U</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Antibodies related to these antigens, like Rh antibodies, are unexpected and may interfere with compatibility testing. Some are immune, 37°C reactive IgG antibodies and clinically significant because they are associated with transfusion reactions (TR) and HDN. Others are naturally occurring, cold reactive IgM antibodies and clinically insignificant because they are not usually associated with in vivo red cell destruction. (Rosse, et al., 1998; Vichinsky, et al., 1994.)

2.7 Consequences of Red Blood Cell Antibodies

Some antibodies, particularly IgM antibodies of the ABO blood group system, are capable of fixing complement to the red cell surface. Complement is a plasma protein
cascade that is activated by some antigen-antibody reactions. Binding of the membrane attack complex disrupts the cell membrane, causing hemolysis. Hemoglobin is released into the plasma, resulting in hemoglobinemia and hemoglobinuria. This process is termed intravascular hemolysis. Red cells coated with IgG antibody that does not fix complement are removed from the circulation following phagocytosis by reticuloendothelial cells. During phagocytosis, heme is metabolized to bilirubin, resulting in icterus. Hemoglobinemia and hemoglobinuria do not occur. This process is termed extravascular hemolysis (Poole, et al., 2007).

2.8 Consequences of Red Blood Cell alloimmunization

According to Powers et al, similar to ABO-mediated hemolytic transfusion reactions, acute and delayed hemolytic transfusion reactions due to non-ABO antibodies may have serious clinical consequences (Powers, et al., 2010). Among the various types of alloimmunization responses, a hemolytic response directed to the newly formed antibody is classified as a delayed hemolytic reaction while an absence of haemolysis is classified as a delayed serologic reaction. Some alloantibodies are hemolytic and may cause, though not invariably, hemolytic transfusion reactions and limit the availability of further safe transfusion (Alves, et al., 2012). In the presence of alloimmunisation, the life span of red blood cells is shortened to less than 120 days, hence the patient's need for blood increases. The development of RBC alloantibodies and/or autoantibodies often complicates transfusion therapy especially when there is a broad diversity of antibodies present. Thus, finding appropriate blood for the patient is almost impossible, and though the person is in need of blood he or she cannot be transfused hence can result to morbidity and mortality.

2.8.1 Alloimmunization and delayed hemolytic transfusion reactions

The incidence of alloimmunization to red blood cell antigens in multitransfused patients with sickle cell disease and cancer is greater than that of the general population. RBC alloimmunization complicates the process of obtaining compatible blood and therefore
results in a high incidence of delayed hemolytic transfusion reactions. During the first exposure to a red cell antigen, the antibody may not be detected for several weeks or months. However, subsequent exposure to the same red cell antigen often leads to a brisk anamnestic response. Due to repetitive antigenic exposure caused by multi transfusion, the antibody level rises rapidly within the first 48-72 hours of the transfusion, and reaches a peak at 7-10 days later (Brecher, 2002). The most serious consequence of RBC alloimmunization in multitransfused patients is the risk of developing a delayed hemolytic transfusion reaction (DHTTR), which can be life-threatening and has a reported incidence of 11% (Talano, et al., 2003). The delayed transfusion reaction mostly occurs 5-20 days after transfusion due to antibodies that are usually not detectable at the time of compatibility testing since the DAT is often negative in sickle cell disease and DHTTR/H syndrome (Talano, et al., 2003). Therefore, this syndrome poses a significant diagnostic challenge. Cox reported a 4% prevalence of DHTTR/H syndrome in patients with sickle cell disease (Cox, et al., 1988). Nevertheless, the syndrome may go unrecognized and may have a much higher incidence. Petz et al described severe cases of DHTR/H syndrome and defined a sickle cell transfusion reaction syndrome with characteristics that include sickle cell pain crisis, laboratory manifestations of hemolysis, life-threatening anemia often more severe than before the transfusion, and reticulocytopenia. The DAT can be negative and the identification of a new red cell antibody is usually absent (Petz, et al., 1997).

In most cases of DHTR, the patient’s hemoglobin level falls below the pretransfusion level after transfusion. This suggests that, in addition to hemolysis of the transfused RBCs, the patient’s own RBCs are also being lysed. This condition is known as hyperhemolysis syndrome. Additional transfusions may therefore exacerbate the hemolysis, worsening the degree of anemia. The destruction of the patient’s own RBCs in DHTR can be partly explained by the presence of autoantibodies that is triggered by alloimmunization. However, DHTR/hyperhemolysis cases have also been reported in the absence of detectable alloantibodies or autoantibodies.
The mechanism in which DHTR occurs involves; antibodies, which are typically IgG, sensitize the transfused red cells in the patient’s circulation causing destruction of these cells. A classic description of a delayed hemolytic transfusion reaction includes the triad of hyperbilirubinemia, fever, and anemia which often occurs around 3-10 days after transfusion (Contreras & Hewitt, 1989). More often, there is a serological evidence of alloimmunization that is demonstrated by a positive direct and/or indirect antiglobulin test that occurs without clinical evidence of red cell destruction. This is reaction is termed as delayed serological transfusion (Shirey, et al., 1990; Singer, et al., 1995). Pineda et al suggests that in order to reduce the occurrence of delayed hemolytic reactions, blood samples should be collected and tested every 72 hours in patients who need multiple transfusions, or a highly sensitive testing method should be used for the pre-transfusion test (Pineda, et al., 1999).

2.8.2 Acute hemolytic transfusion reactions

According to Powers et al, hemolytic transfusion reactions are among the leading causes of transfusion-related morbidity and mortality which are preventable. Several non-ABO antibodies have been implicated in the majority of fatal hemolytic transfusion reactions that were reported to the Food and Drug Authority from 2005 to 2007 (Powers, et al., 2010). Haemolytic transfusion reactions in sickle cell hemoglobinopathies and oncology patients do not differ from those in other patients. Major hemolytic reactions occur primarily with major blood group (ABO) mismatches (Alves, et al., 2012) and must be treated aggressively to maintain blood pressure and glomerular filtration. Most cases can be prevented by avoiding clerical and patient or sample identification errors that occur with cross-matching or transfer of units from donor site to the patient. Minor hemolytic reactions often occur when the amount of antibody in the serum is limiting therefore, unexpected antibodies at low titers may not be detected by pre-transfusion tests. Consequently, they may cause unexpected hemolytic responses due to the transfusion of red blood cells that react with the antibodies which is characterized by decrease in the hematocrit within a period of several days after transfusion with a
consequent appearance of hyperbilirubinemia. Low levels of hematocrit can trigger a
pain crisis. In addition, antibody titers can increase due to secondary immune responses,
which can cause serious hemolytic transfusion complications during a subsequent
transfusion. Atypical form of hyperhemolytic transfusion reaction (HTR) in patients
with sickle cell hemoglobinopathy given compatible blood has been documented and is
characterized by destruction of both donor and recipient cells and is associated with
reticulocytopenia. Continuation of blood transfusion can exacerbate hemolysis.

2.9 Prevention of RBC alloimmunization

Alloimmunization, delayed and hemolytic transfusion reactions in these patients can be
reduced by the following:

1. Acquiring and maintaining adequate records of previous transfusions and
   transfusion complications.
2. Avoiding unwarranted transfusions.
3. Screening for newly acquired antibodies 1-2 months after each transfusion to
detect transient antibodies capable of causing a subsequent delayed hemolytic
reaction.
4. Reducing RBC alloimmunization due to donor/patient antigen mismatch.
   o Typing the patient before the transfusion for antigens of the Rh and Kell
     blood groups and avoiding transfusion of antigen positive blood
     (particularly E, C, and S) if the patient lacks the antigen.
   o More complete antigen matching in RBC alloimmunized patients.

Patients who are alloimmunized to one red blood cell antigen are more likely to develop
antibodies against others. Transfusion of carefully selected units of blood should be
given to patients who are likely to be transfusion dependent for a long period of time.
2.10 Sickle cell disease

Haemoglobinopathies are the most common genetic disorder in the world and about 70% of children born with haemoglobinopathies have SCD (Weatherall, 2011). In 1910, Dr James B. Herrick first described Sickle cell disease when he reported the case of a 20 year-old black student with severe anemia characterized that was by ‘peculiar elongated and sickle-shaped red blood corpuscles’ (Herrick, 1910). SCD is an autosomal recessive genetic disorder that usually presents in childhood with chronic hemolytic anemia that’s often punctuated by crises. The principal genotypes of SCD are homozygous HbSS, HbSC, HbSD, HbS/b0,HbS/b+ (Stuart & Nagel, 2004). In contrast, the sickle cell trait HbAS is a benign condition in which a sickle cell gene is inherited from one parent and a normal gene from another (Serjeant, 1992).

The distribution of malaria and the sickle cell gene are closely linked. The relative protection from severe falciparum malaria possessed by those with the sickle cell trait explains the reason for the maintenance of a high frequency of the sickle cell gene in sub-Saharan Africa (Allison AC, 1954). More than 70% of children with SCD are born in sub-Saharan Africa (Weatherall, 2011, Rees et al., 2010).

2.11 Transfusion in Sickle Cell Disease

Despite the improved patient outcomes observed with the advent of hydroxyurea administration in sickle cell disease patients, indications for chronic transfusions have increased and have resulted to a considerable reduction in morbidity and mortality. The primary goals of transfusion in sickle cell disease are to improve the oxygen-carrying capacity caused by anemia by increasing the total hemoglobin level; to decrease blood viscosity and increasing oxygen saturation by diluting the concentration of sickle hemoglobin and providing RBCs longer circulating half times that do not sickle nor polymerize and to suppress the endogenous production of sickle RBCs by increasing tissue oxygenation; to improve microvascular perfusion by decreasing the proportion of sickle red cells in the circulation.
Sickle cell disease is an inherited chronic hematological disorder that has no established cure to date except in a few patients who have had successful stem cell bone or marrow transplantation. It is the most common genetic hematologic disease in Kenya that is more prevalent in people coming from or living in malaria endemic regions (Aluoch et al., 1993). The complications of this disease are numerous and affect almost every organ and/or tissue in the body. Sickle cell disease clinical manifestations arise from the tendency of the hemoglobin (HbS) to polymerize and deform red blood cells into the characteristic sickle shape. This property occurs due to a single nucleotide change in the β-globin gene leading to substitution of valine for glutamic acid at position 6 of the β-globin chain (β6glu→val or βs) (Han KS, et al., 2006). The homozygous state (HbSS or sickle cell anemia) is the most common form of sickle cell disease. The sickle cells are prematurely removed from the circulation by the spleen, often resulting in hemolytic anemia. The circulating half-life of sickle cells is 16 to 20 days, which is much less than the normal half-life of 120 days for normal RBCs. Overall, RBC transfusions in the patients with sickle cell disease helps improve symptomatic anemia, acute chest syndrome, stroke, splenic sequestration as well as prevent and resolve vaso-occlusive events and preventing first stroke in children (Yazdanbakhsh, et al., 2012).

2.11.1 Transfusion management of Sickle cell disease complications

Intermittent transfusions are generally administered in the acute setting at different times to treat various manifestations of sickle cell disease such as management of severe anemia(Josephson et al., 2007). Acute splenic sequestration and transient red cell aplasia episodes (aplastic crisis) are the most common causes of acute anemia. A third form of acute anemia, called hyperhemolysis, is associated with infection, acute chest syndrome, and particularly malaria. Hemoglobin values of less than 5 gm/dl or a 20 percent fall below the base line during an acute illness are common transfusion triggers, however (Ohene-Fnmpong, et al., 2001). Severe acute splenic sequestration produces hypovolemia and cardiovascular decompensation therefore; patients require immediate transfusion which is indicated when the hemoglobin drops by more than 2 gm/dl to
prevent cardiovascular collapse (Hillyer et al., 2003). Parvovirus B19 is the primary cause of transient red cell aplasia and severe anemia develops to shortened red cell survival without compensatory production of new red cells. RBC transfusion is also used for management of sudden severe illness such as acute chest syndrome which is associated with hypoxia and falling hemoglobin. RBC transfusion may prevent the progression of acute pulmonary disease. Acute multi-organ failure is a devastating complication of sickle cell disease, classically associated with falling hemoglobin and platelet count, as well as progressive multi-organ failure (Hillyer et al., 2003). Transfusions used to improve tissue oxygenation and perfusions are indicated in these seriously ill patients (Josephson et al., 2007). These complications are among the leading causes of death in sickle cell disease. A falling hemoglobin value often accompanies these events.

