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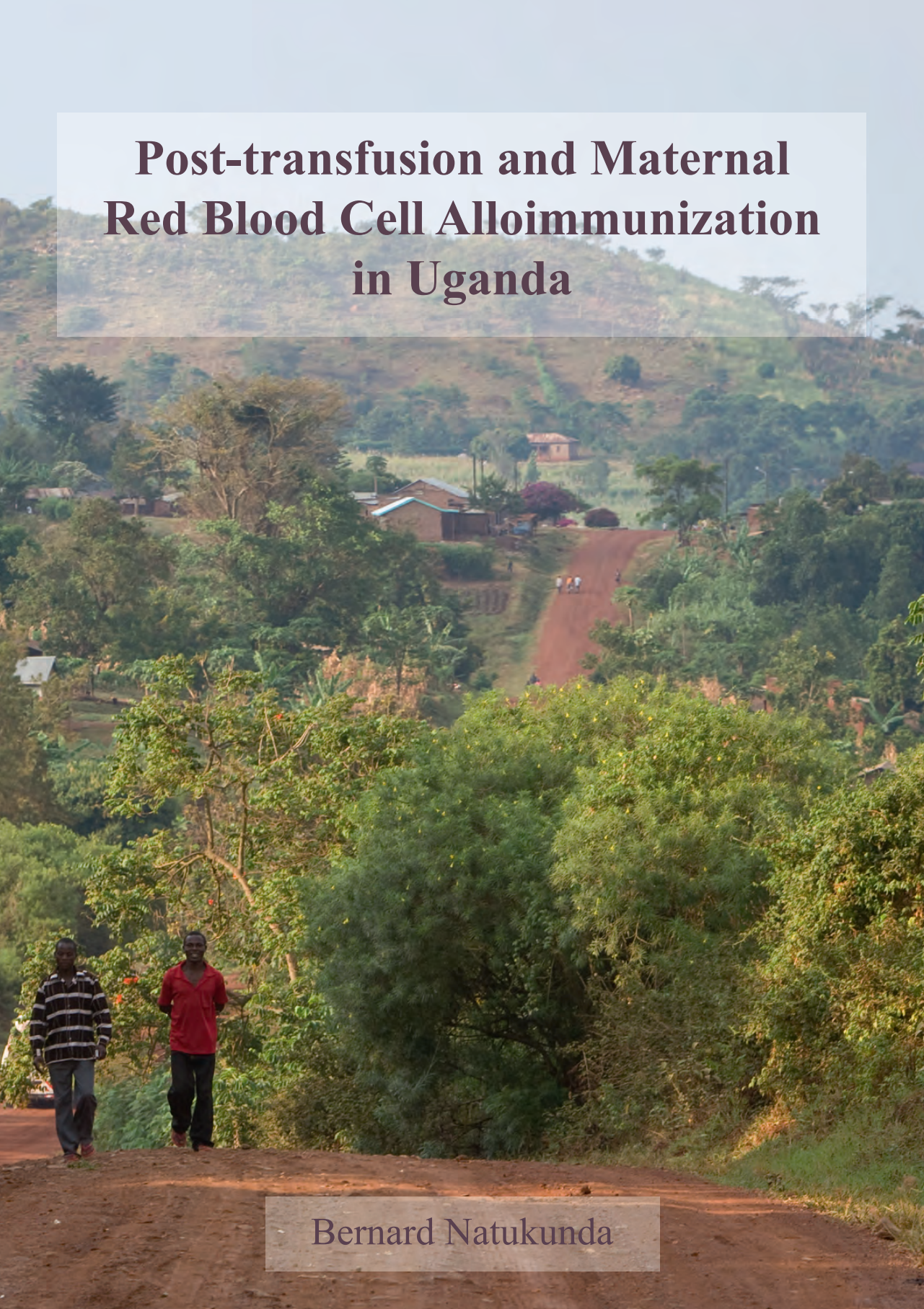


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Post-transfusion and Maternal Red Blood Cell Alloimmunization in Uganda

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**'If a man empties his purse into his head, no man can take it away from him.
An investment in knowledge always pays the best dividends.'**

Benjamin Franklin,
an American statesman and inventor

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GENERAL INTRODUCTION

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- 1.1 Background
- 1.2 Outline of the thesis

1.1 Background

The phenomenon of alloimmunization is an important adverse effect that follows transfusions with allogeneic blood (Walker *et al.*, 1989; Heddle *et al.*, 1995) and pregnancy (Moise, 1993; Moise, 2005). It results from an immune response due to genetic differences between the blood donor and recipient of the same species on the one hand, and the mother and fetus on the other. Immune alloantibodies against red blood cell (RBC) antigens are generally formed early in the course of multiple transfusions, usually before the 10th transfusion (Blumberg *et al.*, 1983; Fluit *et al.*, 1990). Upon further transfusion exposures, patients who have formed antibodies showed a 4 - 5 times increased risk for developing additional alloantibodies (Schonewille *et al.*, 2006; Schonewille *et al.*, 2009). Antibodies may also be formed against class I human leucocyte antigens (HLA) and human platelet antigens (HPA) when whole blood, platelets and granulocytes are transfused. Sickle cell disease (SCD) patients and other multiply transfused (OMT) blood recipients are at high risk of being alloimmunized. Once immunized, obtaining compatible blood for their future transfusions can pose complex serological problems and the need for an RBC typed donor inventory. The antibodies can cause alloimmune haemolysis presenting as haemolytic disease of the fetus and newborn (HDFN) or acute and delayed haemolytic transfusion reactions (HTRs), with potentially serious morbidity and mortality.

No data were available on the frequency of post-transfusion and maternal RBC alloimmunization in Uganda before the commencement of this research project. In the well-resourced parts of the world, the incidence and prevalence of RBC alloantibody formation are reported to be less than 1% up to more than 40% respectively. The frequency of post-transfusion RBC alloimmunization is generally high in patients with haemoglobinopathies, ranging up to 37% in thalassaemia and to 65% in sickle cell anaemia (Wang *et al.*, 2006; Ameen *et al.*, 2009). Factors assumed to influence the rate of alloimmunization are antigen immunogenicity, duration of transfusion therapy, and genetic and environmental factors, but their individual contribution is unknown.

In Uganda, pre-transfusion testing is currently limited to ABO/D typing plus room temperature (RT) saline cross-matches, and most of the patients are given whole blood transfusions instead of blood components. No screening for immune RBC alloantibodies is carried out in antenatal and pre-transfusion settings. Also, Rh immune globulin (RhIG) prophylaxis is not routinely administered to RhD negative mothers who deliver RhD positive babies. Therefore, there was a need to determine the magnitude of the problem of post-transfusion and maternal RBC

alloimmunization in Uganda. In this thesis, recommendations are given on the prevention of RBC alloimmunization and the delivery of improved blood transfusion and related obstetric services in Uganda. The proposed changes in policy and practice are aimed at reducing the morbidity and mortality associated with the consequences of RBC alloimmunization.

1.2 Outline of the thesis

This thesis provides results of a series of studies on the occurrence of RBC alloantibodies in blood transfusion recipients and pregnant women in Uganda.

We tried to answer the following research questions:

- Is there sufficient documentation on the clinical transfusion process and on post-transfusion complications related to RBC alloimmunization in Ugandan hospitals? (Chapter 3)
- What is the frequency and nature of RBC alloimmunization in SCD patients in Uganda? (Chapter 4)
- Does the rate of RBC alloimmunization differ among SCD patients compared to OMT Ugandan blood recipients? (Chapter 5)
- What is the prevalence of RhD negativity and maternal RBC alloimmunization in Ugandan pregnant women? (Chapter 6)
- Is it cost-effective to introduce RBC alloantibody screening as part of pre-transfusion testing in Uganda? (Chapter 7)

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**REVIEW OF THE LITERATURE
ON POST-TRANSFUSION
AND MATERNAL
RBC ALLOIMMUNIZATION**

CONTENTS

- 2.1 A short history of post-transfusion and maternal RBC alloimmunization
- 2.2 Human blood group diversity and function
- 2.3 Pathophysiology of the post-transfusion alloimmune response
- 2.4 Nature of RBC alloantibodies
- 2.5 Pre-transfusion compatibility testing
- 2.6 Prevalence of post-transfusion RBC alloimmunization
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- 2.8 Haemolytic disease of the fetus and newborn

2.1 A short history of post-transfusion and maternal RBC alloimmunization

Alloimmunization to RBC antigens, a consequence of blood transfusion or pregnancy, was recognized following the discovery of the Rhesus (now Rh) blood group system in 1939 by Philip Levine and Rufus Stetson. They reported a case of a woman who delivered a stillborn infant and suffered a severe haemolytic transfusion reaction (HTR) following transfusion with apparently ABO compatible blood from her husband. Her serum was found to contain an alloagglutinin (reacting at 37°C) which agglutinated RBCs of her husband and those of 85% of blood donors. Levine and Stetson showed that this new antigen, which they did not name, was independent of the then known blood groups ABO, MN and P. They postulated that the cause of this case of haemolytic disease of the newborn (HDN) was a maternal antibody entering the fetal circulation leading to fetal RBC destruction (Levine & Stetson, 1939). In 1940, Landsteiner and Wiener made an antibody by injecting Rhesus monkey RBCs into rabbits and guinea pigs (Landsteiner & Wiener, 1941). The resulting antiserum (anti-Rh) agglutinated not only Rhesus monkey RBCs but also those of 85% Caucasians. This specificity appeared identical to that of antibodies in the sera of patients who suffered HTRs after receiving ABO-identical blood (Wiener & Peters, 1940). In 1941, Levine and co-workers reported that the antibody responsible for HDN had the same specificity as the anti-Rh produced by Landsteiner and Wiener, later shown to be anti-LW. Levine and Stetson were indeed describing anti-D although they identified it as anti-Rh in these earliest publications. In 1945, Coombs, Mourant and Race described the use of antihuman globulin (later known as the “Coombs’ test”) to identify “incomplete” antibodies. A year later, they used this test to detect Rh antibodies on RBCs of babies suffering from HDN (Coombs *et al.*, 1946). Thereafter, the versatility of the Coombs’ test in immunohaematology for the detection of post-transfusion and maternal RBC alloantibodies became evident.

2.2 Human blood group diversity and function

Human blood groups are unique, inherited polymorphic structures located on mostly non-polymorphic proteins, glycoproteins, and glycolipids on the extracellular surface of RBCs. Blood groups are detected by a specific alloantibody, implying that the antigens are immunogenic for individuals lacking the blood group. Currently, 33 blood group systems, which include a total of about 339 antigens, have been established by the International Society of Blood Transfusion (ISBT Committee on Terminology for Red Cell Surface Antigens, Cancun 2012). In addition,

antigens not yet fulfilling the requirements for classification into a system have been gathered into collections or series of high- and low-frequency antigens (Daniels *et al.*, 2009). In blood group nomenclature, antigens encoded by the same gene, or cluster of two or more closely linked homologous genes with virtually no recombination events occurring among them, are assigned to the same blood group system.

Each blood group system is genetically discrete from other blood group systems and accommodates from 1-50 antigens. The two most important blood group systems from the point of view of clinical transfusion medicine are ABO and Rh. Rh and MNS are the most complex systems, with 61 and 46 antigens respectively. Most blood group polymorphisms are the result of single nucleotide polymorphisms (SNPs) encoding amino acid substitutions in an extracellular domain of an RBC surface protein. All blood group systems represent a single gene, apart from Rh, Xg and Chido/Rodgers, which have two closely linked genes, and MNS with three genes. In null phenotypes, the whole protein is absent from the membrane usually as a result of a gene deletion or an inactivating mutation. Genes that encode all the blood group systems present on RBCs have been identified (Storry & Olsson, 2004; Storry *et al.*, 2011).

The development of DNA sequencing techniques, and then the polymerase chain reaction (PCR) has paved the way for the rapid molecular characterization of the genes encoding blood group antigens (Beiboer *et al.*, 2005). As the molecular basis of many blood group antigens has been determined (Reid & Lomas-Francis, 2004), it is now feasible to predict the blood group antigen profile of an individual by testing the DNA. Such molecular analyses can be used to overcome the limitations of haemagglutination in clinical transfusion practice e.g. typing of multiply transfused patients, determination of paternal *RHD* zygosity, fetal genotyping from amniocytes or maternal plasma to determine the risk for HDFN, typing of RBCs with a positive direct antiglobulin test (DAT), detection of altered D antigens (weak D or partial D) and screening donor units for antigens (Do^a, Do^b, Js^a, Kp^a, Co^a, Yt^a, etc.) for which there are no commercial reagents (Legler *et al.*, 2001; Reid, 2003; Harper *et al.*, 2004).

The RBC membrane protein structures bearing blood group antigens exhibit diverse functional heterogeneity. The following functions have been attributed to blood group antigens. Some are membrane transporters e.g. band 3 (the Diego antigen) provides an anion channel for HCO₃⁻ and Cl⁻ ions; the Kidd glycoprotein is a urea transporter; the Colton glycoprotein, aquaporin 1, is a water channel; and RhAG is probably a gas channel. The Lutheran, LW, and Indian (CD44)

glycoproteins are adhesion molecules while the Duffy glycoprotein is a chemokine receptor. The Cromer and Knops antigens are markers for decay accelerating factor (CD55) and complement receptor 1 (CD35) respectively (Catron & Colin, 2001; Telen, 2005).

2.3 Pathophysiology of the post-transfusion alloimmune response

2.3.1 *Blood transfusions may lead to either alloimmunization or tolerance induction*

RBC transfusion for anaemia is both the oldest and the most widely employed transplantation procedure. Multiple allogeneic blood transfusions introduce a multitude of foreign antigens and living cells into the recipient that persist for a variable period of time. These can affect the immune response in two opposite ways, leading to either *alloimmunization* or to *tolerance induction*. Alloimmunization is reflected by the development of alloantibodies against RBC antigens (Lostumbo *et al.*, 1966), HLA antigens (Perkins *et al.*, 1966) and other cellularly expressed or soluble antigens; and by T cell activation leading to CD8 positive cytotoxic T cells. T cell receptors specific for alloantigens develop in utero. Intrauterine transfusion of allogeneic blood before the 14th week of gestation may result in tolerance and (transient) establishment of low dose chimerism (Hayward *et al.*, 1998). The full capacity to produce immune antibodies develops slowly after birth. Although anti-HLA antibodies have been reported after non-leucocyte depleted whole blood transfusion (Bedford-Russel *et al.*, 1993), the immunization rate in preterm infants is very low to negligible due to functionally immature B cells (Marshall-Clarke, 2000).

The induction of tolerance is suggested by the enhanced graft survival in transfused versus non-transfused solid organ recipients (Opelz & Terasaki, 1978). Also, recipients of allogeneic blood transfusions have been reported to be at a greater risk of post-operative infections referred to as transfusion-related immunomodulation (TRIM). Moreover, allogeneic blood transfusions have been shown to lead to suppressive effects in immunologic function in recipients *ex vivo* i.e. a decrease in the CD4:CD8 ratio of circulating T cells, reduced natural killer cell function, defective antigen presentation, suppression of lymphocyte blastogenesis, and reduction in delayed type hypersensitivity (Blajchman & Bordin, 1994). In general, contaminating leucocytes are thought to play a pivotal role in the above immunomodulatory effects of blood transfusions; with leucocyte depletion preventing both HLA alloimmunization and tolerance induction (Merryman, 1989).

2.3.2 Pathways for immune recognition of alloantigens

Two recipient T cell recognition mechanisms have been shown to be critical for the initiation of alloimmunity. The *direct pathway* occurs when recipient T helper (Th) cells directly interact with major histocompatibility complex (MHC) class II molecules on donor antigen presenting cells (APCs). The T cell activation by this direct pathway is only exerted by allogeneic class II bearing cells, such as in fetomaternal transfusion and by leukocyte-containing blood products. Approximately 100 times more T cells can be activated by the direct pathway as compared to the *indirect pathway*, which reflects the normal immune response. Indirect recognition occurs when foreign (allogeneic donor) molecules are processed by recipient APCs and presented to self Th cells. Within the context of indirect allorecognition, T cells recognize protein antigens that are degraded or processed within APCs to peptides which combined with MHC molecules are transported to the cell surface and bound within the antigen-binding grooves of either MHC class I or class II molecules (Sayegh *et al.*, 1994). The spectrum of antigen processing ranges from the simple unfolding of conformational determinants to the proteolytic exposure of primary structure by pH-dependent enzymes (e.g. cathepsins). Generally, APCs process exogenously derived proteins via endosomal compartments that shunt the processed peptides to intracellular compartments rich in MHC class II molecules. This pathway is necessary for the activation of CD4⁺ Th cells to provide helper factors for B cell activation and eventual IgG antibody production. Endogenous proteins (e.g. self-proteins or when infected with virally derived proteins), are generally processed by large-molecular-weight proteosomes within the cell cytosol and are subsequently transported to the luminal surface of the endoplasmic reticulum for loading onto MHC class I molecules and foreign molecules can be recognized by CD8⁺ (cytotoxic) T cells. For both direct and indirect pathways, the loaded MHC molecules are expressed on the surface of the APC and are available for presentation to circulating T cells (Watts, 1997). However, after T cell recognition a second signal provided by costimulatory molecules is needed to activate the Th cells.

2.3.3 Role of costimulatory molecules

As depicted above, both antibody production and cytotoxic T cell development depend on the stimulation of the recipient antigen-specific CD4⁺ (helper) T cells. The key requirements for CD4 stimulation are the simultaneous expression of at least two different signals (Schwartz, 1989). The first signal, occupancy of the clonotypic T-cell receptor (TCR), is provided by MHC-

peptide on the APCs. TCR binding leads to a cascade of events culminating in IL-2 expression but not IL-2 secretion (Mueller *et al.*, 1989). A second, costimulatory signal is required for the expression of the IL-2 gene with consequent secretion of IL-2 and cell proliferation (Mincheff & Merryman, 1990). On APC, the B7-1 protein, delivers a costimulatory signal through binding with the CD28 (positive) and CTLA-4 (negative) T-cell receptors, which regulate IL-2 secretion. Lack or impairment of this second signal has been shown to lead to T cell unresponsiveness or anergy (Nossal, 1989). In the case of allogeneic transfusions, the alloantigens on donor class II bearing APCs will be recognized by recipient T cells through the direct pathway (Pouteil-Noble *et al.*, 1991). The immunogenicity of those alloantigens will then be determined by the ability of the donor APCs to present the costimulatory signals to the recipient T cells. After 2 weeks' storage in vitro at 2 - 6°C, APCs lose costimulatory molecules (Mincheff & Merryman 1990). Besides leukocytes, unmodified RBCs often contain large numbers of platelets which are rich in both cell surface and soluble CD40L (Henn *et al.*, 1998). This costimulatory molecule activates B cells and is critical for IgM-to-IgG class switching (Grewal & Flavell, 1996). WBC reduction removes class II bearing donor APCs (e.g. dendritic cells, B-cells, and monocytes) and reduces HLA immunization in particular in case of platelet transfusions, provided that the residual white cell count is less than $1 - 5 \times 10^6$ per unit (Claas *et al.*, 1981, Sirchia *et al.*, 1986; van Marwijk *et al.*, 1991; Oksanen *et al.*, 1991; Saarinen *et al.*, 1993; Blumberg *et al.*, 2003). However, WBC removal has no demonstrable effect on the formation of RBC alloantibodies (Schonewille *et al.*, 2005), indicating that these are mainly elicited through the indirect antigen presentation pathway.

2.3.4 Type 1 and type 2 immune responses

Immunologic responses can become polarized to favour cells and cytokines of Type 1 (Th1) or Type 2 (Th2) responses (Mosmann & Sad, 1996). Type 1 responses involve cytokines such as γ -interferon (IFN- γ), IL-12, and IL-2, enhancing cellular immune responses such as delayed type hypersensitivity. Type 2 responses involve cytokines such as IL-4, IL-5 and IL-10 and enhance humoral immune responses, particularly those involving specific IgG subclasses, as well as IgA and IgE (Romagnani, 1996). Allogeneic leukocyte-containing blood transfusions have been shown to elicit immune deviation favouring Type 2 responses and downregulation of Type 1 responses (Kirkley *et al.*, 1995). This immunological mechanism could account for the unfavourable associations of allogeneic transfusions with the development of alloantibodies to RBCs, WBCs, platelets, and plasma proteins; presumed increased tumour recurrence; and post-

operative bacterial infection, and favourable associations with reduced spontaneous abortions and increased tolerance of solid organ allografts (Blumberg & Heal, 1996).

2.4 Nature of RBC alloantibodies

Alloantibodies against RBC antigens may be “*naturally occurring*” or “*immune*” in nature. Naturally occurring antibodies are most often IgM class, reacting at a temperature optimum below 37°C, but may be partly IgG and are found in individuals who have never been transfused with RBCs or who have not been pregnant with a fetus carrying the relevant RBC antigen. Natural antibodies are not present at birth, but arise early in life presumably due to cross-reactivity with ingested antigens. Immune antibodies are most often IgG but may be IgM or a mixture of IgG and IgM; they may sometimes have an IgA component. An antibody is considered to be *clinically significant* if examples with that specificity are known to have caused HTRs, HDFN or unacceptably short survival of the transfused RBCs (Walker, 1993). RhD is by far the most immunogenic antigen followed by K and c. Development of alloantibodies may compromise the care of chronically transfused patients since the deleterious effects of RBC alloimmunization, including delayed HTRs and HDFN, are increased (Moise, 1993). Antibodies may appear as early as 7 - 10 days after transfusion in primary immunization and within 2 - 7 days in a secondary response. The optimal screening times for the detection of post-transfusion RBC alloimmunization are not known and depend on the nature of the antigen, dose and recipient immunocompetence, although testing after 2 - 4 weeks and 3 - 6 months have been suggested. Schonewille *et al.* (2006) reported that anti-Jk^a and anti-Jk^b were predominant antibodies found in patients tested within one month, whereas anti-K and anti-Fy^a were most encountered after more than 3 months following blood transfusion.

Antibodies against HLA class I may cause confusion in RBC immunohaematologic testing. HLA class I antigens are widely distributed and, in general, can be detected on all nucleated cells. In peripheral blood, platelets and RBCs (which lack nuclei in their mature forms) can also express HLA class I antigens (Rosenfield *et al.*, 1967), sometimes referred to as Bg (Bennet-Goodspeed) antigens on RBCs. The numbers of molecules expressed per platelet have been estimated to be in the range of 14,000 to 82,000 (Kao *et al.*, 1986), being far fewer on RBCs, with a range of 40 to 550 per cell (Giles *et al.*, 1990). In contrast, the number of HLA class I molecules on T lymphocytes is about 100,000 per cell and on B lymphocytes, there are about

260,000 molecules per cell (Everett *et al.*, 1987; Mollison *et al.*, 1997). In view of the low number of HLA class I molecules on most RBCs, there has been speculation whether they are integral membrane components or they are acquired on the membrane by adsorption from plasma, which contains both membrane-shed and secreted forms (Krangel, 1987). It has been reported that RBCs do not synthesise HLA per se, but HLA class I molecules are produced by their nucleated precursor cells (Rivera & Scornick, 1986). Three principal antigens – Bg^a, Bg^b and Bg^c – have been defined and correlated with HLA class I antigens B7, B17 and A28 respectively (Morton *et al.*, 1969). Unwanted positive results in cross-matching due to HLA are common because antibodies to HLA-A28 and HLA-B7 are frequently present in sera and anti-Bg^a has been found in more than 10% of multiply transfused patients and can be the cause of HTRs (Nordhagen & Aas, 1978; Panzer *et al.*, 1987; Mollison *et al.*, 1997).

2.5 Pre-transfusion compatibility testing

The goal of pre-transfusion compatibility testing is to provide the patient with a beneficial and safe transfusion (Shulman *et al.*, 2001; Lieb & Aldridge, 2005). The transfused blood components should have acceptable survival *in vivo*. Pre-transfusion testing, including the antiglobulin phase, is very important because adverse effects of accelerated RBC destruction can be severe. Even recipient's RBCs, albeit less frequently, sometimes undergo accelerated destruction (bystander or autoantibody mediated). Most HTRs result from errors in patient or sample identity; and in some cases blood group alloantibodies to private antigens are not detected by standard serological techniques using RBC panels. When testing samples from antenatal patients and patients transfused within the last 3 months, a fresh sample is obtained for compatibility testing if more than 3 days have elapsed since the original sample was collected. If performed properly, pre-transfusion testing will ensure that a patient is issued the designated blood components, it will verify that the blood is ABO compatible and will detect the most clinically significant unexpected antibodies.