Chronic transfusion therapy programs are indicated primarily on a scheduled basis to improve anemia, reduce or prevent the occurrence of complications or their progression. It is also indicated for several conditions in which the potential medical complications outweigh the risks of alloimmunization, infection and iron overload. The goal of these programs is to maintain the hemoglobin S at 30-50 percent, depending on the specific disorder (Josephson et al., 2007). Transfusions are usually repeated every 3-4 weeks. The STOP trial demonstrated that chronic transfusion therapy reduces the occurrence of first stroke in children with a rate of high blood flow through the circle of Willis cerebral arteries, as measured by Doppler ultrasonography (Lee et al., 2006). Chronic transfusion therapy reduces the rate of recurrent vaso-occlusive stroke rate from 90 percent to less than 10 percent and is indicated for all victims of this complication of sickle cell disease. Anecdotal reports suggest that early exchange transfusions may improve perfusion and oxygenation to brain tissue, thus limiting damage (Lee et al., 2006). Chronic transfusion therapy has been used to decrease the recurrence of pulmonary events in patients experiencing severe acute chest syndrome therefore patients with proven pulmonary hypertension and chronic lung disease should receive long-term chronic transfusion therapy (Josephson et al., 2007). A small
percentage of patients suffer from unusually protracted and severe pain episodes. These patients have a very poor quality of life and are unable to engage in ordinary daily activities. Transfusions are sometimes suggested for a number of conditions in which efficacy is unproven, such as in management of priapism (Rackoff, 1992). Recurrent priapism often produces impotence thus transfusions are done to prevent this complication.

Several methods of transfusion are available, including simple transfusion, partial exchange transfusion, or erythrocytapheresis (Thakral et al., 2008). There should be a comprehensive transfusion protocol that includes accurate records, the patient's red cell phenotype, alloimmunization history, number of units received, serial hemoglobin S percentages, and results of infectious and iron overload monitoring results (Erkman et al., 2001). Although the implementation of RBC transfusion has served to reduce complications and improve quality of life among patients with sickle cell disease, it is not without adverse effects. Thus, both appropriate and judicious use of transfusion therapy is important (Josephson et al., 2007).

2.12 Transfusion management in cancer patients

Transfusion support is vital to the management of patients with hematologic disorders and malignancies who tend to be dependent on transfusion for a longer period (Schonewille et al., 1999). This is due intensive marrow depression. Almost all patients who receive high-dose chemotherapy and radiotherapy need platelets and red blood cell transfusions in order to overcome the pancytopenic period (Barrett-Lee et al., 2000). Anemia is one of the major complications of cancer which is a common indication for RBC transfusions. The pathogenesis of anemia in cancer is multifactorial, its incidence in malignant diseases depending on the type of underlying malignancy, the duration and stage of the disease, the regimen and intensity of therapy and possible intercurrent infections or surgical interventions. Anemia affects more than 50% of all cancer patients regardless of the treatment received. Approximately 20% of all patients undergoing
chemotherapy will require red blood cell transfusion (Johnston et al., 1998). It is a frequent condition in metastatic bone disease and multiple myeloma while it is almost an invariable feature in hematological cancers (Skilling et al., 1995). Chronic disease, bone marrow infiltration tumor cells, shortened red cell (RBC) life span, and inappropriate erythropoietin response play important roles in the development of anemia although myelosuppressive chemotherapy is a main factor (Mercadante et al., 2000).

Bone marrow replacement is the most direct effect of cancer since metastases within the bone marrow usually displace and destroy stem cells and progenitor cells. This impairs the production of hematopoietic growth factors or induces the production of cytokines. Solid tumors, such as breast and prostate cancer, also invade the bone marrow thereby, reducing the marrow space. Among patients with solid tumors, those with ovarian cancer and lung cancer are reported to have the highest frequency of anemia therefore; they require the highest rate of transfusion requirements (Mercadante et al., 2000). Patients with hematological malignancies or myelodysplastic syndromes have the highest occurrence of anemia. Leukemia, lymphomas and multiple myeloma are the most frequent causes of change in the release of mature blood cells, producing a picture with immature cells (Nowrousian et al., 1996). A shortened red cell survival, a lymphoproliferative state with failure of the bone marrow to increase erythropoiesis to meet the demand and restore the deficiency, and a defect in iron re-utilization from bone marrow macrophages have all been reported as causes of anemia although the mechanisms involved are not completely defined (Faquin et al., 1992).

Bone marrow stem cells have a poor capacity to repair radiation damage. Chemotherapy may also cause transient and/or sustained anemia. Mechanisms of drug-induced anemia in patients with cancer include stem cell death, blockage or delay of hematopoietic factors, oxidant damage to mature hematopoietic cells, long-term myelodysplasia, immune-mediated hematopoietic cell destruction, microangiopathy, and plasma volume expansion with dilutional anemia. Drugs other than chemotherapy may also cause immunological destruction of red cells (Mercadante et al., 2000). Numerous secondary
disorders can contribute to the development of anemia such as large gastrointestinal resections may induce malnutrition and deficiencies in iron or folic acid will also predispose the occurrence of anemia (Mercadante et al., 2000). A low hemoglobin level is a significant independent prognostic factor associated with poor survival of cancer patients whether treated with chemotherapy or radiotherapy (Barrett-Lee et al., 2000).

2.12.1 Indication for transfusion in cancer patients

Transfusion in cancer patients is mainly indicated depending on manifestation and severity of anemia which is among the main complications in cancer. A common practice is to order RBC transfusions when hemoglobin levels drop to 8 g/dL. RBC transfusions are used to improve the oxygen supply to the tissues by raising the oxygen content of the blood as well as to achieve symptomatic relief such as fatigue (Freitas et al., 2012) and other symptoms such as tachycardia, orthostatic hypotension and dyspnea to normalize parameters in cancer patients.

2.12.2 Alloimmunization in cancer patients

It has been observed that patients undergoing chemotherapy, especially those with leukemia, exhibit a lesser antibody response than do other patients. Impairment of the immune status as a result of the malignant process or transient immunosuppression due to intensive chemotherapy could enable patients to develop a tolerance for or unresponsiveness to incompatible transfusions. (Dinardo, et al., 2013).

2.13 Complications of RBC alloimmunization in Sickle cell disease and cancer

As compared to the general hospital population, sickle cell disease and cancer patients are dependent on transfusion for a longer period. This means that they are at an increased risk of RBC alloimmunization due to the multiple transfusions involved in the course of their treatment (Dinardo, et al., 2013). In the presence of RBC alloimmunization, the life span of red blood cells is shortened to less than 120 days therefore, the patient's need for red blood cell increases. RBC alloimmunization results
to development of alloantibodies and/or autoantibodies that complicates the process of obtaining compatible blood (Nakutunda, et al., 2010). This results in a high incidence of acute and delayed hemolytic transfusion reactions, hemolysis as well as life-threatening events occasionally. It has been observed that in the recent years hematopoietic stem cell transplantation as well as other preparative regimens is increasingly being applied in this group of patients (Mulumba, & Wilson, 2015). However, the presence of RBC alloantibodies in the recipient against incompatible RBCs in the graft can create technical obstacles. This might deprive the patients of the only chance they had to get cured or to receive proper management and eventually will contribute to morbidity and mortality.

2.14 Frequency of RBC Antibodies in Multi-Transfused Patients

In the general population the most frequently detected antibodies in order of decreasing frequency are; D>K>E>DC>Fya>Jk\(^a\)>c>C>cE>e>DE>V>Jk\(^b\) (Aygun et al., 2002). Even components containing very few red blood cells, such as pooled random donor platelet concentrates can stimulate RBC antibody formation (Thakral, et al., 2008 ). Blood type alloantibodies have been detected in up to 0.8% of blood donors (Hamilton, 2009). Approximately 1.5-2.0% of hospital patients have detectable alloantibodies to red cell antigens caused by previous transfusion or pregnancy (Blumberg et al., 1983; Castellino et al., 1999). The prevalence of alloimmunization following the transfusion of a single unit of blood is has been estimated to be 1 to 2% of hospitalized patients. Multiply transfused patients have much higher incidences of RBC antibody formation. In patients with disorders that often require multiple blood transfusions the rate of RBC alloimmunization has been reported in the range of 5–30% (Seyfried et al., 1990; Redman et al., 1996; Schonewille et al., 1999; Stiegler et al., 2001). In multiple transfused patients who receive chronic transfusion therapy such as sickle cell disease, beta-thalassemia, or cancer, multiple studies have shown that alloimmunization risk increases with an increasing number of transfusions (Hamilton, 2009, Winters et al., 2001; Alves et al., 2012). This range is even wider, approximately 3–76 in patients
diagnosed with other hemoglobinopathies (Singer et al., 2000; Olujohungbe et al., 2001). Overall, approximately 10% of patients transfused with multiple units of red blood cells form antibodies against some of the non-ABO, non-D antigens. In those patients who produce RBC antibody, about 33% will produce additional antibodies (Thakral et al., October 2008; Yazdanbakhsh et al., 2012). The frequency of RBC alloimmunization often varies with age and disease state (Yazdanbakhsh, et al., 2012).

The prevalence of blood type alloantibodies in patients with a history of blood transfusion is approximately 2–9%. The highest blood type alloantibody rates, range from 9 to 30%. Various frequencies of RBC alloimmunization in sickle cell disease patients range from 2.6% to 76% have been reported in a number of studies. According to a study carried out in Uganda, the prevalence of alloimmunization in malignancies was 8.3% (Natukunda, et al., 2010) while for sickle cell disease the prevalence was about 6.1% (Natukunda, et al., 2010).

**2.15 Types of blood products that should be used**

Standard bank blood is appropriate for the patient with sickle cell disease. The age of the blood (time since collection) is usually not important as long as it is within limits set by the transfusion service. Exchange transfusion with blood less than 5 days old (less than 3 days old in the small infant) helps in acute situations requiring immediate correction of the oxygen-carrying capacity (Yazdanbakhsh, et al., 2012). All blood should be screened for the presence of sickle hemoglobin and confirmed to be negative. A solubility test is adequate for screening in this situation. Patients with sickle cell disease should not be transfused with RBCs containing Hgb S.

The antigenic phenotype of the red cells (at least ABO, Rh, Kell, Duffy, Kidd, Lewis, Lutheran, P, and MNS groups) should be determined in all patients older than 6 months of age. A permanent record should be maintained in the Blood Bank, and a copy of the record should be given to the patient or family. All patients with a history of prior transfusion should be screened for the presence of alloantibodies. The efficacy of a
chronic transfusion regimen should be assessed periodically by determining the proportion of hemoglobin S by quantitative hemoglobin electrophoresis as well as the hemoglobin concentration or hematocrit (Rosse, et al., 1998).

The high prevalence of alloimmunization in patients with sickle cell disease and cancer likely has several causes. Lack of phenotypic compatibility between the donor and recipient doubtless is a major factor. All patients undergoing multiple transfusions should receive limited phenotypic matching for antigens E, C and Kell (Alves, et al., 2012). Pre-storage leuko-depletion of red cells is standard practice aimed to reduce febrile reactions, platelet refractoriness, infections, and cytokine-induced complications. Washed red cells should be reserved for patients with a history of allergic reactions following transfusion. Irradiated blood products should be considered in possible bone marrow transplantation candidates. Relatives should not be used as blood donors especially for children who could be candidates for bone marrow transplantation. Autologous blood transfusions for these patients should be avoided. Red cell substitutes are experimental and generally not indicated. Extensive phenotypic matching is recommended for patients who have formed alloantibodies.
CHAPTER THREE
MATERIALS AND METHODS

3.1 Study site

Part of the study that involved patient recruitment and sample collection was carried out at the Kenyatta National Hospital (KNH) which is the largest referral and main teaching hospital in Kenya. The hospital has specialized clinics for cancer and sickle cell disease patients. Laboratory analysis was carried out at the Institute of Transfusion Medicine, Hannover Medical School in Hannover, Germany.

Laboratory description

Hannover Medical School operates an Institute for Transfusion Medicine consists of three divisions: a blood donation service that includes all relevant donation techniques (whole blood donations and the complete spectra of apheresis donations), a center for immunogenetics that carries out the relevant organ transplantation diagnostics (HLA typing procedures based on PCR SSP and direct sequencing techniques, HLA antibody testing) and a center for red cell immunohematology that performs roughly 20,000 complete blood group determinations (AB0, Rh [CcDEe] and K), about 100,000 red cell cross match procedures and a red cell antibody laboratory that performs about 120,000 red cell antibody screens (nearly all are done automatically) and about 300 antibody specifications per year. For all immunohematological investigations the Diamed (now Biorad) ID gel card micro typing system is applied, either automatically by using a Tecan pipetting roboter plus the Diamed ID reader system (including a centrifuge and an automatic evaluation system) or manually. In particular, the red cell antibody specifications are done manually. Briefly, manual tube and gel card tests are performed in red cell alloantibody testing and specification using the Diamed-ID system and commercial specification panels of different manufacturers (e. g. Diamed [Biorad], Ortho, Medion-Grifols [8-11 test cells] and Sanquin [16 test cells]).
3.2 Study design

This was a Descriptive Cross-sectional study.

3.3 Sampling design

The sampling design involved consecutive sickle cell disease and cancer patients being attended at the KNH and who qualified to be included in the study were told about the study and asked to sign consent form.