2.6 Prevalence of post-transfusion RBC alloimmunization

2.6.1 RBC alloimmunization in sickle cell disease patients

In heavily transfused SCD subjects, the RBC alloimmunization rate may approach 30% (Orlina *et al.*, 1978). Acute or delayed HTRs may occur if an alloimmunized patient is exposed to the same foreign antigen during subsequent transfusion. In the Cooperative Study of Sickle Cell

Disease, Rosse *et al.* (1990) reported an overall rate of RBC alloimmunization of 18.6% in 1,814 multiply transfused SCD patients. They identified a positive linear correlation between the number of SCD patients sensitized and the number of RBC exposures. Seventeen percent of the alloimmunized patients demonstrated four or more antibodies with a predominance of anti-C, anti-E and anti-K. In the same study, children less than 10 years old had a lower rate of alloimmunization than those in older age groups. In SCD, nulliparous women are more likely to become sensitized to RBC antigens than multiparous females (Reisner *et al.*, 1987). The suggested mechanisms underlying the increased incidence of alloimmunization in SCD patients include an altered immune response, increased frequency of certain HLA antigens, or lack of phenotypic compatibility between donor and recipient (Ambruso *et al.*, 1987; Cox *et al.*, 1988). Alarif *et al.* (1986) found a significant association between RBC alloimmunization and HLA-B35 among SCD patients. Caccese *et al.* (1987) demonstrated an increased functional activity in monocytes from patients with SCD reflecting an ongoing inflammatory state, when compared with monocytes derived from normal individuals.

When the distribution of antigens is different in the donor and recipient populations, greater alloimmunization may be expected. In a retrospective study, Vichinsky *et al.* (1990) found that racial and ethnic blood group antigen profiles between donor and recipient groups contribute to RBC alloimmunization in SCD. The frequencies of Duffy and Rh blood group system antigens are known to be distinctly different in Blacks and Caucasians. Despite the fact that 68% of Blacks lack the Fy^a and Fy^b and 99.9% of Caucasians have one or both antigens (Daniels, 2002), very few of the Fy(a-b-) Blacks form Duffy antibodies no matter how often they are transfused with Fy(a+) and/or Fy(b+) blood. This is because individuals of the Fy(a-b-) phenotype do not recognize the Duffy antigen as 'foreign' due to the presence of the Duffy glycoprotein on their tissue cells (Issitt & Anstee, 1998). In Brazil, where less heterogeneity between the donor and recipient groups exists, RBC alloimmunization rates in SCD are still substantial, suggesting that other mechanisms may be operative (Moriera *et al.*, 1996).

Following alloimmunization, antibodies to the Rh and Kell system antigens are most often detected, followed by antibodies to antigens of the Duffy and Kidd systems, while transfusion-induced antibodies to other RBC antigens are rarely found (Schroeder, 1999). To prevent the occurrence of alloimmunization, Davies *et al.* (1986) recommended extended RBC phenotyping of all SCD patients at the beginning of their transfusion therapy. This helps in deciding what

blood should be transfused and also aids in the identification of any antibodies that might develop. Thereafter, blood for transfusion should be matched for at least C, E and K antigens (Murphy, 2001). The stroke prevention trial (Vichinsky *et al.*, 2001) demonstrated that when SCD patients were given WBC-reduced RBCs that were matched for C, E and K antigens, the alloimmunization rate dropped from 3% to 0.5% per unit and HTRs dropped by 90%.

There is growing evidence that alloimmunization may lead to the production of autoantibodies and vice versa (Aygun *et al.*, 2002). Castellino *et al.*, (1999) reported the frequency of autoantibody formation as approximately 7.6% in a review of a large series of multiply transfused children with SCD. They also reported a strong association between autoantibody formation and the presence of RBC alloantibodies. The etiology behind the formation of these autoantibodies is poorly understood and not much information exists to suggest ways in which to lower the incidence of autoantibody formation. However, Ahrens *et al.*, (2007) found that blood transfusion appears to play a role in the majority of cases of autoantibodies associated with RBC alloimmunization. Clinically, it is important to recognize that post-transfusion haemolysis in which both autologous and transfused RBCs are destroyed may occur in patients with SCD.

2.6.2 RBC alloimmunization in other multiply transfused patients

In a retrospective study undertaken by Blumberg *et al.* (1983) on patients with disorders that often require multiple transfusions, the rate of alloimmunization (i.e. the proportion of patients with new antibodies) to RBC antigens was 11% in the aplastic anaemia, and 16% in the chronic myeloid leukaemia (CML) disease groups. In the same study, other groups of multitransfused patients had similar rates of alloantibody formation e.g. patients with renal failure (14%) and those with gastrointestinal bleeding (11%). Patients receiving chemotherapy for CML did not seem to be suppressed in terms of their ability to produce blood group alloantibodies when the D antigen was respected in the selection of the donor units. In contrast, none of the 99 patients with chronic lymphocytic leukemia (CLL) followed up for over a 10-year period produced blood group antibodies. Lymphoid leukemia patients are generally characterized by a lack of immunologic response and alloimmunization to RBC antigens following multiple transfusions, in this setting, is uncommon (Han *et al.*, 1981; Fluit *et al.*, 1990). There is often hypogammaglobulinaemia in lymphoid leukemia, which may be attributed to impaired functionality of B cells that are unable to upregulate HLA class II and costimulatory molecules

(Marshall-Clark, 2000) as is also observed in multiply transfused infants during the first few months of life (Ludvigsen *et al.*, 1987; Strauss *et al.*, 1999). Similarly absence of alloimmunization has been reported in some RhD negative AIDS patients receiving RhD positive RBC transfusions (Boctor *et al.*, 2003). This may be attributable to the decrease in CD4⁺ T lymphocytes in AIDS. However, the hypergammaglobulinaemia associated with a positive DAT (Levine & Liebman, 1995) and the persistent immune activation (Eggena *et al.*, 2005) in the course of HIV infection may explain the post-transfusion alloimmunization that is occasionally reported. Seyfried and Walewska (1990) found that the highest rate of immune response to RBC antigens occurred in multitransfused patients (defined as 3 or more blood transfusions) with autoimmune haemolytic anaemia (28%), liver cirrhosis (31.5%) and the myelodysplastic syndromes (40.9%). In a retrospective study, Fluit *et al.* (1990) found that 22 out of 186 (11.8%) multitransfused patients with haematological disorders developed antibodies over a 3-month period, after receiving at least six RBC transfusions. Anti-E and anti-K were the antibodies most frequently found; they were detected in 12 and 15 patients respectively. In patients with transfusion-dependent thalassaemia, the rate of allo-immunization to RBC antigens was found to range from 5 to 37%, with a lower prevalence in children starting transfusions before an age of two years (Spanos *et al.*, 1990; Coles *et al.*, 1981; Wang *et al.*, 2006). A female preponderance of RBC alloimmunization upon transfusions is controversial (Raki 1999; Blumberg *et al.* 1984; Redman *et al.* 1996). In a recent review of literature, Verduin *et al.* (2012) observed a higher RBC alloimmunization rate in transfused females with SCD only and not in other diseases that require multiple blood transfusions.

As discussed in section 2.3.2, HLA class II antigens present blood group peptides to CD4 T cells. Some peptides may be more optimally presented by the antigen-presenting groove formed by particular class II molecules. Reviron *et al.* (2005) and Chu *et al.* (2009) reported associations of anti-Jk^a alloimmunization with particular HLA-BRB1 alleles and anti-Mi^a alloimmunization with HLA-DRB1*0901 allele respectively, suggesting a role for MHC restriction in some cases of RBC antigen presentation (Picard *et al.*, 2009; Hoppe *et al.*, 2009).

2.7 Haemolytic transfusion reactions

Haemolytic transfusion reactions are one of the recognized consequences of post-transfusion RBC alloimmunization. An acute HTR is defined as the haemolysis of donor RBCs, within 24

hours of transfusion, by preformed alloantibodies in the recipient. Clerical errors (mislabelling of blood or misidentification of patients) account for 80% of acute HTRs, confirmed by national haemovigilance schemes which have been operational for several years in Europe and North America (Goodman *et al.*, 2003; SHOT, 2009). Symptoms and signs of acute HTRs are non-specific and include fever, chills, rigors, chest/back/abdominal pain, pain at the infusion site, nausea, vomiting, dyspnoea, hypotension, haemoglobinuria, oliguria/anuria, and disseminated intravascular coagulation (DIC). Most frequently, the offending antibodies are high titer IgM anti-A and/or anti-B although complement-fixing IgG antibodies in the recipient may be responsible as well. Immune-mediated haemolytic reactions can also rarely occur because of RBC antibodies in the plasma of the transfused product, be it in RBCs, fresh frozen plasma (FFP) or platelets. Cases of HTRs after transfusion of group O plasma containing products such as platelets, with high titer anti-A or anti-B to non-group O patients have been reported (Larsson *et al.*, 2000; Lozano & Cid, 2003; Josephson *et al.*, 2004).

Delayed HTRs are more common but usually less severe than acute haemolysis. Delayed reactions occur when a patient previously sensitized by pregnancy or blood transfusion receives “incompatible RBCs” because the low titers of circulating alloantibodies (typically against Rh and Kidd system antigens) escape detection by pre-transfusion testing. However, there is a rapid anamnestic response after transfusion of antigen-positive RBCs, leading to haemolysis. Delayed HTR often go unrecognized because they occur several days (usually within 5 - 10 days) after transfusion, which often means after hospital discharge. Delayed serologic transfusion reactions (DSTRs) are reactions identified serologically but not clinically. Delayed HTRs and DSTRs occur in approximately 1 in 1500 transfusions, with DSTRs being detected at rates two to fourfold higher than delayed HTRs (Ness *et al.*, 1990; Pineda *et al.*, 1999; Hendrickson & Hillyer, 2009). Obtaining a transfusion history and selecting offending antigen-negative RBCs for transfusion of patients with a history of clinically significant RBC alloantibodies is critical in decreasing the risk of delayed HTRs or DSTRs (Hendrickson & Hillyer, 2009). Patients with SCD or other major haemoglobinopathy syndromes who are chronically transfused are at greatest risk of alloantibody formation to RBC antigens and consequent HTRs. About 25% of the clinically significant RBC alloantibodies become undetectable over time, potentially confounding future transfusions and placing the patient at risk of an anamnestic antibody production and severe delayed HTRs (Rosse *et al.*, 1990; Schonewille *et al.*, 2000).

Approximately 40% of SCD patients who are alloimmunized have or will experience a delayed HTR (Knowles, 2001). Importantly, delayed HTRs can mimic various complications of SCD and should be suspected when patients present with appropriate symptoms (e.g. pain, fever, accelerated haemolysis) after a recent transfusion (Diamond *et al.*, 1980).

Another complication of RBC alloimmunization is the hyperhaemolysis syndrome which has a reported incidence of 4 - 11% (Aygun *et al.*, 2002; Talano *et al.*, 2003). In patients with SCD, clinical findings in the hyperhaemolysis syndrome occur approximately 1 week after the RBC transfusion and include the onset of increased haemolysis associated with pain and profound anaemia. The haemoglobin level often drops to below pre-transfusion levels. In many reported adult cases, the DAT remains negative and no new alloantibody is detected as the cause for these transfusion reactions (Talano *et al.*, 2003). Continuation of blood transfusion may be lethal, as this can further exacerbate haemolysis (Friedman *et al.*, 1993). It has been suggested that transfusion be withheld in severe haemolytic episodes, until hemolysis has faded spontaneously or after treatment with corticosteroids, high dose intravenous immunoglobulin or rituximab, a monoclonal antibody against B cells. The exact pathophysiologic mechanism of this syndrome is not well understood. A bystander haemolytic mechanism and transfusion suppression of erythropoiesis have been proposed (Petz *et al.*, 1997; King *et al.*, 1997). There is a broad clinical spectrum of autoantibody formation in association with red blood cell transfusions and reactions range from asymptomatic serologic detection to severe, life-threatening haemolysis (Sosler *et al.* 1989; Zumberg *et al.*, 2001; Garratty (2004).

2.8 Haemolytic disease of the fetus and newborn

Haemolytic disease of the newborn was first described in 1609 in a set of twins by a French midwife called Louise Bourgeois: the first twin was oedematous and stillborn, and the second was deeply jaundiced and subsequently died of what is now called kernicterus (Bowman, 1988). Over the centuries, this clinical picture was recognized and reported as two separate conditions. Diamond *et al.* (1932) realized that congenital anaemia, icterus gravis and hydrops, were manifestations of the same disease, which they named *erythroblastosis fetalis*. The identification of the cause of the haemolysis had to await the discovery of the Rh system (Landsteiner & Wiener, 1940) and the determination soon thereafter that HDFN occurred in an RhD-positive

fetus carried by an RhD-negative woman who had been immunized by the transplacental passage of RhD-positive RBCs during a prior pregnancy (Levine *et al.*, 1941).