3.4 Study population

The study was conducted on confirmed sickle cell disease and cancer patients; who were on treatment for various malignancies. The participants who were selected were those who had received at least one allogeneic blood transfusion in their lifetime and were being managed at the Hematology and Oncology clinics respectively at Kenyatta National Hospital. Of note, all he cancer patients were undergoing treatment at the time of enrollment into the study. A group of 51 donors were included to give a picture of profile for baseline of alloantibody distribution in individuals who have not received any transfusion.

3.4.1 Inclusion criteria:

- Sickle cell disease patients and cancer (all types of cancers) who were being attended at the Kenyatta National Hospital and had received at least one allogeneic blood transfusions.

- Patients had received ABO/D compatible packed RBC, whole blood as well as platelet transfusions.
3.4.2 Exclusion criteria:

- Patient declines to give consent

3.5 Sample size

The prevalence RBC alloimmunization in the two multi transfused patient population is estimated at 6.1% and 8.3% for sickle cell haemoglobinopathy and oncology patients respectively. This was according to two parallel studies conducted in Uganda; Red blood cell alloimmunization in sickle cell patients in Uganda (Natukunda, 2010) as well as Prevalence and specificities of red blood cell alloantibodies in transfused Ugandans with different diseases (Natukunda, 2010) since no study has been carried out in Kenya. Furthermore, Uganda shares similar geographical and socio-demographic predispositions as Kenya therefore the prevalence reported in Uganda could reflect the situation in Kenya.

The Fisher’s formular was be used for calculating the sample size (Kasiulevičius, et al., 2006)

\[ n = \frac{Z^2 \times (p) \times (1 - p)}{d^2} \]

Where

- \( n \) = sample size,
- \( Z \) = \( Z \) statistic for a level of confidence, 95% confidence interval
- \( p \) = expected prevalence or proportion and
- \( d \) = precision (in proportion of one; if 5%, \( d = 0.05 \)).

Sample size for sickle cell haemoglobinopathy

\[
N=1.69 \times 1.96 \times [0.061 \times (1-0.061)] / (0.05 \times 0.05)
\]

\[
N=88
\]

Sample size for malignancy
N=1.69*1.96 [0.083 (1-0.083)] / (0.05*0.05)
N=117

3.6 Consenting process

Permission will be sought from the doctor and nurse in charge of the hematology and oncology clinics. The patients were captured after seeing the doctor. The research assistant introduced himself and explained what was to be done and asked some questions regarding the inclusion criteria. A consenting form was then administered (appendix II) for those patients who satisfied the inclusion criteria. The patients were briefly informed on the title of the study, purpose of the study, the benefits, the risks, confidentiality issues and the procedure as well as elaborated and answered any questions that were raised. Patients were also be briefed on exportation of samples obtained from them explaining why the laboratory analysis will be done out of the country.

The patients and/or guardians signed the consenting form as a sign that they understood what the study was about and that they were willing to participate in the study and that they accepted their samples to be exported for analysis. Demographic data was obtained from the files after the patient had accepted to be enrolled into the study.

3.7 Laboratory procedures

After consent, 2-4 mls of blood was drawn into ethylenediaminetetraacetic acid (EDTA) tubes for laboratory investigations. Plasma and red blood cells were separated and the plasma was stored at -18\(^\circ\)C while the red cells were stored at 2-6\(^\circ\)C and preserved with a drop of CPDA. The samples were then shipped to the Transfusion Medicine Institute at Hannover medical school for immuno-haematological studies.

The samples were packaged as Biological substance, Category B which was packaged and transported in compliance with IATA Packing Instruction 650. The packaging was done as follows: A primary receptacle: that consisted of the vial, containing the
specimen surrounded by an absorbent material (cotton balls and paper towels) to prevent leakage. A secondary packaging that included the vial holders used to hold the primary receptacle: An outer packaging for shipping was filled with ice pellets and both the primary and secondary packaging were placed into it. On the external surface of the outer packaging, a text box with the words: “BIOLOGICAL SUBSTANCE, CATEGORY B” was indicated. The guidelines for shipping Biological specimen are discussed in Appendix VI.

3.7.1 Serologic Testing

On arrival of the RBCs and plasma to the Institute of transfusion, the samples were sorted out according to the numbers on the label and the plasma was stored at -180°C while the red cells were stored at 2-60°C and analyzed in batches.

Antibody screening:

Plasma samples were screened for the presence of RBC alloantibodies by use of a standard 3-cell panel of reagent group O RBCs at 20°C using NaCl gel cards for cold agglutinins, at 37 °C phase for warm reacting antibodies using low ionic strength saline (LISS) gel cards so as to enhance antigen-antibody interactions. This screen provides cells that are homozygous for the antigens to which individuals most frequently make antibodies. The screening cells are supplied as three vials, each containing a suspension of human group O red blood cells derived from a single donor. This helps ensure the detection of weakly reactive antibodies. The results of these typings are provided on a piece of paper known as an Antigram. A “+” in the column under the antigen indicates the cell has been tested and found to be positive for the antigen, a “0” indicates the cell has been tested and found to be negative for the antigen. If a patient’s plasma sample showed agglutination of reagent screening cells, an autocontrol was performed by reacting the patient’s red cells with his/her own plasma. This was to distinguish whether the positivity of the reaction was due to auto-antibodies or allo-antibodies. For samples that showed agglutination, subsequent alloantibody identification was carried out with
an 11-cell panel using LISS/Coombs gel cards. Antibodies were identified using the DiaMed gel system (DiaMed-ID®; Bio-Rad Laboratories, DiaMed GmbH, Cressier FR Switzerland) or Diana 11-cell panel and sometimes additional methods such as Sanquin 16-cell panel were used. Identification was confirmed by positive homozygous expression of RBC antigens. Antigens were D, C, E, c, e, K, k, Fy^a, Fy^b, Jk^a, Jk^b, Le^a, Le^b, P1, M, N, S, and s.

Antigram attached in appendix VII.

**Direct antiglobulin test (DAT):**

The DAT is used to detect sensitized red cells or IgG or C3 bound to the surface of the red cell. A positive DAT due to IgG is seen most frequently in patients with warm autoantibodies. DAT was performed using polyclonal gel cards consisting of anti-IgG, anti-IgA, anti-IgM, anti-C3c and anti-C3d. Samples that were DAT positive, an acid eluate was prepared

**Elusion:**

When the DAT is positive due to coating with IgG it is important to determine the specificity of the antibody by performing an elution. Elutions are performed to remove antibody from IgG coated red blood cells. The eluate is then tested against reagent red blood cells to determine the specificity.

The elusion was screened using the standard 3-cell panel. Those that were positive were then screened for the specificity using 11-cell panel.

Patients were considered to be alloimmunized if antibodies to one or more RBC antigens could be identified. Patients with antibodies not detected via the screening cells were not deemed alloantibody formers.
3.8 Flow of laboratory analysis

300 samples plasma and red blood cell samples

Plasma samples screened using a three cell panel antibody screen

38 reactive

Autocontrol = Corresponding patient's RBCs and plasma

32 samples reactive

Typed with 11 cell panel

DAT

6 showed alloab specificity

IgG pos 16/32, IgG+C3d/C3c pos 6/32, C3c only 7/32, IgM only 1/32, IgA only 2/32.

acid eluate

eluate screened using 11 cell

7 were reactive. Only 1 was specific, auto-antibodies others were polyspecific
3.9 Quality assurance

The tested samples included positive and negative controls. Some random samples were performed automatically.

3.9.1 Pre-analytical quality assurance

This involved: Test ordering process – the principal investigator ordered the test using the correct code.

Patient preparations for the test – the patient were informed about the study and consent was obtained from the patient before collection of sample. Specimen collection procedures – the sample was collected in EDTA tubes. During sample collection, the collection tube was adequately filled to the full volume. Proper mixing of the sample was ensured immediately after collection. All the collected specimens were labeled using a code number and date of collection.

Careful handling of samples and prompt transportation was done to ensure sample integrity. Transport of samples to the laboratory was ensured that samples were kept in cold packs.

3.9.2 Analytical step

Involved: Reviewing sample acceptability; the samples did not have clots and most of it was sufficient enough.

Ensured all tests performed employed methods, techniques or procedures that followed the standard operating procedure

All reagents and methods conformed to manufacturers' instructions and alid test reagents were used.
The reagents were controlled each day of use and were visually inspected for haemolysis and/or discoloration. The results of the visual inspection, reagent lot number, expiry date, date of the inspection and the individual performing the inspection was documented. The expiry date was checked on each reagent used. Reagents beyond expiry date were not used.

Centrifuges used for testing of red blood cell agglutination underwent revolutions per minute (RPM) and timer checks quarterly. Functional calibration that determines optimal centrifugation was performed prior to initial use, after adjustments for repairs was documented. The procedure specified the speed and duration of centrifugation.

3.9.3 Post-Analytical activities
This was done by ensuring proper recording and documentation of test results.

3.10 Ethical Considerations

Approval was sought from the KNH/UoN Ethical regulations committee. The proposal was presented at JKUAT department of Human Pathology and Laboratory Medicine and had been accepted. Consent was obtained from patients who meet the inclusive criteria and 4mls of blood was drawn aseptically ensuring minimal risk to the study subject. The results of this study were given to the respective hospital departments to assist in better management of the patient in future. Any information obtained during the study will be kept confidential.

3.11 Statistical methods

Statistical software packages Excel 5.0 (Microsoft, Redmond, CA) and Statistical Package for the Social Sciences 12.0 (SPSS Inc., Chicago, IL) were used for data management and analysis respectively. Descriptive statistics were reported as the number and percentage, the mean and range. Categorical variables of possible associations between RBC alloimmunization and gender, units of blood transfusion, diagnosis of SCD or solid and hematological malignancy were compared using the Chi-
squared test. Groups were assumed to differ significantly when the probability level was less than 0.05. The student t-test was used for continuous variables with normal distribution.
CHAPTER FOUR

RESULTS

4.1 Patient Data

Of the samples from 300 patients who met the inclusion criteria, 72 samples could not be evaluated for the following reasons: insufficient sample because of leakage during shipment \((n = 40)\); samples breaking in the centrifuge whilst processing \((n = 20)\); and lack of proper labeling \((n = 12)\). A total of 228 samples were analysed, including 137 from SCD patients, 48 from HM patients and 43 from SM patients, with a median number of two to three transfusions per group (Table 4.1). Of these, 117 (51.3\%) were women, of whom 22 (18.8\%) had a history of pregnancy. Overall, the mean age at the time of blood transfusion was aged 17.2 years (range: 1–93). Indeed, the majority of patients were children aged 16 years or younger \((n = 159; 70\%)\); 14\% were aged three years or younger (Figures 4.1 and 4.2).

FIGURE 4.1: Transfused sickle cell disease patients by age, sex and seropositivity at Kenyatta National Hospital, Nairobi, Kenya, 2014.
FIGURE 4.2: Cancer patients by age, sex and seropositivity at Kenyatta National Hospital, Nairobi, Kenya, 2014.
TABLE 4.1: Characteristics of transfused sickle cell disease and cancer patients at Kenyatta National Hospital, Nairobi, Kenya, 2014

<table>
<thead>
<tr>
<th>Variables</th>
<th>All patients</th>
<th>SCD patients</th>
<th>Cancer patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>All cancer patients</td>
</tr>
<tr>
<td>Characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>228</td>
<td>137</td>
<td>91</td>
</tr>
<tr>
<td>Mean Age (range)</td>
<td>17.2 (1-93)</td>
<td>8 (1-36)</td>
<td>31.1 (1.5-93)</td>
</tr>
<tr>
<td>Female / male ratio</td>
<td>1.05</td>
<td>1.01</td>
<td>1.17</td>
</tr>
<tr>
<td>Transfusions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>685</td>
<td>331</td>
<td>354</td>
</tr>
<tr>
<td>Mean (range)</td>
<td>3 (1-19)</td>
<td>2.4 (1-8)</td>
<td>3.9 (1-19)</td>
</tr>
<tr>
<td>Whole blood</td>
<td>532</td>
<td>308</td>
<td>224</td>
</tr>
<tr>
<td>Packed red blood</td>
<td>85</td>
<td>23</td>
<td>62</td>
</tr>
<tr>
<td>cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>68</td>
<td>0</td>
<td>68</td>
</tr>
<tr>
<td>Immunisation</td>
<td>-</td>
<td>20.4%</td>
<td>11.0%</td>
</tr>
</tbody>
</table>

SCD, Sickle cell disease; HM, Haematological malignancy; SM, Solid malignancy.