Alloimmunization to the D surface antigen is the commonest cause of HDFN, which, before the introduction of anti-D immunoprophylaxis affected 1% of all newborns and was responsible for the death of one baby in every 2200 births (Kumar & Regan, 2005). By the 1970s, routine antenatal care in well-resourced countries included screening of all expectant mothers to select Rh-D negative cases and giving preventive treatment with anti-D after birth of a Rh-D positive child. This led to a dramatic decrease in the incidence of HDFN, particularly severe cases that were responsible for stillbirths and neonatal deaths (Mollison *et al.*, 1997). Despite the widespread use of this prophylaxis, a significant number of women still become alloimmunized for a variety of reasons, including no administration or insufficient dosage of RhIG in case of unrecognized miscarriage, leakage of fetal RBCs into the maternal circulation late in pregnancy, large fetomaternal hemorrhage (FMH) or exposure to traumatic deliveries including Caesarean sections, manual removal of the placenta, stillbirths and intrauterine deaths, blunt abdominal trauma during the third trimester, twin pregnancies (at delivery), external cephalic version, chorionic villous sampling, antepartum haemorrhage, ectopic pregnancy and an unexplained hydrops fetalis (Sebring & Polesky, 1990; Bowman, 1997). FMH involves smaller amounts: in 3% of the women, fetal RBCs are detectable in the maternal circulation during the first trimester of pregnancy; in 12% during the second trimester; in 46% during the third trimester; and in 64% of the women after delivery, usually in amounts less than 20 ml of fetal blood (Bowman *et al.*, 1986). It is not uncommon for there to be silent leaks of RBCs from the fetus into the mother (with no pain or bleeding), especially in the third trimester. The maternal IgG antibodies traverse the placenta to the fetal circulation during gestation and cause RBC destruction with complications before birth, or anaemia and hyperbilirubinaemia after birth, or both. In its most severe form, HDFN produces hydrops fetalis, which is characterized by total body oedema, hepatosplenomegaly and heart failure, and can lead to intrauterine death. HDFN may also follow blood transfusion with antigen positive blood that is incompatible with the mother. Virtually all alloantibodies reactive by the IAT have been implicated in HDFN in different populations. The prevalence of D-negativity varies in different ethnic groups with 15% of Caucasians, 8% of Blacks and 1% of Asians being D-negative (Reid & Lomas-Francis, 2004). The D antigen accounts for about 50% of cases of maternal alloimmunization; the remainder is mainly due to

incompatibility to K, c, C/G, E, and Fy^a antigens and to low incidence antigens in the Rh, MNS, and Diego blood group systems (Hedde *et al.*, 1993). Anti-D formation is more frequent in D positive individuals of African descent than in Europeans, which is probably a result of the high frequency of aberrant *RHD* alleles belonging to the three African D clusters i.e. DIVa, weak D type 4 and DAU in some African populations (Touinssi *et al.*, 2009). Also, because the number of copies of the D antigen on each RBC is higher in the R₂ haplotype (range: 14,000 to 16,000) than in the R₁ haplotype (range: 9,000 to 14,600), fetuses whose RBCs are R₂ have more severe anemia than their R₁ counterparts (Mollison *et al.*, 1997). Ulm *et al.* (1999) reported that male fetuses were 13 times more likely to develop hydrops than female fetuses, and perinatal mortality was 3 times higher in male fetuses. However, this was not confirmed by other investigators (Ramsey & Sherman, 1999).

From the perspective of prevention an initial step is to estimate fetal risk by establishing paternal *RHD* zygosity. After paternal testing has revealed the possibility of a heterozygous state for *RHD*, fetal testing is indicated. Fetal D antigen determination through noninvasive DNA testing from maternal plasma is now routine practice in some countries (Lo *et al.*, 1998; Daniels *et al.*, 2004). If the maternal race is black, then the presence of a maternal pseudogene or *Ccde^s* gene should be considered in the scheme of fetal testing (Faas *et al.*, 1997; Singleton *et al.*, 2000). The presence of one of these genes in the fetus can lead to a false positive molecular diagnosis yet the fetus would be found to be D-negative by serology after birth, leading to unnecessary fetal interventions such as antenatal RhIG administration. Whenever an IAT-reactive antibody is detected during pregnancy, a cord blood sample at birth should be tested by DAT and if positive, the haemoglobin (Hb) and bilirubin levels monitored to initiate treatment (BCSH Guidelines, 1996). Monitoring maternal anti-D during pregnancy is very important to predict the severity of antenatal HDFN. In most centres, a critical titre for anti-D between 8 and 32 is usually used (Moise, 2005; Moise, 2008). Because the titre is not very reliable predicting fetal hemolysis, functional assays such as the monocyte monolayer assay (MMA) or antibody-dependent cellular cytotoxicity (ADCC) are performed in reference laboratories. Once sensitization has occurred the fetus should be monitored by preferentially non-invasive echo-doppler techniques to estimate the degree of fetal anemia. The following therapeutic options are open: controlled early delivery or intrauterine transfusion of which the latter has the best prognosis but requires a specialized centre (Urbaniak & Greiss, 2000; van Kamp *et al.*, 2004).

Interestingly, a fetus that is ABO incompatible with the maternal anti-A/B is less likely to have HDFN due to anti-D, presumably due to rapid removal of the ABO-incompatible RBCs by the naturally occurring anti-A/B. Although maternal-fetal ABO incompatibility is common, in general haemolysis is mild and the clinical course is relatively benign needing only phototherapy (Grundbacher, 1980; Drabik-Clary *et al.*, 2006). In cases of ABO incompatibility between the mother and the fetus, group O mothers are more likely to become sensitized (Ozolek *et al.*, 1994). This process occurs to a much less extent in group A or B neonates who are born to heterospecific A or B mothers, because in this situation, the respective anti-A or anti-B immunoglobulin is predominantly IgM and therefore unable to cross the placenta (Kaplan *et al.*, 2009).

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**ASSESSMENT OF THE CLINICAL
TRANSFUSION PRACTICE AT A
REGIONAL REFERRAL HOSPITAL
IN UGANDA**

Bernard Natukunda, Henk Schonewille and Cees Th. Smit Sibinga. Assessment of the clinical transfusion practice at a regional referral hospital in Uganda. *Transfusion Medicine*, 2010;20:134-139.

SUMMARY

The aim of this study was to determine the indications for transfusion, blood ordering practices and post-transfusion complications at Mbarara Regional Referral Hospital (MRRH) in Mbarara, Uganda; and to assess the clinical transfusion practice at MRRH. There are no guidelines on the appropriate use of blood at MRRH. Therefore, there was need to assess the local clinical transfusion practice. Patients' hospital files were studied for evidence of blood transfusions in 2008. All five wards were reviewed and details on the transfusion process recorded. One thousand seven hundred and thirty patients (median age, 19.0 years; range, 1 day to 88 years; female-to-male ratio, 1.4), for whom blood was cross-matched, were studied. Of these, 1674 (96.8%) patients actually received transfusions, which were as whole blood in 58.4% of recipients. The mean number of units per recipient was 1.7 and the crossmatch-to-transfusion ratio was 1.3. The three most frequent indications for transfusion were malaria (38.8%), bleeding (27.1%) and other infections (16.1%). There were no records for pre-transfusion haemoglobin, compatibility testing, transfusion start-times and vital signs in 30.2%, 51.8%, 21.5% and 97.6% of recipients, respectively. Transfusion reactions were recorded for ten (0.6%) patients. Although there was no evidence of blood wastage, inadequacies were noted in the documentation of the transfusion process. There is need to train staff in blood transfusion and to design a 'blood transfusion form' for easy monitoring and evaluation. A hospital transfusion committee and guidelines on the appropriate use of blood should be put in place at MRRH.

Key words: Appropriate use, Clinical transfusion practice, Documentation, Hospital Transfusion Committees, Uganda.

INTRODUCTION

According to the World Health Organization (WHO), Transfusion Medicine is defined as that part of the health care system which undertakes the appropriate provision and use of human blood resources (WHO, 1992). Transfusion Medicine practitioners must maintain quality and work to increase the safety of blood and blood products. The specialty is unique because it links one sector of the community (the donors) with another (the patients) in an altruistic and potentially life-saving activity. The transfusion process includes a series of events comprising of ordering of blood or blood components for transfusion, taking pre-transfusion blood samples, laboratory practices, collection and administration of blood or blood components, monitoring of the transfused patient, managing adverse events and documenting the transfusion events and outcomes.

Blood transfusion is an essential and integral part of patient care: when used correctly, it saves lives and improves health. However, transfusion carries a potential risk of acute and/or delayed transfusion reactions and transfusion transmitted infections. WHO recommends that national health programs should develop policies and strategies to reduce the need for blood transfusion, minimize unnecessary transfusions and ensure the safe and appropriate use of blood and blood components (WHO, 2002). The effective development and maintenance of satisfactory standards of Transfusion Medicine practice requires an organization-wide approach and adoption of a quality management system. In the absence of a quality infrastructure, errors will occur with potential outcomes such as wastage of blood, hypoxia or coagulopathy as a result of delays or incompatible transfusions that may be fatal (Knowles, 2001). Safe transfusion therapy depends on a complex process that requires integration and coordination among multiple hospital services including laboratory medicine, nursing, anaesthesiology, surgery, clerical support, and transportation. The multidisciplinary hospital transfusion committee has been traditionally charged with oversight of transfusion safety. Transfusion errors are usually rooted in the failure to follow clerical or technical procedures and/or in the breakdown in professional practice or judgement (Dzik *et al.*, 2003).

In Uganda, regional blood banks of the Uganda Blood Transfusion Service (UBTS) collect blood from voluntary non-remunerated donors; routinely screen the donated blood for HIV, syphilis, hepatitis B and C viruses; perform ABO/Rh D typing; and distribute the blood to hospitals free of charge in accordance with the national transfusion policy (Ministry of Health, Uganda, 2005).

So far, only the Central regional blood bank at the UBTS headquarters in Kampala is able to produce blood components for transfusion. The Ministry of Health, with assistance from the United States President's Emergency Plan for AIDS Relief (PEPFAR) project, meets the financial needs of the UBTS. Even in times of increased demand for blood products e.g. during malaria seasons, the UBTS does not recruit paid or replacement donors. However, there are no specific written guidelines on the appropriate use of blood in hospitals. The aim of this study was to assess the clinical transfusion practice at a regional referral hospital in Uganda.

STUDY DESIGN AND METHODS

Study setting and design

A one-year retrospective study was conducted at Mbarara Regional Referral Hospital (MRRH), the teaching hospital for Mbarara University of Science and Technology (MUST) Medical School. MRRH is a 330-bed hospital and it serves as the regional referral centre for South Western Uganda. The hospital transfusion laboratory receives its blood supply from the Mbarara Regional Blood Bank located about 2 kilometres away. The study was approved by the Research and Ethics Committee at MUST.

Patients and methods

Medical records for patients admitted at MRRH in 2008 were reviewed for evidence of having received blood transfusion(s). Patients of both sexes and all ages admitted to the five main wards at MRRH (i.e. Paediatric, Accident and Emergency [A&E], Surgical, Obstetric and Gynaecological [OBGY], and Medical), for whom blood was ordered and crossmatched, were included in the study. The medical and transfusion histories were studied and the following data collected: age and sex of the recipient; ward of admission; date of transfusion; clinical diagnosis; pre-transfusion haemoglobin level; indication(s) for blood transfusion; number of units of blood ordered; number of units of blood transfused; number of transfusion episodes; type of blood component transfused; presence of compatibility form(s) in the patient's file; record of times for the beginning of the transfusion; record of vital signs during the transfusion; and a record of any adverse events. Laboratory practices were not included in this review.

Statistical analysis

The information on the patients' transfusion history and laboratory tests was recorded using data collection forms. Statistical software package Excel 5.0 (Microsoft, Redmond, WA, USA) and Statistical Package for the Social Sciences 12.0 (SPSS, Inc., Chicago, IL, USA) were used for data management and analysis, respectively. The chi-square test was used to test for differences in frequencies between groups of recipients. Groups were assumed to differ significantly when the p-values were less than 0.05.