Patients received ABO/Rhesus D compatible and non-leucocyte-depleted whole blood units (n = 532), packed RBC transfusions (n = 85) and platelet transfusions (n = 68), totaling 685 units of blood in 593 transfusion events (i.e., 1 transfusion unit per transfusion episode or a mean of 2.7 transfusion units per patient). Of the SCD patients, 90% were transfused because of severe anaemia – haemoglobin less than 5–6 g/dL according to World Health Organization guidelines. Transfusion in malignancy patients was mainly a result of anaemia caused by intensive chemotherapy, by the disease process and/or surgical interventions. All cancer patients were receiving chemotherapy at the time of enrollment into the study. HM patients had the following types of
malignancies: acute lymphoblastic leukaemia (n = 15), chronic lymphoblastic leukaemia (n = 10), Hodgkin’s lymphoma (n = 9), multiple myeloma (n = 6), acute myeloid leukaemia (n = 5) and chronic myeloid leukaemia (n = 3). SM patients had the following types of malignancies: abdominal tumour (n = 1), bladder (n = 2), breast (n = 8), cervical (n = 4), colon (n = 3), hepatocellular (n = 2), mouth (n = 1), nasopharyngeal (n = 1), pancreatic (n = 2), prostate (n = 4), rectum (n = 4), rhabdomyosarcoma (n = 4), sinonasal tumour (n = 1), squamous cell carcinoma (n = 2) and stomach cancer (n = 4). The \( P \)-value for the number of blood units transfused was 0.004, which was statistically significant as cancer patients received more transfusions.

4.2 Serological results

The overall prevalence of RBC immunisation was 16.7\%, with 38 of the 228 patients testing positive for antibody screening. The prevalence of RBC immunisation amongst SCD patients was 20.4\% (28 of 137 patients) and amongst malignancy patients, 11.0\% (DAT+, n = 8 plus alloAb+, n = 2; altogether 10 out of 91 patients).

4.2.1 RBC alloantibody identification

Only 38 patients were positive for the antibody screening, and RBC alloantibodies were detected in only 6 of 228 patients (2.6\%) (Table 4.2). The rate of alloAb formation amongst SCD patients was 2.9\% (4 of 137) and 4.2\% (2 of 48) amongst HM patients, whereas the prevalence amongst SM patients for alloAb identification was 0. The specificities of the alloAbs from the SCD patients were anti-\( C^w \), anti-S, anti-Co\( ^b \) (probably immune in nature) and anti-M (probably naturally occurring). In addition, there was one anti-K (immune) and one anti-Le\(^a\) (natural) in two HM patients, whereas the SM group showed no RBC alloimmunisation. The rate of alloimmunisation was 6.14\% for men versus 8.33\% for women; the difference was not statistically significant (\( P = 0.25 \)).
TABLE 4.2: Serological results of transfused sickle cell disease and cancer patients at Kenyatta National Hospital, Nairobi, Kenya, 2014.

<table>
<thead>
<tr>
<th>Variables</th>
<th>All patients</th>
<th>SCD patients</th>
<th>Cancer Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HM patients</td>
</tr>
<tr>
<td>Number</td>
<td>228</td>
<td>137</td>
<td>48</td>
</tr>
<tr>
<td>AlloAb</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Immune†</td>
<td>3‡</td>
<td>1§</td>
<td>0</td>
</tr>
<tr>
<td>Naturally occurring‡</td>
<td>1††</td>
<td>1‡‡</td>
<td>0</td>
</tr>
<tr>
<td>DAT positive</td>
<td>32</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>IgG+</td>
<td>16</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>IgG +, C3d/C3c</td>
<td>6</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>C3c only</td>
<td>7</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>IgM only</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>IgA only</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

SCD, Sickle cell disease; HM, Haematological malignancy; SM, Solid malignancy; AlloAb, Alloantibody; DAT, Direct antiglobulin test
†Immune antibodies are formed after immunisation through pregnancy or previous transfusions.
‡AlloAb specificities: anti-C<sup>w</sup>; anti-S, anti-Co<sup>b</sup>.
§ AlloAb specificity: anti-K.

4.2.2 Red cell autoimmunisation

Of the 228 patients, 32 patients (14.0%) presented a positive DAT (Table 4.2). Fifty per cent of these patients (16 of 32) were positive for anti-IgG alone, whereas 18.8% (6 of 32) showed reactions to anti-IgG plus anti-C3c or C3d. Out of the subset of 21 IgG-positive patients, the acid eluate was reactive in seven, thereby indicating a true RBC autoAb prevalence of 3.1% for this population of patients (7 of 228) and 33.3% (7 of 32) amongst the DAT-positive patients. RBC autoAb prevalence was 5.1% (7 of 137) amongst SCD patients, whereas there were no RBC autoAbs amongst patients with malignancies. Moreover, we observed a few cases (3 of 32) with isolated IgM or IgA reactivity. The majority (24 of 32) of the DAT-positive reactions with anti-IgM and anti-
IgA were observed in the SCD group. Eighteen per cent (24 of 137) of the SCD group were DAT positive compared with 8.8% (8 of 91) in the HM/SM group.

### 4.2.3 Comparison of combined RBC allo- and autoimmunisation in sickle cell versus cancer patients

The prevalence of RBC immunisation (demonstration of an immune alloAb and a positive DAT) amongst SCD patients was 19.7% (27 of 137) versus 9.9% (9 of 91). Immune alloAbs were found in 2.2% (3 of 137) of the SCD patients versus 1.1% (1 of 91) of the patients with malignancies. With one exception (polyspecific in eluate, but auto-anti-e in serum), these autoAbs showed polyspecificity only. We also performed a comparison for demographic and transfusion variables between patients with and without serological reactivity (Table 4.3). We did not find a significant link between patients’ sex, age or number of units of blood transfused and the positivity of the antibody screening.
TABLE 4.3: Characteristics of immunised and non-immunised transfused sickle cell and cancer patients at Kenyatta National Hospital, 2014

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Immunised</th>
<th>Non-immunised</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients (n, %)</td>
<td>33 (14.47)</td>
<td>195 (85.53)</td>
<td></td>
</tr>
<tr>
<td>Age (n, range)</td>
<td>17 (1–60)</td>
<td>17.2 (1–93)</td>
<td>0.946</td>
</tr>
<tr>
<td>Female to male ratio</td>
<td>1.28</td>
<td>1</td>
<td>0.458</td>
</tr>
<tr>
<td>Number of units transfused</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>93</td>
<td>594</td>
<td>0.496</td>
</tr>
<tr>
<td>Mean (range)</td>
<td>2.8 (1–7)</td>
<td>3.05 (1–19)</td>
<td></td>
</tr>
</tbody>
</table>

†P-values less than 0.05 were considered to be statistically significant

4.2.4 Healthy donor phenotypes

Amongst the blood donor samples, there were no serological peculiarities. Fifty-one donors were phenotyped for Rhesus antigens C/c, D, E/e and for the antigen K (Table 4.4). Of these, 29 donors (57%) showed the Rh phenotype ccD.ee, the other phenotypes were ccD.Ee (n = 13), CcD.ee (n = 5), ccddee (n = 2) and single cases of Ccddee (n = 1) and CcD.De (n = 1). None of the 51 donors were Kell positive.
TABLE 4.4: Rhesus and Kell phenotypes amongst 51 healthy Kenyan blood donors at Kenyatta National Hospital, 2014.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ro ro</td>
<td>29</td>
<td>57</td>
</tr>
<tr>
<td>R2r</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td>R1r</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Rr</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>r'r</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>R1R2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>R2R2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K negative</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>51</td>
<td>100</td>
</tr>
</tbody>
</table>

K, Kell
CHAPTER FIVE

DISCUSSION

The risk of alloimmunisation is a concern that needs to be addressed and managed, especially amongst patients requiring multiple blood transfusions, such as those with SCD and malignances.

5.1 RBC alloimmunisation among SCD and cancer patients

In this study, the frequency of alloimmunisation across all patients was determined to be 2.6%; and the rate of alloAb formation was 2.2% amongst patients with malignancies and 2.9% amongst SCD patients. Moreover, the specificities of the demonstrated alloAbs do not occur often in daily laboratory results. Anti-C\textsuperscript{w} and anti-S are comparatively rare Rh- and MNS antibodies, respectively; and anti-Co\textsuperscript{b} is a very rare RBC alloAb specificity of the Colton system. The one example of anti-K that we detected was the only common RBC alloAb specificity. Other common RBC alloAb specificities, such as anti-D, anti-E, anti-c, anti-C, belonging to the Rhesus system, or those of the Kell system (other than anti-K), the Duffy or the Kidd blood group systems, were not found in our study population. This lead to the phenotyping of blood from the healthy blood donors to establish the frequencies of Rhesus phenotypes in the population.

5.2 RBC autoimmunisation among SCD and cancer patients

In this study, a significant proportion of patients had some degree of RBC autoimmunisation, as shown by a positive DAT. In this study, a positive DAT was found in 32 (14.0%) patients, with a subgroup of seven IgG warm autoAbs (true RBC autoantibodies, indicated by a positive acid eluate). The total RBC autoAb prevalence for this population of patients (7 of 228) was 3.1% and 33.3% (7 of 32) amongst the DAT-positive patients. RBC autoAb prevalence was 5.1% (7 of 137) amongst SCD patients, whereas there were no RBC autoAbs amongst patients with malignancies. These rates are higher compared to a study by Datta \textit{et al.}, among 500 thalassaemia
patients, with whom only 5 (1%) developed autoantibodies (Datta, et al., 2015) but slightly lower to those of a study by Schnowille et al., where the rates of autoimmunization in 167 paediatric and 62 adult SCD patients was 8% and 9.7%, respectively. (Schonewille, et al., 1999).

In other studies, in 319 thalassemia patients, 90 (28.2%) patients developed autoantibodies (Dhawan, et al., 2014). This rate was high because 43 out of 90 (53.1%) auto positive patients had undergone splenectomy. It is known that splenectomized patients are more prone to autoimmunization as compared to patients who are not splenectomized (Singer, et al., 2000). In a similar study by Singer, et al., reported autoimmunization rate of 25% in 64 thalassemia patients, 9 of whom had undergone a splenectomy. In our study, no patient had undergone a splenectomy and this could partially explain the low autoimmunization levels particularly in the Sickle cell group.

The rate of autoimmunization in this study’s SCD group could induce significant clinical autoimmune haemolysis. However, no information about the presence of autoimmune haemolytic anaemia in these patients was sought, because this can be clinically asymptomatic and the reaction can be masked by the severity of the underlying disease and lack of adequate post-transfusion records.

5.3 RBC immunization in Sickle cell disease

Regularly transfused SCD patients, are known to be at high risk for RBC alloAb formation. (Natukunda, et al., 2010; Rosse, et al., 1990; Olujohungbe, et al., 2001). However, SCD patients in this study group had among the lowest values for of allo- and autoantibody formation as compared to other studies (Natukunda, et al., 2010; Schonewille, et al., 1999; Stieger, et al., 2001). The RBC alloimmunisation rate of 2.9% amongst the SCD patients is comparable to a study in a Jamaican cohort, (Olujohungbe, et al., 2001) where the rate was 2.6% amongst 115 transfused SCD patients and 1.6% amongst the total number of 190 patients. However, this rate differs considerably from that reported in a Ugandan study of 428 SCD patients, where the prevalence rate was
6.1%. (Natukunda, et al., 2010). Although the mean number of transfusions was three blood units in all of these studies, 21 of the 26 alloimmunised patients in the Ugandan study had received up to 10 blood units. This is marginally higher than the maximum number ($n = 8$) of transfusion observed in one patient in this study where most SCD patients received a mean of 2.4 units of transfusions, thus were exposed to minimal antigenic challenge. Numerous studies have reported that the rate of RBC alloimmunisation increases with the number of transfusions. (Natukunda, et al., 2010; Rosse, et al., 1990; Olujohungbe, et al., 2001; Hmida, et al., 1994; Sarnaik, et al., 1986; Mohsin, et al., 2013). This could explain the low alloimmunisation rate amongst this study’s participants compared with their counterparts in developed countries who received more transfusions.

Secondly, many other studies have reported higher percentages of RBC alloimmunisation in haemoglobinopathies, such as SCD or thalassaemia, including Uganda (Natukunda, et al., 2010) (SCD, alloAb 6.1% amongst 428 patients), Tunisia (Sarnaik, et al., 1986) (SCD and thalassaemia, alloAb 7.8% amongst 309 patients), Italy (Sirchia, et al., 1985) (thalassaemia, alloAb 5% amongst 1435 patients) and Brazil (Murao, et al., 2005) (SCD, alloAb 9.9% amongst 828 patients). These studies included a significantly higher number of patients; thus, the relatively low number of patients in this study might be a limiting factor. The low rates in this study also differ from studies conducted in populations where there is high heterogeneity between donors and patients. In a study by Rosse et al., involving 1814 SCD patients with an RBC alloimmunisation rate of 18.6%, the donors were of European-American ancestry and the SCD patients were of African-American ancestry (Rosse, et al., 1990).

Thirdly, patients in this study were predominantly children aged 16 years or younger ($n = 159; 70$%), 14% were ≤ 3 years of age. Studies of paediatric patients have reported lower RBC alloimmunisation rates. Aygun et al. (Aygun, et al., 2002) and Sarnaik et al. (Sarnaik, et al., 1986) concluded that children with SCD who were hypertransfused had a lower frequency of alloimmunisation as compared with adults. (Sarnaik, et al., 1986).
Another study involving 167 paediatric and 62 adult SCD patients supported this observation, where the rates of allo- and autoimmunisation in children and adults were 29% and 8%; 47% and 9.7%, respectively. (Schonewille, et al., 1999) Other authors advocate that transfusion started when patients are young (aged 1–3 years) may induce immune tolerance against alloimmunisation (Singer, et al., 2000) The fact that 14% of this study’s patients were aged ≤ 3 years could have contributed to the low rate of RBC alloAb formation that we observed.

5.4 RBC immunization among cancer patients

The prevalence of RBC alloimmunisation amongst this study’s cancer patients was low (2.2%), with only two HM patients and no alloAbs amongst SM patients. This is lower than that in the Ugandan study, where the rate was 8.3% amongst cancer patients. Mohsin et al. (Mohsin, et al., 2013) studied 150 cancer patients who had at least five transfusions and found the prevalence rate of alloAbs to be 6%. In this study, HM patients received a mean of 4.7 units and SM patients received a mean of 3.0 units thus were also exposed to low antigenic challenge. In a study by Seyfried and Walewska (Seyfried, et al., 1990) of 1502 multi-transfused patients, the overall incidence of alloAbs was 5.7%, with the lowest rate found amongst patients with lymphoproliferative syndromes (1.8%)(Seyfried, et al., 1990). Of note, all the cancer patients in our study were undergoing chemotherapy at the time of transfusion. It has been observed that patients with progressive malignancies undergoing intensive chemotherapy tend to have a low antibody formation response to foreign antigens. (Schonewille, et al., 1999; Lichtiger, et al., 1992; Quijada et al., 1996).

5.5 Rhesus phenotypes among blood donors

A majority (57%) of this study’s donor population expressed the Rh formula of ccD.ee, which could partly explain why no RBC alloantibodies directed against highly immunogenic antigens such as D or E were found. A study by Badjie et al., (Badjie, et al., 2011) conducted amongst 800 donors from various ethnic groups, found the
prevalence of the ccD.ee phenotype to be 81.9% in East Africa and a study by Baby et al. (Baby, et al., 2010) found a prevalence of 67.9% in West Africa (Mali). These results suggest that a large proportion of donors – exceeding 50% – and transfusion recipients in Africa share equal Rh phenotypes, so that Rh antibodies may be less frequently induced than in other parts of the world. This view is also supported by the low numbers for the ‘rr’ (Rhesus-D negative) phenotype amongst this study’s donor group (only 4%). This phenomenon might be also true for Kell antibody formation, as no Kell positive individuals were found amongst this study’s donors. It has been reported that more than 98% of black Africans are Kell negative (M‘baya, et al., 2010).

In this study, a low rate of RBC allo- and autoimmunisation amongst both SCD and cancer patients was observed. The low numbers of transfusions and transfusion events that are currently being applied at KNH and the relatively homogeneous distribution of Rh-/K-RBC alloantigens amongst Kenyan donors provide an explanation for the low alloAb and autoAb frequency amongst Kenyan transfusion recipients. At the current stage of the Kenya Health Care System, routine antibody screenings or extended RBC antigen matching do not seem to be justified, as the relatively homogenous RBC alloantigen distribution of Kenyan blood donors provides at least some protection from immune RBC alloAb and autoAb formation. However, with improvements in health care, more SCD and haemato-oncology patients are likely to receive a more intensive transfusion treatment, which could lead to an increased risk of RBC alloimmunisation. Therefore, further development of the healthcare system in Kenya will require a thorough reconsideration of the pretransfusion laboratory practice, in particular, if transfusion frequencies increase and/or donor groups change.
CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Despite the racial homogeneity between blood donors and blood recipients in Kenya and moderate number of transfusions, there is significant RBC immunization to RBC antigens especially in SCD patients. In this study, we observed that RBC-immunization in oncology patients did not differ from other multiply transfused patients’ thus extended RBC antigen matching between donor and recipients is deemed not to be necessary. However, with improvements in health care, more SCD and hemato-oncology patients are likely to receive intensive transfusion treatment leading to an increased risk of RBC alloimmunization. These patients could be candidates for hematopoietic stem cell transplantation therefore, they may benefit from extended antigen matching in order to prevent RBC antibody formation with consequences thereof for RBC support.

6.2 Recommendations

- Extended phenotype matching is costly and will be impossible for all patients, it is important to limit antigen matching to patients who showed to be responders to prevent additional alloantibody formation in the already immunized patients.
- Transfusion guideline should be implemented and further studies of phenotypes should be conducted in the African population.
REFERENCES


Baby, M., Fongoro, S., Cissé, M., Gakou, Y., Bathily, M., Dembélé, A. K., ... & Diallo, D. A. (2010). Frequency of red blood cell alloimmunization in polytransfused patients at the university teaching hospital of Point G, Bamako,


APPENDICES

Appendix I Consent form

INFORMED CONSENT FORM

Study Title: Prevalence of allo- and auto-antibodies in transfused sickle cell disease and cancer patients at Kenyatta National Hospital.

Introduction:

You are invited to be in a research study of ‘The prevalence of red cell allo and auto-immunization (antibody development) in transfused SCD and malignancy patients at Kenyatta National Hospital’. You were selected as a possible participant because you are sickle cell/ malignancy patient or guardian of any of these patients being managed at KNH. We ask that you read this form and ask any questions you may have before agreeing to be in the study.

Background Information:

The purpose of this study is to determine the development of allo-and auto-antibodies after receiving allogeneic transfusions.

Procedures:

If you agree or if you allow your child to be in this study, we will ask you to do the following things:

1. Read the consent form and answer a questionnaire.

2. 4 mls of blood will be drawn from your or your child’s cubital vein for analysis. This may be uncomfortable because of the needle prick but efforts will be
made to ensure that only an experienced person draws the blood and that this will be
done in the safest way possible.

**Risks and Benefits of Being in the Study:**

One of the risks of this study is the little pain and discomfort on the drawing of blood
from you or your child. We will be testing for antibodies in your blood or your child’s
blood. This information may be important in the way you or your child will be managed
in the future.

There are no direct benefits of this study to you or your child but the information will be
used to help in the management of other SCD and malignancy patients.

**Confidentiality:**

The records of this study will be kept private. In any sort of report we might publish, we
will not include any information that will make it possible to identify a participant.
Your record for the study may, however, be reviewed by any member of the research
team, the KNH ethical committee and to that extent, confidentiality is not absolute.

**Financial Considerations:**

There are no financial benefits from this study.

**Voluntary Nature of the Study:**

Your decision whether or not to participate in this study will not affect your current or
future relations with Kenyatta national Hospital/University of Nairobi hematology or
oncology clinic. If you decide to participate in this study, you are free to withdraw at any
time for any reason without affecting those relationships.
Contacts and Questions:

The researcher conducting this study is:

Caroline Mangare Mobile No: 0725358196

If you have any questions or concerns about the way you were treated as a participant in this research study, please contact Prof A.N Guantai, Chairperson KNH/UoN- ERC. Even though he may ask your name, information will be kept confidential.

You will be given a copy of this form to keep for your records.
Appendix II: Statement of Consent:

The information about the proposed research study and consent has been explained to you by:

__________________________________    ___________________________________

Name of Principal Investigator    Signature of Principal Investigator

When you sign this form, you agree that you understand the above description of this research. You also agree that your questions have been answered, and that you will want to take part in this research study.

_________________________________________   ___________________________

Signature of Patient/ guardian    Date
Appendix III: Questionnaire:

Study No:_________

Date of Birth:__________________ / __ / __________________

Sex:________________________ M F (No of previous pregnancies)___

Date of interview:__________ / __ / __________________

Diagnosis:

Any other underlying conditions:

Number of transfusions:________________________ Any transfusion reactions?____

Yes No

Did Alloimmunisation occur ______________

Reasons for transfusions: Date of transfusion

a) _______________________________ / / /

b) _______________________________ / / /

Any complications: a) _______________________________

b) _______________________________

Date of interview:__________________________________________

Name of interviewer: ________________________________________
Appendix IV: Laboratory procedure

Antibody identification using Diamed ID-Micro Typing System.

The requirements for antigen configuration are stringent: it must allow the safe detection of all clinically significant antibodies. For the Rh system, MNSs, Duffy and Kidd, the antigens must be in homozygous form. Due to higher sensitivity of the indirect antiglobulin test (IAT) with new procedures such as the DiaMed-ID Micro Typing System, some scientists in various countries have formed the opinion that the enzyme test has become somewhat less important.

The test cell reagents are specially designed for the ID-Micro Typing System.

For antibody screening, single donors, blood group O:

ID-DiaCell I-II R1 wR1+R2R2 for IAT and NaCl test

ID-DiaCell I-II-III R1 wR1+R2R2+rr for IAT and NaCl test

ID-DiaCell I-II-III P papainized, for enzyme technique

ID-DiaCell Pool R1R1+R2R2 (2 pooled cells for donor screening)

ID-DiaCell I-II-III Asia R1R1+R2R2+cell of the GP.MUR phenotype, for IAT and NaCl test

For antibody identification, single donors, blood group O:

ID-DiaPanel 11 test cells for IAT and NaCl test

ID-DiaPanel P 11 test cells papainized, for enzyme technique

Special antigens:

These cells are for use as an adjunct with other routine antibody screening cell sets.
ID-Dia (Diego) positive

ID-I negative cell

Additional reagent required

- ID-Card “LISS Coombs + enzyme test” 3 microtubes with polyspecific anti-human globulin (AHG) serum and 3 microtubes containing neutral gel (Id-no: 50581).

- ID-Card “LISS/Coombs” 6 microtubes with polyclonal AHG serum (Id-no: 50531).

- ID-Card “Coombs Anti-IgG” 6 microtubes containing rabbit anti-IgG (Id-no: 50540).

- ID-Card “NaCl, Enzyme test and cold agglutinins” 6 microtubes containing neutral gel (Id-no: 50520).

- During the working procedures, the test cell reagents should remain in suspension. If there is settling of the cells, resuspend again.

- For the ID-System, precise pipetting is of importance. Use the ID-Pipetors for serial pipetting.

- Avoid contamination of the test cell reagents.

- Ensure that the lot number of the antigen table corresponds with the lot number of the reagent vials when recording the reactions.

- After use, close the vials and replace them in the refrigerator.

Interpretation of the results
**Principle**

Positive: Agglutinated cells forming a red line on the surface of the gel or agglutinates dispersed in the gel.

Negative: Compact button of cells on the bottom of the microtube.

**Note:** Test cell reagents for antibody screening containing pooled cells can show a double cell population appearance depending on the antibody present. This is considered to be a positive result.
Appendix V: Declaration letter

Dear Ms Mangare,

This is to certify that the blood specimens referred to herein are being sent to the Institute for Transfusion Medicine, Hannover Medical School, Carl-Neuberg-Str. 1, D-30625 Hannover, Germany, for further analysis/experimentation. They will be in the custody of the Institute for Transfusion Medicine, and I hereby confirm that they will be utilized for the purpose stated in this request form, and I accept full responsibility and control over the usage of these samples.

Name of Department: Institute for Transfusion Medicine, Hannover Medical School
Institution Head: Prof. Dr. med. Rainer Blasczyk
Red Cell Antibody Laboratory: PD Dr. med. Hans-Gert Heuft

Signature: [Signature]
Date: 2013-10-23

PD Dr. Hans-Gert Heuft
### Antigen-Table / Antigen-Table / Table d'antigènes / Tabela antigênicos / Tabela de antígenos / Tabela de antígenos

<table>
<thead>
<tr>
<th>Rh-antigen</th>
<th>Rh-hr</th>
<th>Kell</th>
<th>Duffy</th>
<th>Kidd</th>
<th>Lewis</th>
<th>P</th>
<th>MNS</th>
<th>Lut H</th>
<th>Xg</th>
<th>Special Antigens</th>
<th>Resultat</th>
<th>Enzym</th>
<th>4°C</th>
<th>Special Antigens</th>
<th>Resultat</th>
<th>Enzym</th>
<th>4°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>CCCWD+</td>
<td>R1W+</td>
<td>R1r</td>
<td>R1R1</td>
<td>R1R1</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td></td>
<td>IAT</td>
<td>Enzym</td>
<td>4°C</td>
<td></td>
<td>Resultat</td>
<td>Enzym</td>
<td>4°C</td>
</tr>
<tr>
<td>II</td>
<td>ccD+D+</td>
<td>r+</td>
<td>r+</td>
<td>r+</td>
<td>r+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>ccdde+</td>
<td>r+</td>
<td>r+</td>
<td>r+</td>
<td>r+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Lot**

- I: 00845.50.x (Japan: 00845.50.x)
- II: 00645.50.x (Japan: 00645.50.x)
- III: 00615.50.x (Japan: 00615.50.x)

**Set I-III**

- 45184.50.x (Japan: 45184.50.x)

**Set IP-II**

- 45194.50.x (Japan: 45194.50.x)

**V.I.P. Software**


**Remarks**

- Bitte geben Sie hier Ihre Beobachtungen an.
- Voir les remarques au verso / Ver le note consulte il retro / Ver observaciones en el reverso / Ver observaciones no verso
Appendix VII: Ethical review committee approval

This is to inform you that the KNU/EoN-Ethics & Research Committee (KNU/EoN-ERC) has reviewed and approved your above proposal. The approval period is 4th February 2014 to 3rd February 2015.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNU/EoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNU/EoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNU/EoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period, (Attach a comprehensive progress report to support the renewal).f) Clearance for export of biological specimens must be obtained from KNU/EoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an executive summary report within 90 days upon completion of the study.