RESULTS

A total of 1,730 patients (median age, 19.0 years; range, 1 day to 88 years; female-to-male ratio, 1.3), for whom blood was ordered and cross-matched, were studied. Of these, 1,674 (96.8%) patients were actually transfused.

Table 1. Patients whose blood was cross-matched and those who received transfusions on each ward at MRRH in 2008

Number of patients	Paediatric	Medical	OBGY	A&E	Surgical	Total
Cross-matched	726	453	346	180	25	1,730
Transfused (%)	724 (99.7)	453 (100)	318 (91.9)	158 (87.8)	21 (84.0)	1,674

Table 1 shows the number of patients for whom blood was cross-matched and transfused on the five wards at MRRH. No autologous blood transfusions were recorded in the whole year. One hundred and nine (6.5%) patients received emergency blood transfusions at the A&E ward due to surgery or trauma.

The three most frequent indications for blood transfusion were: malaria (38.8%), bleeding (27.1%), and other infections (16.1%). The indications for blood transfusion at MRRH in 2008 are shown in Table 2.

Table 2. Indications for blood transfusion with the respective crossmatch-to-transfusion (CT) ratios at MRRH in 2008

Indication for transfusion	Patients (n, %)	Units cross- matched (n, %)	Units transfused (n, %)	CT ratio
Malaria	672 (38.8)	1022 (27.8)	918 (33.1)	1.1
Bleeding	468 (27.0)	1245 (33.9)	823 (29.6)	1.5
Non-malarial infections	278 (16.1)	715 (19.5)	533 (19.2)	1.3
Cancer	115 (6.6)	287 (7.8)	217 (7.8)	1.3
Organ disorders (kidney, heart, liver)	56 (3.2)	155 (4.2)	118 (4.2)	1.3
Trauma	50 (2.8)	101 (2.7)	63 (2.3)	1.6
Surgery	48 (2.7)	97 (2.6)	55 (1.9)	1.8
Malnutrition	35 (2.0)	39 (1.1)	39 (1.4)	1.0
Sickle Cell Disease	8 (0.5)	12 (0.3)	11 (0.4)	1.1
Total	1,730	3,673	2,777	1.3

There were 672 recipients with malaria, 556 (82.7%) of whom were children admitted to the Paediatric ward. Figure 1 shows the number of patients with severe anaemia due to malaria and other non-malarial indications who received blood transfusions in each month of 2008. Obstetric haemorrhage accounted for 55.7% of all bleeding patients at MRRH who received transfusions; and for 75.4 % of all recipients at the OBGY ward.

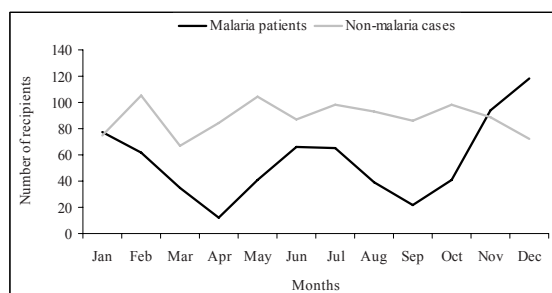
**Figure 1** Patients at Mbarara Regional Referral Hospital for whom blood transfusions were ordered because of severe malarial anaemia (n=672) and other non-malarial indications (n=1058) in each month of 2008. Two malaria seasons in the months of May - August and November - February are shown.

Table 3 shows a breakdown of the types of haemorrhage for the 468 recipients at MRRH whose indication for transfusion was '*bleeding*'. The most frequent bacterial infections among transfused patients were tuberculosis (3.9%) and septicaemia (3.5%). One hundred and nine (24.1%) recipients on the Medical ward had AIDS and 48 (44.0%) of them presented with severe anaemia following anti-retroviral therapy (ART) regimens containing the nucleoside analog reverse transcriptase inhibitor, zidovudine (AZT).

Table 3. Nature of haemorrhage for the 468 patients at MRRH
whose indication for blood transfusion was '*bleeding*'

Type of bleeding	Patients (n; %)
Obstetric haemorrhage	261 (55.7)
Gastrointestinal bleeding	99 (21.2)
Penetrating soft tissue injury	31 (6.6)
Epistaxis	27 (5.8)
Umbilical cord bleeding	22 (4.7)
Cephalhaematoma	15 (3.2)
Others [including Purpura, Haemoptysis, Tooth bud (' <i>false teeth</i> ') extraction]	13 (2.8)

All the 453 transfused patients on the Medical ward received whole blood transfusions. There were five additional units of random donor platelet transfusions in two recipients (0.4%). No other platelet transfusions were given on other wards. Overall, 58.4% of all recipients at MRRH were given whole blood transfusions. Approximately 97.0% of the Paediatric ward recipients were transfused with packed red blood cells (RBCs). Overall a total of 3,673 units of whole blood and RBCs were ordered and out of these, 2,777 units (75.6%) were transfused.

The mean number of units given per recipient was 1.7 and the overall crossmatch-to-transfusion (CT) ratio was 1.3. Three hundred and sixty seven (38.6%) adult transfusion patients received only one unit of blood. Figure 2 shows the utilization of blood at MRRH in each month of 2008. There were no records for compatibility testing and monitoring of vital signs in 51.8% and 97.6% of the recipients' files respectively.

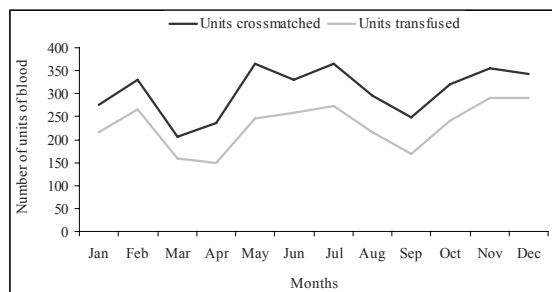


Figure 2 Blood utilization at Mbarara Regional Referral Hospital in each month of 2008. Total units ordered and crossmatched = 3,673; total units transfused = 2,777.

Transfusion start-times were documented in 78.5% of the cases. In 1,208 patients (69.8%), pre-transfusion haemoglobin levels had been estimated and recorded in the hospital files. Absence of pre-transfusion haemoglobin records was strongly associated with transfusions in states of acute blood loss (i.e. bleeding, surgery and trauma) compared to other indications ($p < 0.0001$) and with surplus ordering of blood i.e. CT ratios greater than unity (385/1208 versus 217/522 patients; $p = 0.0001$).

Table 4. Transfusion practices and outcomes in the five wards at MRRH in 2008

Variables assessed in patients' files	OBGY (n=346)	Medical (n=453)	Surgical (n=25)	Paediatric (n=726)	A&E (n=180)
Mean units of blood ordered (range; total)	2.7 (1-13; 920)	3.1 (1-20; 1421)	2.0 (1-3; 50)	1.7 (1-7; 893)	2.2 (1-10; 389)
Crossmatch-to-transfusion (CT) ratio	1.7	1.3	1.8	1.0	1.5
Compatibility form present in file	167 (48.3)	266 (58.7)	16 (64.0)	271 (37.3)	114 (63.3)
Record of pre-transfusion haemoglobin	181 (52.3)	371 (81.9)	19 (76.0)	544 (74.9)	93 (51.7)
Record of patients' vital signs	1 (0.3)	25 (5.5)	1 (4.0)	14 (1.9)	0 (0)
Record of transfusion start-times	241 (69.7)	440 (97.1)	14 (56.0)	526 (72.3)	137 (76.1)
Record of adverse events	2 (0.6)	5 (1.1)	0 (0)	2 (0.3)	1 (0.6)
Additional transfusions after ≥ 48 hour	15 (4.3)	62 (13.7)	0 (0)	98 (13.5)	5 (2.8)

Data presented as number (n) and % unless stated otherwise

Acute transfusion reactions were observed in ten patients (0.6%) in twelve months. In 180 recipients (10.4%), additional blood transfusions were given 48 hours or more after completion of the initial transfusion episode and this was not significantly associated with any transfusion

indication. A comparison of the transfusion practices and outcomes in the five wards at MRRH is shown in Figure 3 and Table 4.

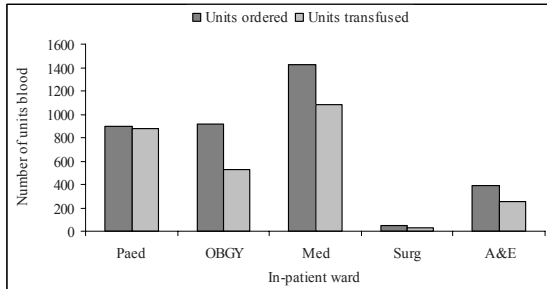


Figure 3 Blood ordering and transfusion practices in five wards at Mbarara Regional Referral Hospital (Paediatric, Obstetric & Gynaecological [OBGY], Medical, Surgical, and Accident & Emergency [A&E]) in 2008. Total units of blood ordered = 3,673; total units transfused = 2,777; overall crossmatch-to-transfusion ratio = 1.3.

DISCUSSION

In a period of twelve months, 2,777 units of whole blood and RBCs were transfused to 1,674 patients on the five major wards at MRRH. Records at MRRH showed that there were approximately 16,000 patients admitted in 2008. Therefore, 10.5% of all in-patients at MRRH received blood transfusion support as part of their treatment and care. Of these, 42.0% were children admitted with severe anemia largely due to malaria (76.6%). Rates of blood transfusion for malaria were highest in the two malaria seasons of May - August and November - February (Figure 1). The overall CT ratio was 1.3 indicating that there was no significant wastage of blood resources at MRRH in 2008. However, there might have been shortages of blood for transfusion since 367 (38.6%) adult patients received only one unit of blood. Due to the absence of records for post-transfusion haemoglobins and/or haematocrits, we were unable to determine rates of under-transfusion or over-transfusion. The low number of blood recipients on the Surgical ward is explained by the fact that 109 (6.5%) patients received blood transfusions at the A&E ward following surgery or trauma.

Our data indicate that a significant number of children received blood transfusions because of severe malarial anaemia compared to adult patients. This was expected because malaria is a leading cause of morbidity and death in childhood in Sub-Saharan Africa (WHO, 2009). In contrast to children, adults were transfused mainly because of bleeding and bacterial or viral infections ($p < 0.0001$). While the CT ratios for both the Medical and Surgical sections of MRRH were within normal limits (Table 4), surgeons (CT ratio; 1.8) and obstetricians (CT ratio; 1.7)

ordered significantly more units of blood that were not transfused ($p<0.0001$). However, more patients on the Medical ward received additional transfusions 48 hours or more after completion of the initial transfusion episodes as compared to all the other wards combined ($p<0.0001$). The fact that a total of 180 patients (10.4%) at MRRH received additional blood transfusion(s) 48 hours or more after completion of the initial transfusion episode(s) may partly suggest that there could have been shortage of blood for immediate additional transfusions; that there might have been delays in post-transfusion evaluation by the clinical and/or laboratory staff or that the patients could have experienced delayed haemolytic transfusion reactions. Since there are no pre- or post-transfusion alloantibody detection tests carried out at MRRH (and in the whole of Uganda), it is difficult to examine the occurrence of haemolytic transfusion reactions in this setting. However, 33 (18.3%) of such patients had been transfused primarily because of bleeding problems and the bleeding might have recurred.

There were inadequacies regarding the documentation of pre-transfusion tests and bedside monitoring of the transfused patients: there were no records for pre-transfusion haemoglobin levels, compatibility testing, transfusion start-times, and vital signs in 30.2%, 51.8%, 21.5% and 97.6% of all recipients respectively. Missing pre-transfusion haemoglobin records were strongly associated with transfusions in states of acute blood loss i.e. following bleeding, trauma and surgery compared to other indications ($p<0.0001$) and with surplus ordering of blood i.e. CT ratios >1.0 ($p=0.0001$). Thus, in the absence of haemoglobin results, clinicians made decisions to transfuse basing on clinical symptoms and signs. However, Bates *et al.* (2001) reported that when clinicians relied entirely on clinical judgement to guide transfusion practice, significant numbers of inappropriate transfusions were observed at a district hospital in Malawi. Our data also indicate that there were limitations on the availability of blood components (apart from packed RBCs) for transfusion at MRRH. This was illustrated by the finding that on the Medical ward, for example, all recipients were given whole blood transfusions except two patients (0.4%) that received additional five units of random donor platelets. Yet on the same ward, 7.7% and 23.4% of the recipients respectively had haematological malignancies and bleeding problems and might have required transfusions with platelet concentrates and/or fresh frozen plasma if available. Almost half of the blood recipients with AIDS on the Medical ward had AZT-related anaemia. This underscores the important role of blood transfusions in this category of patients, given a 5.4% national prevalence of HIV infection among adults (UNAIDS, 2008) plus the

increasing availability and use of ART. Ten patients (0.6%) were reported to have developed immediate transfusion reactions during transfusion. Apart from the record in the case notes that *'the patient reacted and blood was stopped'*, there were no details on the nature of these transfusion reactions and on their management. Although we did not have comprehensive data from other hospitals in Uganda, the inadequacies herein outlined most likely apply to other clinical settings in the country. Similarly, Kajja *et al.* (2008) and de Graaf *et al.* (2009) have respectively observed poor blood ordering practices and lack of appropriate guidelines for blood transfusion at Mulago National Referral Hospital in Kampala, Uganda.