For more details consult the KNU/EoN ERC website www.uonbi.ac.ke/activities/KNU/EoN.
Appendix VIII: Shipping approval

Caroline Eunice Mangare
Dept. of Human Pathology and Laboratory Medicine
JKUAT

Dear Caroline

Re: Approval of shipment of samples – study titled – The prevalence of red cell alloimmunization in two multi-transfused patient populations: a cross-sectional study at Kenyatta National Hospital, Kenya (P364/07/2013)

Reference is made to your communication of 11th February 2014.

The KNH/UON-ERC has reviewed and approved shipment of 1ml of plasma and 1ml of red cells of about 200 samples for further analysis in Germany.

The samples will be under the custodian of the following:

Caroline Eunice Mangare
Institute of Transfusion medicine,
Hannover Medical School Laboratory
Red cell Antibody laboratory
Telephone +49511532-6703
+49511532-6701, +49511532-2079
Caroleunice2000@gmail.com
Carl-neuberg-strabel, 30625 Hannover Germany
+49511532-0. Heuft.hans-gert@mh-hannover.de
www.mh-hannover.de

Yours sincerely

Prof. A.N. Guantai
Chairperson, KNH/UON-ERC

Cc. The Deputy Director CS, KNH
The Principal, College of Health Sciences, UoN

"Protect to Discover"
Appendix IX: Shipping guideline

Packaging Guidelines for UN 3373 Shipments

Follow these instructions for packaging, marking and labeling Biological Substance, Category B (UN 3373) shipments for FedEx Express® services.
Requirements for Biological Substance, Category B (UN 3373) Shipments

This guide outlines the requirements for shipping with FedEx Express. In addition, all shipments must comply with all applicable local, state and federal laws governing packing, marking and labeling. Blood, urine, fluids, and other specimens containing or suspected of containing infectious substances must be shipped according to applicable government, International Air Transport Association (IATA) and International Civil Aviation Organization (ICAO) regulations.

Customers who ship Biological Substance, Category B (UN 3373) shipments must comply with local, state and federal laws governing identification, classification, packaging and package markings (which may be in label form). FedEx Express strictly adheres to the IATA, ICAO and U.S. government guidelines for materials categorized as Biological Substance, Category B (UN 3373).

General Packaging Requirements

For Biological Substance, Category B (UN 3373) shipments, cushioning material is required for both liquid and dried specimens. You must also include four layers of packaging:

1. **Primary watertight inner receptacle.** Use primary receptacles made of glass, metal or plastic with a positive means of ensuring a leakproof seal; skirted stopper or metal crimp seal must be provided; screw caps must be reinforced with adhesive tape. For liquid specimens, the primary receptacle must not contain more than 1 L. For dried specimens, the primary receptacle must not exceed the outer packaging weight limit.

2. **Absorbent material.** Place absorbent material between the primary and secondary receptacles, using enough material to absorb the entire contents of all primary receptacles. Absorbent material is required for Biological Substance, Category B (UN 3373) shipments containing liquids. Acceptable absorbent materials include cellulose wadding, cotton balls, super-absorbent packets and paper towels.

3. **Secondary watertight inner receptacle.** Use a secondary container that is leakproof for liquid specimens or spillproof for dried specimens. Choose only secondary containers certified by the manufacturer for Biological Substance, Category B (UN 3373) prior to use. Either your primary or secondary receptacle must be able to withstand, without leakage, an internal pressure differential of not less than 95 kPa in the range of -40 C to 55 C (-40°F to 130°F). To prevent contact between multiple fragile primary receptacles, individually wrap or separate them inside the secondary container.

4. **Sturdy outer packaging.** Use rigid outer packaging constructed of corrugated fiberboard, wood, metal or plastic, appropriately sized for the contents. Chipboard or paperboard boxes are unacceptable outer packaging. Limit the total volume for liquid samples to 4 L and the total weight of dried samples to 4 kg per outer container. The minimum outer container size in the smallest overall external dimension is 4". Completed packages must be able to withstand a 4' (1.2 m) impact test as outlined in IATA 6.6.1 regulations. Before sealing the outer packaging, you must make an itemized list of the contents of the package and enclose the list between the secondary packaging and outer packaging.

Acceptable Primary Receptacles

- Taped plastic container
- Taped glass or plastic jar
- Taped glass or plastic vial

Acceptable Absorbent Materials

- Cellulose wadding
- Cotton balls
- Super-absorbent packets
- Paper towels
**Acceptable Secondary Receptacles**

- Sealed plastic bag
- Plastic container
- Screw-cap can

**Biological Substance, Category B (UN 3373) Marking Requirements**

- Clearly mark “Biological Substance, Category B” in 6-mm-high text on the outer package adjacent to a properly sized UN 3373 diamond-shaped marking. If you prefer, package markings may be in the form of a label.
- If you use the FedEx® UN 3373 Pak, duplicate all required dangerous goods markings on each package inside the overpack.
- The name and telephone number of a person responsible must be marked on the package or provided on the airbill.
- The name and address of the shipper and recipient must be marked on the package.

“Biological Substance, Category B” must appear in 6-mm-high text on the outer package adjacent to a diamond-shaped mark like the one shown here. The UN 3373 marking must be in the form of a square set at an angle of 45 degrees. Each side of the UN 3373 diamond should measure a minimum of 2" (50 mm). The width of the diamond rule line must be a minimum of 2 mm, and the letters and numbers must be at least 8 mm high.

**FedEx UN 3373 Pak**

For your convenience, we offer the FedEx UN 3373 Pak as an overwrap for your Biological Substance, Category B (UN 3373) shipments. We recommend the FedEx UN 3373 Pak for use when the sturdy outer packaging of your properly packaged shipment is smaller than 7" x 4" x 2" (minimum acceptable size).

To help increase your operational efficiencies and clearly identify this type of shipment, the FedEx UN 3373 Pak is preprinted with the required IATA UN 3373 marking, the proper shipping name and the OVERPACK marking.

The FedEx UN 3373 Pak can only be used to ship Biological Substance, Category B (UN 3373) shipments. If you need an overwrap for exempt clinical and environmental test sample shipments, use the FedEx® Clinical Pak.

To order the FedEx UN 3373 Pak, call 1.800.GoFedEx 1.800.463.3339.
Packaging Restrictions

- Foam boxes, plastic bags and paper envelopes are unacceptable outer containers.
- The FedEx® Envelope, FedEx® Tube, FedEx® Pak, FedEx® Padded Pak and FedEx boxes, including FedEx brown packaging offered at FedEx shipping locations, are not acceptable as outer containers for Biological Substance, Category B (UN 3373) shipments.
- The FedEx Clinical Pak cannot be used to ship Biological Substance, Category B (UN 3373) shipments.
- Only shipments classified as Biological Substance, Category B (UN 3373) can be shipped in the FedEx UN 3373 Pak.
- Biological Substance, Category B (UN 3373) shipments that are shipped refrigerated, frozen, on dry ice, or in liquid nitrogen must comply with current IATA and ICAO regulations.

If you have questions about whether your shipments require a biohazard label, consult the Occupational Safety and Health Administration (OSHA) for the applicable regulations.

NOTE: Never place a Biological Substance, Category B (UN 3373) shipment in a FedEx Express® Drop Box. Call 1.800.GoFedEx 1.800.463.3339 to be directed to a FedEx location that can accept these shipments.

FedEx Office and FedEx World Service Center® locations do not accept shipments classified as Biological Substance, Category B (UN 3373). Call 1.800.GoFedEx 1.800.463.3339 to schedule a FedEx Express pickup, or to be directed to a FedEx location equipped to handle these shipments.

FedEx Packaging Services

FedEx Packaging Services offers package development consultation services. The FedEx Packaging Services lab does not test packaging containing Biological Substance, Category B (UN 3373) materials.

Contacts and Resources

- How to Pack guidelines at fedex.com/packaging.

NOTICE:

FedEx Express will refuse to accept packages that do not meet FedEx Express, government, or IATA and ICAO requirements. This brochure is in no way intended to replace requirements mandated by 49CFR and IATA. This is for informational purposes only.

NOTICE: This packaging brochure is provided to FedEx customers to help reduce loss or damage due to improper packaging. It is NOT intended to be a comprehensive guide for packaging items we accept for transport. We make no warranties, expressed or implied, regarding this information. Proper packaging is the sole responsibility of the shipper. For more information and comprehensive guidelines, contact the FedEx Dangerous Goods/Hazardous Materials Hotline at 1.800.GoFedEx 1.800.463.3339; press “8” or say “dangerous goods.” Refer to the current FedEx Services Guide for terms, conditions and limitations applicable to FedEx® delivery services.

© 2011 FedEx. All rights reserved.
Appendix X: Publication
Red cell allo- and autoimmunisation in transfused sickle cell and cancer patients in Kenyatta National Hospital, Nairobi, Kenya

Background: Currently, no data are available on the prevalence of red blood cell (RBC) antibody formation amongst Kenyan patients with multiple transfusion needs, such as patients with sickle cell disease (SCD) or haematological malignancies (HM) and solid (SM) malignancies.

Objectives: We determined the prevalence and specificities of RBC alloantibodies and autoantibodies in two patient groups with recurrent transfusion demands at Kenyatta National Hospital, Nairobi, Kenya.

Method: Between February and August 2014, 300 samples from SCD, HM and SM patients were collected and screened for alloantibodies. Samples from 51 healthy blood donors were screened for irregular antibodies and phenotyped.

Results: Amongst the 228 patients with viable samples (SCD, n = 137; HM, n = 48; SM, n = 43), the median transfusion frequency was two to three events per group, 38 (16.7%) were RBC immunised and 32 (14.0%) had a positive direct antiglobulin test. We identified specific alloantibodies in six patients (2.6%). Four of these six were SCD patients (2.9%) who had specific RBC alloantibodies (anti-C\textsuperscript{b}, anti-M, anti-Co\textsuperscript{b}, anti-S); amongst HM patients one had anti-K and one had anti-Le\textsuperscript{a}. RBC autoantibody prevalence was 3.1% (7/228). Amongst the healthy blood donors, the R\textsubscript{r}, ccD.\textsubscript{ee} and R\textsubscript{r}, ccD.Ee phenotypes accounted for 82% of the Rhesus phenotypes and all were Kell negative.

Conclusion: The numbers of transfusions and the rates of RBC alloantibodies are low and the most important RBC alloantibody-inducing blood group antigens are relatively homogeneously distributed in this population. A general change in the Kenyatta National Hospital pre-transfusion test regimen is thus not necessary. The current transfusion practice should be reconsidered if transfusion frequencies increase in the future.

Introduction

Blood transfusion constitutes an important supportive modality in the management of patients with sickle cell disease (SCD) and cancer, because of longer periods of treatment and increased survival rates. SCD is the most prevalent haematologic genetic disease in Kenya\textsuperscript{1} and cancer is an increasingly important challenge for the Kenyan public health system.\textsuperscript{2} Red blood cell (RBC) allo- and autoimmunisation often develop as a result of transfusions with allogeneic blood and occur because of the response of recipients’ immune systems to foreign RBC antigens from donors.\textsuperscript{3} Some of the facets involved in these immunological reactions are: recipient age; sex; history of pregnancy; number of blood units transfused; and diagnosis- and treatment-related impairment to the recipient’s immune system.\textsuperscript{4,5}

RBC alloimmunisation is associated with clinical complications, such as morbidity resulting from acute and delayed haemolytic transfusion reactions. The former can mimic a sickle cell crisis. Furthermore, alloimmunisation creates difficulties for laboratories, including expensive and time-consuming laboratory workups to determine compatible blood, especially for cases with multiple alloantibodies (alloAb). AlloAbs can become undetectable over time and/or be boosted as an anamnestic response after another transfusion. RBC alloAb against incompatible RBC in an allogeneic bone marrow transplant may require procedures for RBC reduction.\textsuperscript{6,7} The development of alloAb has been associated with that of autoantibodies (autoAb),\textsuperscript{8,9} which can shorten the lifespan of recipients’ own RBCs and/or transfused RBCs and potentially cause haemolysis. Because of this, these patients may require several transfusions and may need interventions, such as drugs to suppress the immune system and/or splenectomy.\textsuperscript{9} These challenges need to be considered when handling patients who are likely to be transfusion-dependent, as well as those who could benefit from haematopoietic stem cell transplantations.
Decreasing the risk of RBC alloimmunisation by implementing strategies to avoid allogeneic blood transfusions (e.g., erythropoietin administration in cancer patients) or extensive phenotypic matching of RBC blood group antigens, such as the Rhesus, Kell, Duffy, Kidd and MNS blood groups, has been advocated previously.\textsuperscript{10,11} However, this is costly and impractical in many health settings, particularly in developing countries.