In conclusion, there is need to improve the clinical practice of Transfusion Medicine in Uganda. It is therefore recommended that an in-hospital quality management system for blood transfusion should be developed through the creation of awareness on appropriate clinical use of blood. A standard form (a 'Blood Transfusion Form') for documenting the entire transfusion process should be designed. This would be incorporated in patients' hospital files to allow easy monitoring and evaluation of blood transfusions and to provide a basis for the development of evidence based clinical transfusion practice in Uganda. To increase the availability of platelets and other blood components, arrangements should be made to process whole blood locally at the regional blood banks. Currently, platelets are only processed and supplied from the UBTS headquarters - about 300 kilometres away - in Kampala. The UBTS should encourage the design and implementation of clinical guidelines for the appropriate use of blood as well as the set up of Hospital Transfusion Committees or clinical review groups. The latter will monitor and evaluate the usage of blood and transfusion outcomes so as to improve the overall quality of clinical transfusion practice in Uganda. Above all, there is need to train nursing, biomedical and medical students and clinical staff on the significance of safe and appropriate blood transfusions in the management and care of patients.

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**RED BLOOD CELL
ALLOIMMUNIZATION IN
SICKLE CELL DISEASE PATIENTS
IN UGANDA**

Bernard Natukunda, Henk Schonewille, Christopher Ndugwa and Anneke Brand. Red blood cell alloimmunization in sickle cell disease patients in Uganda. *Transfusion*, 2010;50:20-25.

ABSTRACT

BACKGROUND: Blood transfusion is an integral part in the management of sickle cell disease (SCD) patients. Alloimmunization is a recognized complication of red blood cell (RBC) transfusions with consequences including delayed hemolytic transfusion reactions and difficulties in getting compatible blood for future transfusions. The objective of this study was to determine the frequency of RBC alloimmunization in SCD patients in Uganda where pre-transfusion screening for alloantibodies is not practiced.

STUDY DESIGN AND METHODS: In a cross-sectional study, SCD patients at Mulago Hospital Sickle Cell Clinic, Kampala, Uganda, were investigated. The demographic characteristics and transfusion history were recorded. Blood samples were drawn from consenting, previously transfused patients and RBC alloimmunization was demonstrated using immunohematological techniques.

RESULTS: There were 428 patients (median age, 12; F/M ratio, 1.0) and they had received a median 3 units in a median 3 transfusion episodes. Twenty six patients (6.1%) possessed RBC alloantibodies and 21 (80.7%) of them had received up to 10 transfusions. A total of 30 alloantibodies was found; 20 (66.7%) and 5 (16.6%) belonged to Rh and MNS blood groups respectively. Five of the alloimmunized patients had multiple antibodies.

CONCLUSION: The rate of RBC alloimmunization in Ugandan SCD patients was 6.1%. The homogeneity between donors and SCD patients plus the low transfusion load may explain this immunization frequency. Nevertheless, our study confirms the significance of RBC alloimmunization as a complication in Ugandan SCD patients. Therefore, there is need to improve immunohematological testing in Uganda so that RBC alloimmunization and its consequences may be prevented.

Keywords: Alloimmunization, Sickle cell disease, Red blood cell, Uganda.

INTRODUCTION

Sickle Cell Disease (SCD) is the most common genetic disease in Uganda where about 5 million people (20% of the total population) have the sickle cell trait and about 25,000 children are born with the disease each year.¹ Blood transfusion is an important therapeutic tool in the management of SCD: it increases the oxygen carrying capacity of the blood by increasing the hemoglobin concentration and decreasing the percentage of sickle hemoglobin by dilution.² However, red blood cell (RBC) alloimmunization is one of the complications of allogeneic blood transfusions and in general the risk increases with the number of blood transfusions, although many patients become alloimmunized early during transfusion therapy.³ Various frequencies of RBC alloimmunization in SCD patients ranging from 2.6-76% have been reported in a number of studies.⁴⁻¹⁶

Alloimmunization may limit the availability of compatible blood for future transfusions and can contribute to perinatal morbidity due to hemolytic disease of the newborn.¹⁷ In addition, delayed hemolytic transfusion reactions can mimic a sickle cell crisis and may be responsible for major morbidity in the SCD patient.^{10, 16, 18} In the Cooperative Study of Sickle Cell Disease in the United States, multiple antibodies were detected in over 50% of alloimmunized subjects.⁸ With time, many of the antibodies become undetectable, potentially confounding future transfusions and placing the patient at risk of anamnestic antibody production and delayed hemolytic transfusion reactions.^{2, 19} The antigens most frequently involved belong to the Rh, Kell, Kidd, Duffy, Lewis and MNS blood group systems.^{7, 8, 10, 11, 13, 20 - 22} Factors implicated in RBC alloantibody formation include recipient gender and age, history of pregnancy, number and timing of blood transfusions, recipient clinical diagnosis and treatment, genetic factors related to the antigenic response, and racial differences between donors and recipients.^{7, 8, 20, 23 - 25}

The prevalence of post transfusion alloimmunization in Uganda, where blood donors and SCD patients are more racially homogeneous and where pretransfusion testing is only limited to ABO/D grouping plus a room temperature saline cross-match, is not known. The objective of this study was to determine the frequency and nature of RBC alloimmunization in SCD patients in Uganda.

MATERIALS AND METHODS

Patients

In a cross-sectional study, patients with homozygous SCD attending the Sick Cell Clinic (SCC) at Mulago National Referral Hospital in Kampala, Uganda, were investigated. The study took place between 1st February and 31st July, 2008. Informed consent was obtained from the patients or their parents/guardians. Eligibility criteria included SCD patients who were at least 2 years of age and had received at least 2 previous allogeneic blood transfusions – the last transfusion episode being longer than 2 weeks before enrollment into the study. These criteria were chosen so as to study a group of patients that were most likely to have become alloimmunized at an appropriate age and time after exposures to RBC antigens. In general, patients received packed RBC transfusions compatible with their ABO and D phenotypes and which were not leukoreduced.

Data collection

Records at the SCC regarding the recruited SCD patients were reviewed for their demographic characteristics and the transfusion history. In cases of incomplete or missing records, older patients or accompanying parents and relatives were asked for additional information on the above history. The number of transfusion episodes, number of units of blood transfused, date of transfusion, indication for transfusion, age of first transfusion and a history of pregnancy were recorded in a data collection form. For patients who were first transfused in childhood and could not recall their exact age at first transfusion, we entered the age of *3 years* in the database for analysis. The study was approved by the research and ethical committees at Mbarara University of Science and Technology and Makerere University Medical School.

Laboratory investigations

After consent, blood was drawn for laboratory investigations. Frozen plasma and buffy coat samples were shipped to the Sanquin Blood Bank in Leiden, The Netherlands, for immunohematological studies. The plasma samples were screened for the presence of RBC alloantibodies by use of a standard 3-cell panel of reagent group O RBCs. For the indirect antiglobulin test (IAT), a LISS-enhanced gel centrifugation technique (DiaMed ID, Micro Typing System, Cressier sur Morat, Switzerland) with polyspecific antihuman globulin (rabbit anti-IgG and monoclonal anti-C3d) was used. When the antibody screening was positive,

antibody identification was performed by testing the plasma samples with commercial panels of reagent RBCs of selected phenotypes. Patients were considered to be alloimmunized if antibodies to one or more RBC antigens were identified. DNA was extracted from buffy coat samples (using the QIAamp DNA Blood Midi Kit, Qiagen) of patients who possessed anti-D alloantibodies and D genotyping using an *RHD* multiplex polymerase chain reaction (PCR) was performed as described by Maaskant-van Wijk and co-workers.²⁶

Statistical methods

Statistical software packages Excel 5.0 (Microsoft, Redmond, CA, USA) and Statistical Package for the Social Sciences 12.0 (SPSS Inc., Chicago, IL, USA) were used for data management and analysis respectively. For univariate analysis of possible associations between alloimmunization and age at the time of enrollment, age at first transfusion, gender, pregnancy history, number of transfusion episodes, number of units received and the indication for transfusion, the Chi-square test or Fisher's exact test were used for discrete variables. Logistic regression analysis was used for continuous variables of a non-Gaussian distribution. Groups were assumed to differ significantly when the probability level was less than 0.05.

RESULTS

Patient data

We recruited a total of 428 transfused SCD patients during the study period. Of these, 217 (51%) were females and among them, 19 (8.8%) had a history of pregnancy. The median age at the time of blood draw was 12 (range, 2-44) years. The patients were transfused with a total of 3,366 (median, 3; range, 2-100) units of blood in 2,463 (median, 3; range, 2-80) transfusion episodes. Twenty six patients (6.1%; 95% CI: 4.0-9.0%) were found to be alloimmunized to RBC antigens; 21 (80.7%) of them having received up to a maximum of 10 blood transfusions. There were 57 patients (13.3%) who had been transfused in childhood and could not recall their exact time when they were first transfused and an age of 3 *years* was used in the analysis as their *age of first transfusion*. The number of units of blood transfused was significantly associated with the rate of alloimmunization ($p=0.02$). There was a trend towards statistical significance between the number of transfusion episodes and the rate of RBC alloimmunization ($p=0.08$). Other demographic and transfusion characteristics of alloimmunized patients were not significantly different to those in the non-immunized group (Table 1).

TABLE 1: Demographic and transfusion characteristics of SCD patients in Uganda

	Alloimmunized patients	Non-immunized patients	<i>p</i> -value
Patients (n, %)	26 (6.1)	402 (93.9)	
Female-to-male ratio	1.8	1.0	NS
Age in years	13 (2-35)	12 (2-44)	NS
History of pregnancy (%)	11.8	8.5	NS
Age of first transfusion < 10years (%)	80.8	89.1	NS
Transfusion episodes	3.5 (2-32)	3 (2-80)	0.08
≤ 10 transfusion episodes (%)	80.7	90.2	NS
Units of blood transfused	5 (2-60)	3 (2-100)	0.02
History suggestive of a 'febrile illness' at the time of transfusion (%)	73.1	70.8	NS
Number of years since the last transfusion	1.0 (0-8)	1.0 (0-8)	NS
Number of years between first and last transfusions	6.0 (0-22)	4.0 (0-41)	NS

Data are presented as median and range unless otherwise stated, NS = *p*-value ≥ 0.1

RBC antibodies

The 26 alloimmunized patients produced a total of 30 RBC alloantibody specificities. This implies that after transfusion with 3,366 RBC units, the alloimmunization rate was 0.9% per RBC unit transfused. Two of the patients possessed panreactive antibodies. Table 2 shows the specificities of the antibodies identified, with 20 (66.7%) belonging to the Rh blood group system. MNS was the next frequent blood group system involved contributing 5 (16.6%) alloantibodies, 4 (80%) of these being of anti-S specificity. Eleven of the alloantibodies (36.7%) presented as multiple antibody combinations. Of the immunized patients with specific antibodies, 19 (79.2%) produced only one antibody while 5 (20.8%) had multiple antibodies.

D genotyping

Seven of the allommunized patients possessed anti-D antibodies and their RHD genotyping revealed the following results: one was D negative; five had partial D (i.e. four patients were of category D^{Va} and one was a probable category D^{IIIb}) and the last had a probable Rh D pseudogene. The D negative individual was a 5-year old girl who had been transfused four times

in the previous two years while the patient with a probable Rh D pseudogene was an 11-year old boy with a history of three blood transfusions since the age of 1 year. Both patients had been typed as D negative by serology. Most likely, they received D positive RBC transfusions despite the local transfusion policy of matching for the D antigen.