Studies conducted on the frequency of RBC alloimmunisation in different patient populations have reported rates of 1\% to 6\% in occasionally transfused patients and up to 30\% in polytransfused patients.\textsuperscript{12} In Europe and the United States, alloimmunisation rates of 5\% to 36\% have been reported amongst transfused SCD patients.\textsuperscript{13} Currently, there are minimal data from Africa regarding transfusion-dependent RBC allo-/autoimmunisation. The few existing studies have reported varied results. A Ugandan study\textsuperscript{14} recently reported an RBC alloimmunisation prevalence rate of 6.1\% amongst 428 SCD patients. An investigation conducted in Egypt amongst 42 SCD patients reported an alloimmunisation rate of 21.4\%,\textsuperscript{14} whereas amongst 130 Tunisian thalassaemia patients, RBC alloimmunisation was 7.7\% and 40\% of these patients developed RBC autoantibodies.\textsuperscript{15} In a study of 108 Ugandan patients with malignancies, alloimmunisation was reported at a frequency of 8.3\%.\textsuperscript{16} There are no data on the prevalence of RBC alloAb/autoAb formation amongst Kenyan patients, where pretransfusion testing is limited to ABO/Rhesus D group typing and crossmatching only. As there is no routine pre-transfusion RBC antibody screening or identification, this study sought to determine the prevalence and specificities of RBC alloAbs and autoAbs amongst two different groups of transfusion recipients at Kenyatta National Hospital (KNH), Nairobi, Kenya. In addition, we screened samples from blood donors for irregular antibodies and phenotyped them for ABO, Rhesus and Kell antigens in order to determine whether there is alloimmunisation in the general population served by KNH.

Methods

Setting and design

Using a cross-sectional design, SCD, haematological malignancy (HM) and solid malignancy (SM) patients attending haematology and oncology clinics at KNH were approached between February and August 2014 and invited to participate in the study. To be eligible for the study, participants had to be KNH patients with SCD, HM or SM who had received at least one allogeneic blood transfusion; 300 patients met the inclusion criteria. Samples from 51 healthy blood donors of African ancestry from KNH’s blood bank were obtained for limited RBC antigen phenotyping. The Kenya National Blood Transfusion Policy defines the criteria for healthy donors as those who are aged 18–65 years; weigh more than 50 kg; have a minimum haemoglobin of 12 g/dL; have normal blood pressure (systolic 120–129 mmHg, diastolic 80–89 mmHg) and a pulse rate of 60–100 beats per minute.\textsuperscript{17}

Data and sample collection

After obtaining informed consent, 2–4 mL of blood was drawn from patients into ethylenediaminetetraacetic acid tubes for laboratory investigations. Patients’ notes were reviewed for: demographic characteristics; recipient age; sex; diagnosis; history of pregnancy; and transfusion history and indications. The number of blood components, units transfused and transfusion episodes were recorded. Healthy blood donor samples were collected from donors who gave consent and met the healthy donor criteria. Documentation of patient ethnicity is a routine requirement in Kenyan medical records or clinical data.

Laboratory investigations

Plasma and RBCs were separated within two hours after collection and the plasma was frozen whilst the red cells were stored at 2 °C – 6 °C. RBCs were preserved by adding a drop of citrate phosphate dextrose anticoagulant. The samples were then shipped on dry ice at a controlled temperature to the Institute for Transfusion Medicine at Hannover Medical School, Hannover, Germany for immunohaematological analysis.

Immunohaematological testing

The Bio-Rad ID gel card system (DiaMed-ID\textsuperscript{18}; Bio-Rad Laboratories, DiaMed GmbH, Cressier, Switzerland) was used with both untreated and papain-treated RBC reagents. Plasma samples were screened for the presence of RBC alloAb by use of a standard three-cell panel of reagent group O RBCs using NaCl gel cards at room temperature for alloAbs with low thermal range and low ionic strength saline (LISS) gel cards (LISS/Coombs) at 37 °C for warm-reacting alloAbs. For samples that showed agglutination, subsequent antibody identification was carried out with at least one 11-cell group O RBC panel (usually Bio-Rad, Switzerland). In instances without immediate determination of alloAb specificity, additional cell panels (e.g., an 11-cell [Grifols Inc., Los Angeles, California, United States] and/or a 16-cell panel [Sanquin, Plesmanlaan, Amsterdam, Netherlands]) were used. If a patient’s plasma sample showed agglutination of reagent screening cells, an autocontrol was also performed by reacting the patient’s RBCs with his or her own plasma. Positive autocontrols were further evaluated by means of a poly-/monospecific direct antiglobulin test (DAT). DAT was performed using monoclonal gel cards consisting of anti-IgG, anti-IgA, anti-IgM, anti-C3c and anti-C3d. From samples that were positive with at least one of these antiglobulins, an acid eluate was prepared. The eluate was screened using the standard three-cell panel. Those that were positive were then analysed for specificity using 11-cell panels containing these antigens: D, C, E, c, e, K, k, Fy\textsuperscript{a}, Fy\textsuperscript{b}, Jk\textsuperscript{a}, Jk\textsuperscript{b}, Le\textsuperscript{a}, Le\textsuperscript{b}, P1, M, N, S and s. In addition, donor blood was screened for irregular antibodies using a panel of three screening cells and then phenotyped for ABO, Rhesus (C, c, D, E, e) and Kell antigens. Plasma samples of 40 blood donors were screened for RBC alloAb.
Patients were considered to be alloimmunised if antibodies to one or more RBC antigens could be identified, whilst autocontrol and DAT screening remained negative. Patients with a positive autocontrol, a positive DAT and a reactive acid eluate were considered to be sensitised to have autoAbs to RBCs. In cases with a positive autocontrol and a positive DAT, but a non-reactive acid eluate, a non-specific loading of the RBC surface with immunoglobulins was assumed.

‘Immune’ antibodies are formed after immunisation through pregnancy and/or previous transfusions. ‘Naturally-occurring’ antibodies are formed as a result of exposure to environmental agents similar to red cell antigens, such as bacteria.

Statistical analyses

Statistical software packages were used: Excel 5.0 (Microsoft, Redmond, California, United States 1993) for data management and Statistical Package for the Social Sciences 12.0 (SPSS Inc., Chicago, Illinois, United States 2003) was used for analysis. Student’s t-test was used for variables with normal distribution. Categorical variables of possible associations between RBC alloimmunisation and sex, units of blood transfusion, diagnosis of SCD or solid and haematological malignancy were compared using the Chi-squared test. Groups were assumed to differ significantly when the probability level was less than 0.05.

Ethical considerations

Ethical approval was obtained from KNH/University of Nairobi Ethics Review Committee. Both oral and written informed consent was obtained from patients or their guardians. Donors completed a questionnaire provided by the blood bank services and signed a consent form.

Results

Patient data

Of the samples from 300 patients who met the inclusion criteria, 72 samples could not be evaluated for the following reasons: insufficient sample because of leakage during shipment (n = 40); samples breaking in the centrifuge whilst processing (n = 20); and lack of proper labeling (n = 12). A total of 228 samples were analysed, including 137 from SCD patients, 48 from HM patients and 43 from SM patients, with a median number of two to three transfusions per group (Table 1). Of these, 117 (51.3%) were women, of whom 22 (18.8%) had a history of pregnancy. Overall, the mean age at the time of blood transfusion was aged 17.2 years (range: 1–93). Cancer patients, in particular SM patients, were significantly older than SCD and HM patients (P < 0.001). Indeed, the majority of patients were children aged 16 years or younger (n = 159; 70%); 14% were aged three years or younger (Figures 1 and 2). There were no significant differences in the female to male ratios between the groups.

| TABLE 1: Characteristics of transfused sickle cell disease and cancer patients at Kenyatta National Hospital, Nairobi, Kenya, 2014. |
|---|---|---|---|---|
| Variables | All patients | SCD patients | cancer patients | HM patients | SM patients |
| Characteristics | | | | | |
| Number | 228 | 137 | 91 | 48 | 43 |
| Mean Age (range) | 17.2 (1-93) | 8 (1-36) | 31.1 (1.5-93) | 22.4 (1.5-70) | 48.1 (8-93) |
| Female / male ratio | 1.05 | 1.01 | 1.17 | 1.28 | 1.15 |
| Transfusions | | | | | |
| Number | 685 | 331 | 354 | 227 | 127 |
| Mean (range) | 3 (1-19) | 2.4 (1-8) | 3.9 (1-19) | 4.7 (1-19) | 3.0 (1-10) |
| Whole blood | 532 | 308 | 224 | 147 | 77 |
| Packed red blood cells | 85 | 23 | 62 | 37 | 25 |
| Platelets | 68 | 0 | 68 | 43 | 25 |
| Immunisation | | | | | |
| - | - | - | 20.4% | 11.0% | - |

SCD, Sickle cell disease; HM, Haematological malignancy; SM, Solid malignancy.

FIGURE 1: Transfused sickle cell disease patients by age, sex and seropositivity at Kenyatta National Hospital, Nairobi, Kenya, 2014.

FIGURE 2: Transfused cancer patients by age, sex and seropositivity at Kenyatta National Hospital, Nairobi, Kenya, 2014.

Patients received ABO/Rhesus D compatible and non-leucocyte-depleted whole blood units (n = 532), packed RBC transfusions (n = 85) and platelet transfusions (n = 68), totaling 685 units of blood in 593 transfusion events (i.e., 1 transfusion unit per transfusion episode or a mean of 2.7 transfusion units per patient). Of the SCD patients, 90% were transfused because of severe anaemia – haemoglobin less than 5–6 g/dL according to World Health Organization guidelines.20 Transfusion in malignancy patients was mainly...
Serological results
The overall prevalence of RBC immunisation was 16.7%, with 38 of the 228 patients testing positive for antibody screening. The prevalence of RBC immunisation amongst SCD patients was 20.4% (28 of 137 patients) and amongst malignancy patients, 11.0% (DAT+, n = 8 plus alloAb+, n = 2; altogether 10 out of 91 patients).

RBC alloantibody identification
Only 38 patients were positive for the antibody screening, and RBC alloantibodies were detected in only 6 of 228 patients (2.6%) (Table 2). The rate of alloAb formation amongst SCD patients was 2.9% (4 of 137) and 4.2% (2 of 48) amongst HM patients, whereas the prevalence amongst SM patients for alloAb identification was 0. The specificities of the alloAbs from the SCD patients were anti-C\textsuperscript{w}, anti-S, anti-Co\textsuperscript{w} (probably immune in nature) and anti-M (probably naturally occurring). In addition, there was one anti-K (immune) and one anti-Le\textsuperscript{a} (natural) in the two HM patients, whereas the SM group showed no RBC alloimmunisation. The rate of alloimmunisation was 6.14% for men versus 8.33% for women; the difference was not statistically significant (P = 0.25).

Red cell autoimmunisation
Of the 228 patients, 32 patients (14.0%) presented a positive DAT (Table 2). Fifty per cent of these patients (16 of 32) were positive for anti-IgG alone, whereas 18.8% (6 of 32) showed reactions to anti-IgG plus anti-C3c or C3d. Out of the subset of 21 IgG-positive patients, the acid eluate was reactive in seven, thereby indicating a true RBC autoAb prevalence of 3.1% for this population of patients (7 of 228) and 33.3% (7 of 21) amongst the DAT-positive patients. RBC autoAb prevalence was 5.1% (7 of 137) amongst SCD patients, whereas there were no RBC autoAbs amongst patients with malignancies. Moreover, we observed a few cases (3 of 32) with isolated IgM or IgA reactivity. The majority (24 of 32) of the DAT-positive reactions with anti-IgM and anti-IgA were observed in the SCD group. Eighteen per cent (24 of 137) of the SCD group were DAT positive compared with 8.8% (8 of 91) in the HM/SM group.