TABLE 2: Specificities of RBC alloantibodies identified in 26 SCD patients in Uganda

Blood group system	RBC alloantibody specificity	Number of antibodies (respectively)
Rh	E, D, C, C ^w	10, 7, 2, 1
MNS	S, M	4, 1
Kidd	Jk ^a	2
Kell	K	1
Duffy	Fy ^a	1
Lewis	Le ^a	1
N.A.	Panreactive	2

N.A. = not applicable

DISCUSSION

This is the first ever study carried out to determine the frequency and nature of RBC alloimmunization following blood transfusions in Uganda. It also involves one of the largest numbers of SCD patients ever investigated for RBC alloimmunization, in a cross-sectional survey. We observed an RBC alloimmune response rate of 6.1% in 428 SCD patients (or 0.9% per RBC unit transfused) and multiple antibodies were present in 20.8% of immunized patients with specific alloantibodies. Two patients possessed panreactive antibodies 5 years after their last transfusions. However, we could not rule out the presence of post-transfusion autoimmunization because we lacked autologous RBCs and DATs were not done. The number of immunized patients is lower than that reported in most of the literature on RBC alloimmunization in SCD.^{4, 5, 8-11, 13, 15} The presumed high phenotypic compatibility between blood donors and SCD patients (who were black Ugandans in both cases) and the low transfusion load may explain the low rate in RBC alloantibody formation. Studies need to be

performed to confirm the phenotypic similarities between blood donors and SCD patients in Uganda. The 6.1% rate of alloantibody formation in Ugandan SCD patients is comparable with the 2.6% alloimmunization reported by Olujohungbe *et al.*¹⁴ in a Jamaican cohort of SCD patients where there was less heterogeneity among donors and patients. It also compares well with the 5.3% frequency of RBC alloimmunization reported in a study by Sarnaik *et al.*¹¹ in which children with SCD, who were not on a prophylactic transfusion program and had received a low transfusion load, became alloimmunized. The findings in this study show that the rate of RBC alloimmunization was associated with the number of units of blood transfused and the number of transfusion episodes, although the association was not statistically significant in the latter case (the calculated p values were 0.02 and 0.08 respectively). These findings are consistent with previous reports which have shown an association between RBC alloimmunization and increased number of donor exposures.^{4, 8 - 10}

The SCD patients mainly received acute simple RBC transfusions and they were not heavily transfused (median number of units of blood transfused = 3) compared to their counterparts in the developed world who may be on chronic transfusion programs. Most patients (71%) and/or their parents described having presented with symptoms of fever, body pains, general weakness or severe anemia at the time of hospital admission and prior to blood transfusion. Unfortunately, the true picture of this 'febrile illness' could not be ascertained from the available medical records but it might have been a sickle cell painful crisis, a delayed hemolytic transfusion reaction (since 11.2% of the patients received repeat transfusions within 2 weeks of a prior transfusion episode), a bacterial infection or even malaria. The role of such an underlying pathophysiology vis-à-vis the rate of alloimmunization needs to be explored in a future prospective study. Recently, inflammation was reported to be associated with increased RBC alloimmunization, in a murine model²⁷. Owing to the fact that the patients were not being monitored for alloantibody formation, the rate of RBC alloimmunization may actually be higher than what was observed. This being a cross-sectional study, some RBC antibodies may have been missed because up to 25% of alloantibodies have been reported to disappear within a median 10 months of follow up.¹⁹

Eighty percent of the detected alloantibodies corresponded to the Rh and MNS system antigens C, D and E; and S respectively (Table 2). Interestingly, 7 (23.3%) of the patients formed anti-D alloantibodies notwithstanding the local clinical transfusion practice in which ABO/D group

compatible blood is transfused. Moreover, 5 (71%) of the patients who produced anti-D were females within the age range of 5 – 19 years and with no history of pregnancy. Since some of these patients had been typed as D positive by serology, we decided to investigate the molecular bases underlying these observations. Using an *RHD*-specific multiplex PCR, D genotyping of the 7 SCD patients who were D-alloimmunized revealed that one of them was D negative, five had partial D (D^{Va} in four patients and a probable D^{IIIb} in the other), and the last one had a probable Rh D pseudogene (D negative by serology; all amplified exons present). The patient who was probably D^{IIIb} had all the six *RHD* exons (3, 4, 5, 6, 7 and 9) tested and had been found to be D positive by serology. We could not proceed to perform DNA sequencing for confirmation of the probable RhD pseudogene or the D^{IIIb} category because there was no more DNA available. These findings underscore the need to use monoclonal anti-D reagents that are capable of detecting D variants among blood donors and recipients and to improve the standards of immunohematological testing in Uganda. The antibodies encountered in other series^{3, 4, 5, 10, 28} have most commonly been of C, E or K specificities. To prevent alloimmunization in SCD patients in the United States and Europe, the standard practice is to perform antigen matching for C, E, and K antigens for patients without prior alloantibody formation.²⁹ Our findings indicate that anti-K is rare (3.3%) while anti-S is more common (13.3%) among alloimmunized SCD patients in this study. This is presumably because of a difference in the distribution of Kell and MNS phenotypes in Caucasian and Black populations. Accordingly, anti-S should be borne in mind in case a program of *limited* phenotype matching (i.e. for C, E and S antigens) to improve the care of already alloimmunized SCD patients in Uganda is to be considered in future.

The effects of RBC alloimmunization in SCD patients may be examined in the context of the policy on laboratory and clinical transfusion practice in Uganda. This study has revealed the presence of clinically significant IgG alloantibodies in plasma of transfused SCD patients. Transfusion-acquired antibodies have been implicated in immediate and delayed transfusion reactions;^{2, 10, 16} some patients with multiple antibodies are difficult to cross-match and to transfuse;^{8, 21} others develop autoantibodies in addition to being alloimmunized;^{28, 30} and five nulliparous females in the present study were alloimmunized to the D antigen. The current transfusion practice in Uganda does not involve the detection or monitoring of alloantibody formation and the clinical consequences thereof. Besides ABO/D grouping and a saline cross-match at room temperature, no other compatibility testing is performed. Therefore, we

recommend a change in the policy of the Uganda Blood Transfusion Service to include laboratory and clinical guidelines on the prevention and management of immunological complications of allogeneic blood transfusions, including RBC alloimmunization in SCD patients.

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**PREVALENCE AND SPECIFICITIES OF
RED BLOOD CELL ALLOANTIBODIES
IN TRANSFUSED UGANDANS
WITH DIFFERENT DISEASES**

Bernard Natukunda, Henk Schonewille, Leo van de Watering and Anneke Brand. Prevalence and specificities of red blood cell alloantibodies in transfused Ugandans with different diseases. *Vox Sanguinis*, 2009;98:167-171.

Abstract

Background and Objectives: Alloantibody formation against red blood cell (RBC) antigens is a common complication of transfusion therapy. However, the prevalence of RBC alloimmunization is hardly known in Black Africans. In Uganda, the practice is to transfuse ABO/D compatible blood without screening for immune antibodies. The aim of this study was to determine the prevalence and specificities of RBC alloantibodies in transfused Ugandans with different diseases.

Materials and methods: Using a cross-sectional design, transfused patients at Mulago Hospital in Kampala, Uganda, were investigated. Demographic characteristics and transfusion histories were recorded. EDTA blood samples were obtained from consenting patients and RBC alloimmunization was demonstrated using standard immunohematological tests.

Results: A total of 214 transfused patients (mean age, 30.3 years; F/M ratio, 1.0) were investigated. Thirteen patients (6.1%) possessed RBC alloantibodies whose specificities were 6 anti-E; 3 anti-S; one each of anti-D, -K and -Le^a; and two samples were pan-reactive. Eleven (84.6%) of the alloimmunized patients had experienced up to 10 transfusion episodes. The number of units of blood transfused and the transfusion episodes were significantly associated with the RBC alloimmunization rate ($p=0.01$).

Conclusions: The prevalence of RBC alloimmunization in transfused Ugandans was 6.1% and was associated with the number of donor exposures. This immunization rate is similar to that observed in Caucasians, although patients with malaria were less likely to develop RBC antibodies. Alloantibodies were mainly against E and S antigens. We recommend the introduction of pre-transfusion antibody tests in Uganda depending on the recipient's diagnosis.

Key words: RBC alloantibodies, Transfusion, Cancer, Malaria, Uganda.

Introduction

Red blood cell (RBC) alloimmunization is an important adverse effect that follows repeated transfusions with allogeneic blood [1]. It results from an immune response due to the genetic differences between blood donors and recipients. Immune anti-RBC antibodies are generally formed early in the course of multiple transfusions, usually before the 10th transfusion [2, 3]. In patients with disorders that often require multiple blood transfusions the rate of RBC alloimmunization has been reported in the range of 5 - 30% [2 - 10]. This range is even wider (3-76%) in patients with hemoglobinopathies [11 - 15]. Risk factors for RBC alloimmunization include female sex, a history of pregnancy, recipient clinical diagnosis and treatment, and racial differences between transfusion recipients and blood donors [16 - 18].

Patients receiving chemotherapy for myeloproliferative disorders do not seem to be suppressed in terms of their ability to produce blood group alloantibodies. However, patients with lymphoproliferative disorders are generally characterized by an impaired immunologic response and therefore alloimmunization to RBC antigens following multiple transfusions is less common [2, 3, 19]. Absence or low rates of alloimmunization have been reported in some D negative AIDS patients receiving D positive RBC transfusions and in multiply transfused AIDS patients with AZT-associated anemia [20, 21]. Some reports indicate that multitransfused females register higher rates of alloantibody formation than males [18]. However, studies by Blumberg *et al.* [19] and Redman *et al.* [6] showed no difference in gender regarding alloimmunization rates. RBC alloimmunization is generally detected when patients need a blood transfusion. During pre-transfusion testing, the patient's blood sample is usually screened for unexpected alloantibodies [24]. Antibodies against antigens of the Rh and Kell blood group systems are the specificities most frequently found in alloimmunized patients in Western Europe and the United States [3, 5, 6, 9]. In Uganda, no such pre-transfusion screening for immune antibodies is carried out on blood transfusion recipients. The aim of this study was to determine the prevalence and specificities of RBC alloantibodies in transfused Ugandan patients with different diseases.

Materials and methods

Patients

Using a cross-sectional design, transfused patients with different diseases admitted at Mulago National Referral Hospital in Kampala, Uganda, were investigated. The initial part of the study

involved patient recruitment and blood sample collection and took place between 1st February and 31st July, 2008. Eligibility criteria included inpatients that were at least 2 years of age and had received at least 2 previous allogeneic blood transfusions – the second transfusion episode being more than 2 weeks before enrolment into the study. This was intended to enrol a group of participants that were likely to have become alloimmunized at an appropriate age and time after exposures to blood transfusions. Patients with hemoglobinopathies were being investigated in a parallel study and were excluded. Obstetric patients were also excluded from this study. In general, patients received ABO/D compatible and non-leucocyte depleted whole blood or packed RBC transfusions.

Data collection

Patients' charts were reviewed for demographic characteristics and the transfusion history. In cases of incomplete or missing records, patients or their attendants were asked for additional information on the above history. The number of transfusion episodes, number of units of blood transfused, date of transfusion, indication for transfusion, age of first transfusion and for oncology patients whether they had received anti-cancer chemotherapy; were recorded in a data collection form. The study was approved by the research and ethical committees at Mbarara University of Science and Technology, Mbarara, Uganda, and Makerere University Medical School, Kampala, Uganda.

Laboratory investigations

After consent, blood was drawn into ethylenediaminetetraacetic acid (EDTA) tubes for laboratory investigations. Plasma and buffy coat samples were removed and stored frozen at the Joint Clinical Research Center (JCRC) in Kampala, Uganda, until they were shipped to the Sanquin Blood Supply in Leiden, The Netherlands, for immunohematological studies.

Plasma samples were screened for the presence of RBC alloantibodies by use of a standard 3-cell panel of reagent group O RBCs. In the indirect antiglobulin test (IAT), a LISS-enhanced gel centrifugation technique (DiaMed ID, Micro Typing System, Cressier sur Morat, Switzerland) with polyspecific antihuman globulin (rabbit anti-IgG and monoclonal anti-C3d) was used. When the antibody screening was positive, antibody identification was performed by testing the plasma samples with commercial panels of reagent RBCs, of selected phenotypes, by similar or additional methods whenever needed. Patients were considered to be alloimmunized if

antibodies to one or more RBC antigens could be identified. For one patient who possessed anti-D alloantibodies, an RHD multiplex PCR was carried out to determine the genotype and hence the molecular basis of this observation.