Comparison of combined RBC allo- and autoimmunisation in sickle cell versus cancer patients
The prevalence of RBC immunisation (demonstration of an immune alloAb and a positive DAT) amongst SCD patients was 19.7% (27 of 137) versus 9.9% (9 of 91). Immune alloAbs were found in 2.2% (3 of 137) of the SCD patients versus 1.1% (1 of 91) of the patients with malignancies. With one exception (polyspecific in eluate, but autoanti-e in serum), these autoAbs showed polyspecificity only. We also performed a comparison for demographic and transfusion variables between patients with and without serological reactivity (Table 3). We did not find a significant link between patients’ sex, age or number of units of blood transfused and the positivity of the antibody screening.

Healthy donor phenotypes
Amongst the blood donor samples, there were no serological peculiarities. Fifty-one donors were phenotyped for the Rhesus antigens C/c, D, E/e and for the antigen K (Table 4). Of these, 29 donors (57%) showed the Rh phenotype ccD.ee.

### Table 2: Serological results of transfused sickle cell disease and cancer patients at Kenyatta National Hospital, Nairobi, Kenya, 2014.

<table>
<thead>
<tr>
<th>Variables</th>
<th>All patients</th>
<th>SCD patients</th>
<th>Cancer patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>228</td>
<td>137</td>
<td>81</td>
</tr>
<tr>
<td>AlloAb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immune\textsuperscript{†}</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Naturally occurring\textsuperscript{¶}</td>
<td>-</td>
<td>3\textsuperscript{¶}</td>
<td>1\textsuperscript{¶}</td>
</tr>
<tr>
<td>DAT positive</td>
<td>32</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>IgG+</td>
<td>16</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>IgG+, C3d/C3c</td>
<td>6</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>C3c only</td>
<td>7</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>IgM only</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>IgA only</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

SCD, Sickle cell disease; HM, Haematological malignancy; SM, Solid malignancy; AlloAb, Alloantibody; DAT, Direct antiglobulin test\textsuperscript{†}, Immune antibodies are formed after immunisation through pregnancy or previous transfusions; \textsuperscript{¶}, AlloAb specificities: anti-C\textsuperscript{w}, anti-S, anti-Co\textsuperscript{w}; \textsuperscript{¶}, AlloAb specificity: anti-K; \textsuperscript{¶}, Naturally-occurring antibodies are formed as a result of exposure to environmental agents similar to red cell antigens, such as bacteria; \textsuperscript{††}, AlloAb specificity: anti-M; \textsuperscript{‡‡}, AlloAb specificity: anti-Le\textsuperscript{a}.

### Table 3: Characteristics of immunised and non-immunised transfused sickle cell and cancer patients at Kenyatta National Hospital, 2014.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Immunised</th>
<th>Non-immunised</th>
<th>P-value\textsuperscript{†}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
<td>All patients (n, %)</td>
<td>33 (41.47)</td>
<td>195 (85.53)</td>
</tr>
<tr>
<td>Age (n, range)</td>
<td>17 (1–60)</td>
<td>17.2 (1–93)</td>
<td>0.946</td>
</tr>
<tr>
<td>Female to male ratio</td>
<td>1.28</td>
<td>1</td>
<td>0.458</td>
</tr>
<tr>
<td>Number of units transfused</td>
<td>93</td>
<td>594</td>
<td>0.496</td>
</tr>
<tr>
<td>Mean (range)</td>
<td>2.8 (1–7)</td>
<td>3.05 (1–19)</td>
<td>-</td>
</tr>
</tbody>
</table>

\textsuperscript{†}, P-values less than 0.05 were considered to be statistically significant.
TABLE 4: Rhesus and Kell phenotypes amongst 51 healthy Kenyan blood donors at Kenyatta National Hospital, 2014.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>R, R, R</td>
<td>29</td>
<td>57</td>
</tr>
<tr>
<td>R, cCcD.De</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td>R, cCcD.ee</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>r, rCcD.cee</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>r, rCcD.ee</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>R, R, cCcDe</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>R, R, cCcD.ee</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K negative</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>100</td>
</tr>
</tbody>
</table>

the other phenotypes were ccD.Ee (n = 13),CcD.ee (n = 5), cccddee (n = 2) and single cases of Ccddee (n = 1) and CcD.De (n = 1). None of the 51 donors were Kell positive.

Discussion

The risk of alloimmunisation is a concern that needs to be addressed and managed, especially amongst patients requiring multiple blood transfusions, such as those with SCD and malignances. This investigation sought to determine the magnitude of RBC immunisation and to identify antibodies amongst two transfused patient groups. In this study, we detected a significant proportion of patients with some degree of RBC autoimmunisation, as shown by a positive DAT in 14.0% of the patients, seven of whom had true RBC autoantibodies. Eighteen per cent of SCD patients were DAT positive compared with 8.8% of HM/SM patients. In contrast, RBC alloAb formation was low, at only 2.6%. Moreover, the specificities of the demonstrated alloAbs do not occur often in daily laboratory results. Anti-Cw and anti-S are comparatively rare Rh- and MNS antibodies, respectively; and anti-Ce is a very rare RBC alloAb specificity of the Colton system. The one example of anti-K that we detected was the only common RBC alloAb specificity. Other common RBC alloAb specificities, such as anti-D, anti-E, anti-c, anti-C, belonging to the Rhesus system, or those of the Kell system (other than anti-K), the Duffy or the Kidd blood group systems, were not found in our study population. In this study, the frequency of alloimmunisation across all patients was determined to be 2.6%; and the rate of alloAb formation was 2.2% amongst patients with malignancies and 2.9% amongst SCD patients.

There may be several reasons for these unexpected findings. Firstly, the total numbers of transfusions and the numbers of transfusion events were low, never exceeding the mean values of three transfusion events per patient. This was particularly true for the SCD patients, who are known to be at high risk for RBC alloAb formation. However, SCD patients showed the lowest values for transfused RBC units per patient and transfusion events per patient in our study. The RBC alloimmunisation rate of 2.9% amongst our SCD patients is comparable to a study in a Jamaican cohort, where the rate was 2.6% amongst 115 transfused SCD patients and 1.6% amongst the total number of 190 patients. However, this rate differs considerably from that reported in a Ugandan study of 428 SCD patients, where the prevalence rate was 6.1%. Although the mean number of transfusions was three blood units in all of these studies, 21 of the 26 alloimmunised patients in the Ugandan study had received up to 10 blood units. This is marginally higher than the maximum number (n = 8) of transfusions observed in our study. Our SCD patients received a mean of 2.4 units of transfusions, HM patients received 4.7 units and SM patients received 3.0 units. Therefore, all groups were exposed to minimal antigenic challenge. Numerous studies have reported that the rate of RBC alloimmunisation increases with the number of transfusions.

Secondly, many other studies have reported higher percentages of RBC alloimmunisation in haemoglobinopathies, such as SCD or thalassaemia, including Uganda (SCD, alloAb 6.1% amongst 428 patients), Tunisia (SCD and thalassaemia, alloAb 7.8% amongst 309 patients), Italy (thalassaemia, alloAb 5% amongst 1435 patients) and Brazil (SCD, alloAb 9.9% amongst 828 patients). These studies included a significantly higher number of patients; thus, the relatively low number of patients in our study might be a second limiting factor. The low rates in our study also differ from studies conducted in populations where there is high heterogeneity between donors and patients. In a study by Rosse et al., involving 1814 SCD patients with an RBC alloimmunisation rate of 18.6%, the donors were of European-American ancestry and the SCD patients were of African-American ancestry.

Thirdly, patients in our study were predominantly children aged 16 years or younger (n = 159; 70%), 14% were aged ≤ 3 years. Studies of paediatric patients have reported lower RBC alloimmunisation rates. Aygun et al. and Sarnaik et al. concluded that children with SCD who were hypertransfused had a lower frequency of alloimmunisation as compared with adults. Another study involving 167 paediatric and 62 adult SCD patients supported this observation, where the rates of allo- and autoimmunisation in children and adults were 29% and 8%; 47% and 9.7%, respectively. Other authors advocate that transfusion started when patients are young (aged 1–3 years) may induce immune tolerance against alloimmunisation. The fact that 14% of our study patients were aged ≤ 3 years could have contributed to the low rate of RBC alloAb formation that we observed.

Fourth, the prevalence of RBC alloimmunisation amongst our cancer patients was low (2.2%), with only two HM patients and no alloAbs amongst SM patients. This is lower than that in the Ugandan study, where the rate was 8.3% amongst cancer patients. Shahida et al. studied 150 cancer patients who had at least five transfusions and found the prevalence rate of alloAbs to be 6%. In a study by Seyfried

and Walewska\textsuperscript{30}\textsuperscript{31} of 1502 multi-transfused patients, the overall incidence of alloAbs was 5.7\%, with the lowest rate found amongst patients with lymphoproliferative syndromes (1.8\%).\textsuperscript{31} Of note, all the cancer patients in our study were undergoing chemotherapy at the time of transfusion. It has been observed that patients with progressive malignancies undergoing intensive chemotherapy tend to have a low antibody formation response to foreign antigens.\textsuperscript{26,27,28}

Finally, a majority (57\%) of our donor population expressed the Rh formula of ccD.ee, which could partly explain why we did not find RBC alloantibodies directed against highly immunogenic antigens such as D or E. A study by Badjie et al.,\textsuperscript{29} conducted amongst 800 donors from various ethnic groups, found the prevalence of the ccD.ee phenotype to be 81.9\% in East Africa and a study by Baby et al.\textsuperscript{30} found a prevalence of 67.9\% in West Africa (Mali). These results suggest that a large proportion of donors – exceeding 50\% – and transfusion recipients in Africa share equal Rh phenotypes, so that Rh antibodies may be less frequently induced than in other parts of the world. This view is also supported by the low numbers for the ‘rr’ (Rhesus-D negative) phenotype amongst our donor group (only 4\%). This phenomenon might be also true for Kell antibody formation, as we found no Kell positive individuals amongst our donors. It has been reported that more than 98\% of black Africans are Kell negative.\textsuperscript{30,31}

We found a positive DAT in 32 (14.0\%) patients, with a subgroup of seven IgG warm autoAbs, which can induce significant clinical autoimmune haemolysis. We did not seek information about the presence of autoimmune haemolytic anaemia in these patients, because this can be clinically asymptomatic and the reaction can be masked by the severity of the underlying disease and lack of adequate post-transfusion records.

Limitations

It has been reported that 25\% of alloantibodies become undetectable within a median of 10 months of follow-up, which may lead to the underestimation of the prevalence of antibodies formed.\textsuperscript{30,32} This can result in a patient receiving RBCs and consequently experiencing a secondary immune response that may compromise the benefit of the following transfusion.\textsuperscript{30} Because this was a cross-sectional study, some RBC alloantibodies might have been missed, since they have been reported to disappear with time.\textsuperscript{32,33} Other factors that might also be responsible for the disparity in results include: the fact that the majority of the study patients were children; low mean of transfused units; inability to meet optimal transfusion needs for these patient groups; and the frequency of testing.

Conclusion

In this study, we observed a low rate of RBC alloimmunisation amongst both SCD and cancer patients. The low numbers of transfusions and transfusion events that are currently being applied at KNH and the relatively homogeneous distribution of Rh-\textsuperscript{K}/RBC alloantigens amongst Kenyan donors provide an explanation for the low alloAb frequency amongst Kenyan transfusion recipients. At the current stage of the Kenya Health Care System, routine antibody screenings or extended RBC antigen matching do not seem to be justified, as the relatively homogenous RBC alloantigen distribution of Kenyan blood donors provides at least some protection from immune RBC alloAb formation. However, with improvements in health care, more SCD and haematology patients are likely to receive a more intensive transfusion treatment, which could lead to an increased risk of RBC alloimmunisation. Therefore, further development of the healthcare system in Kenya will require a thorough reconsideration of the pretransfusion laboratory practice, in particular, if transfusion frequencies increase and/or donor groups change.

Trustworthiness

This study reflects the findings obtained from laboratory testing and analysis as observed by the technical group.

Reliability and validity

The experimental design and procedures used in this study are reliable and valid as they have been used previously in other studies, most of which are cited in this article. The results of the experiments in this article were obtained using specimens collected in various clinics at Kenyatta National Hospital, Kenya and were analysed using standard procedures in Hannover, Germany.

Acknowledgements

The authors thank the staff of the Hematology and Oncology Clinics and Blood Transfusion Unit at KNH, Kenya for their help in recruiting patients and donors respectively. The authors also thank KNH Research and Programs department for their monetary support. The authors thank the staff at the Institute for Transfusion Medicine, Hannover Medical School, Hannover, Germany, for providing laboratory reagents, space and technical and medical advice.

Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors’ contributions

C.M. (Jomo Kenyatta University of Agriculture and Technology) was the project leader, developed the proposal, obtained ethical clearance for the study, collected and shipped specimens, performed most of the experiments and developed the manuscript. A.M. (Jomo Kenyatta University of Agriculture and Technology) made conceptual contributions. P.M. and J.R. (Kenyatta National Hospital/
References