Statistical methods

Statistical software packages Excel 5.0 (Microsoft, Redmond, CA, USA) and Statistical Package for the Social Sciences 12.0 (SPSS Inc., Chicago, IL, USA) were used for data management and analysis respectively. For univariate analysis of possible associations between RBC alloimmunization and gender, history of pregnancy, whole blood transfusion, diagnosis of malignancy, anti-cancer therapy and HIV positivity, the Chi-square test or Fisher's exact test were used. Logistic regression analysis was used for continuous variables of a non-Gaussian distribution i.e. the age at the time of enrolment, the number of units of blood transfused, the number of transfusion episodes and the number of years since the last transfusion. Groups were assumed to differ significantly when the probability level was less than 0.05.

Results

Patient data

We recruited 214 transfused inpatients at Mulago National Referral Hospital during the study period. Of these, 113 (52.8%) were females and among them, 77 (68.1%) had a history of pregnancy. The mean age at the time of blood collection was 30.3 (median, 29.5; range, 2-80) years. The patients had been transfused with a total of 1,869 (mean, 8.7; median, 5.0 and range, 2-65) units of blood in 1,285 (mean, 6.0; median, 4.0 and range, 2-57) transfusion episodes. Half of the patients studied (108; 50.6%) had malignant disorders while 62 (29%) had infectious diseases (Table 1).

Thirteen patients (6.1%; 95% CI: 3.0-10.0%) were found to be alloimmunized to RBC antigens; 11 (84.6%) of them having experienced up to a maximum of 10 transfusion episodes. The number of units of blood transfused and the number of transfusion episodes were significantly associated with the rate of alloimmunization ($p=0.01$). Other demographic and transfusion characteristics of alloimmunized patients were not significantly different to those in the non-immunized group (Table 2).

Table 1: Disease groups of the 214 Ugandan transfused patients and proportions of those who were alloimmunized in each group

Disease group	Patients (n; %)	RBC Alloimmunized (n; %)
<i>Malignancies</i>	<i>108 (50.6)</i>	<i>9 (8.3)</i>
• Hematologic	64	4
• Solid tumors	44	5
<i>Infectious diseases</i>	<i>62 (29.0)</i>	<i>2 (3.2)</i>
• Malaria	34	1
• AIDS	24	1
• Bacterial infections	4	0
<i>Others</i>	<i>44 (20.4)</i>	<i>2 (4.5)</i>
• Gastrointestinal disease	12	1
• Renal disease	10	0
• Trauma	10	0
• Heart disease	7	1
• Diabetes mellitus	3	0
• Burns	2	0

RBC antibodies

Eleven of the alloimmunized patients produced a total of 12 RBC alloantibodies of known specificity. The remaining two patients possessed pan-reactive antibodies. The specificities of the alloantibodies identified were: anti-E, 6; anti-S, 3; and 1 each of anti-D, -K and -Le^a. In one patient (9.1%), two alloantibodies, anti-E plus anti-K, presented as a combination; the rest of the alloantibodies were as single specificities. D genotyping on DNA isolated from the buffy coat of the patient whose plasma contained alloanti-D revealed that this patient had partial D of the R₀^{Har} category.

Discussion

This cross-sectional study was undertaken to determine the prevalence and specificities of RBC alloantibodies in Ugandan patients who received blood transfusions. Out of 214 patients studied, 13 (6.1%; 95% CI: 3.0-10.0) possessed RBC alloantibodies. This overall RBC alloimmunization

prevalence is comparable with rates previously reported on patients receiving transfusion support for chronic renal failure and hematological disorders in developed countries [2, 3, 5-10]. The antibody specificities (anti-E, 6; anti-S, 3; anti-D, 1; anti-K, 1; anti-Le^a, 1; and 2 pan-reactive) are also similar to the ones commonly detected and reported [3, 6, 9]. The only difference is the finding of a slight increase in the rate of alloimmunization against the S antigen.

Table 2: Demographic variables and transfusion characteristics of alloimmunized and non-immunized Ugandan patients who received blood transfusions

	Alloimmunized (n = 13)	Non-immunized (n = 201)	<i>p</i> -value
Mean age in years (median; range)	34.8 (35.0; 2-75)	30.0 (28.0; 2-80)	NS
Sex ratio (F/M)	1.6	1.0	NS
History of pregnancy (%)	62.5	67.6	NS
Mean transfusion episodes (median; range)	11.8 (4.0; 2-57)	5.6 (4.0; 2-50)	0.01
≤ 10 transfusion episodes (%)	84.6	90.5	NS
Mean units of blood transfused (median; range)	16.2 (8.0; 2-65)	8.3 (5.0; 2-60)	0.01
Transfusion with whole blood (%)	92.3	88.1	NS
Patients with malignancy (%)	69.2	46.2	NS
Patients received anti-cancer chemotherapy (%)	55.6	50.5	NS
HIV positive patients (%)	23.1	30.3	NS
Mean years since the last transfusion (median; range)	2.54 (0; 0-29)	1.36 (0; 0-49)	NS

NS = *p*-value > 0.05

Also, we recently found an increased number of alloantibodies (13.3% of all antibody specificities identified) with anti-S specificity in a parallel study on RBC alloimmunization in Sick Cell Disease patients in Uganda [unpublished data]. There was a mixture of antibodies (anti-E and -K) in one of the alloimmunized patients which is comparable to the frequency of multiply alloimmunized patients in other studies [2, 9]. The patient who was alloimmunized to the D antigen – a 50 year old female with no history of pregnancy – had apparently been typed as D positive by serology. Using an *RHD* multiplex PCR [25], D genotyping was carried out on this patient and a partial D of the R₀^{Har} category was found. This emphasizes the requirement that transfusion laboratories should be supplied with blood grouping reagents that can detect D variants among blood donors (typed as D positive) and patients (typed as D negative).

Analysis of the transfusion characteristics revealed that the rate of RBC alloimmunization was significantly associated with the number of units of blood received and the number of transfusion episodes ($p=0.01$ in both cases) i.e. the alloimmunization risk increased with the number of donor exposures. This observation is in agreement with retrospective analyses by Fluit *et al.* [3] and Schonewille *et al.* [9]. According to Blumberg *et al.* [2], although the formation of RBC alloantibodies is influenced by the number of transfusions, most of the alloimmunization occurs early in the course of blood transfusion. This was also the case in 84.6% of alloimmunized patients in the present study. Female sex and a history of pregnancy were not associated with RBC alloimmunization. Our data indicate that 9 (8.3%) of 108 patients with malignant disorders became immunized to RBC antigens following blood transfusion and this prevalence is comparable with what has been observed in other studies on RBC alloimmunization in oncologic disorders [9]. Malaria is a serious disease and patients usually present with hemolytic anemia that may be severe. The effect of malaria infection on the pathogenesis of RBC alloimmunization remains to be elucidated. Recent reports by Hendrickson *et al.* demonstrated suppression of RBC alloimmunization by a bacterial endotoxin lipopolysaccharide (LPS) and an enhanced magnitude of RBC alloimmunization in a setting of viral-like inflammation, in murine models [26, 27]. In Uganda, severe malaria is a common indication for blood transfusion therapy and the occurrence of RBC alloantibodies in malaria patients in the present study was anticipated. However, only one patient (2.9%) was immunized in 34 transfused patients with severe malaria infection. These data suggest that there seems to be no enhanced RBC alloimmunization in patients with malaria. Absence of alloimmunization as a result of immunosuppressive effects of AIDS or anti-cancer chemotherapy has been reported in previous studies [20, 28]. However, in this study 5 (55.6%) of the 9 alloimmunized patients with cancer had been receiving anti-cancer chemotherapy. Also, one patient with AIDS – a 45 year old female who had received 11 units of whole blood in 3 transfusion episodes – was alloimmunized to the E antigen. However, the fact that anti-E may sometimes be a naturally occurring antibody [29] cannot be excluded in this case.

Thus, alloantibody formation remains a notable adverse effect of blood transfusion in the provision of clinical care even in a setting of immunosuppression, especially for cancer patients. This emphasizes the important role played by eliciting a good clinical history of previous blood transfusion(s) and routine laboratory screening for the presence of unexpected antibodies before transfusion. However, the implementation of routine antibody screening for all transfusion

recipients in Uganda and in most of Sub-Saharan Africa is not currently feasible. This is because of the extra costs involved in the monthly procurement of reagent cell panels; the increased work load and hence additional staffing requirements for hospitals and transfusion services; the apparently low RBC alloimmune response rates in most of the transfusion recipients; the lack of organized administrative systems for laboratory record keeping and retrieval; and the lack of adequately qualified personnel in the fields of Immunohematology and Transfusion Medicine. In case of patients with malignant diseases, hemoglobinopathies or other disorders likely to require repeated blood transfusions there should be special considerations. For these patient categories that are at higher risk of RBC alloimmunization, we recommend the introduction of a *pre-transfusion typing and screening strategy* [24] comprised of the following three tests: ABO/D grouping of the donor and recipient; IAT screening for irregular RBC antibodies in the recipient's serum (a 3-cell panel); and a saline cross-match between the patient's serum and the donor RBCs at room temperature. Any detected antibodies in the patients can be identified accordingly so that transfusion with antigen-negative blood is given. Regarding other transfusion recipients, we recommend the use of a *complete cross-match* [30] before blood transfusion i.e. incubation of the patient's serum with the donor RBCs and addition of antihuman globulin serum. In so doing, we shall go a long way towards the prevention of morbidity related to immunological complications of blood transfusion in Ugandan patients with different diseases.

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**MATERNAL RED BLOOD CELL
ALLOIMMUNIZATION IN
SOUTH WESTERN UGANDA**

Bernard Natukunda, Godfrey Mugenyi, Anneke Brand and Henk Schonewille. Maternal red blood cell alloimmunisation in South Western Uganda. *Transfusion Medicine*, 2011, 21, 262–266

ABSTRACT

Objectives: To determine the prevalence of RhD negativity and the rate of red blood cell (RBC) alloimmunization in Ugandan pregnant women.

Aim: To identify the frequency and nature of maternal RBC alloimmunization in Uganda.

Background: Haemolytic disease of the fetus and newborn (HDFN) results from maternal alloimmunization following exposure to allogeneic RBCs during pregnancy or blood transfusion. The prevalence of maternal RBC alloimmunization in Ugandans is not known.

Materials and methods: Pregnant women at Mbarara Hospital, South Western Uganda, were investigated in a cross-sectional study. Demographics, transfusion and obstetric histories were recorded. Maternal RBC alloimmunization was demonstrated using immunohaematological techniques.

Results: A total of 2001 pregnant women were recruited; 3.6% of them being RhD-negative. Forty five women (2.2%; 95% CI: 1.6-2.9) were found to be alloimmunized to RBC antigens. There were 38 RBC alloantibodies of known specificity including: anti-S, 12; anti-M, 11; anti-Le^a, 6; anti-D, 4; and 1 each of anti-K, -Fy^b, -Jk^a, -Lu^a, and -Kp^a. In two women (4.4%), there were antibody combinations (anti-M+S and -K+Kp^a). Obstetric history, gestational age and previous immunizing events were not significantly associated with the rate of alloimmunization.

Conclusions: This study revealed a maternal RBC alloimmunization rate of 2.2% which was comparable with findings from a Zimbabwean study where the prevalence was 1.7%. Given the 6.0% prevalence of anti-D among RhD-negative women in our study and the high immunogenicity of the D antigen, programs for preventing anti-D alloimmunization and HDFN in Uganda should be considered seriously.

Key words: Maternal alloimmunization, RBC alloantibodies, Pregnancy, Haemolytic disease of the newborn, Uganda.

INTRODUCTION

Rhesus sensitization was first reported in Africans by Zoutendyk in 1947 when he described three cases in South African Bantu (Jacob *et al.*, 1959). Maternal alloimmunization is defined as the presence of irregular red blood cell (RBC) alloantibodies in the blood of a pregnant woman that can theoretically cause haemolytic disease of the fetus and newborn (HDFN). Virtually all alloantibodies reactive by the indirect antiglobulin test (IAT) have been implicated in HDFN in different populations. Most severe HDFN associated with intrauterine death is reported in women with Rh-D, -c and K alloantibodies (van Kamp *et al.*, 2004; Moise, 2008, Koelewijn *et al.*, 2008). In Caucasians, the D antigen accounts for about 50% of cases of maternal alloimmunization; the remainder is mainly due to incompatibility to K, c, C/G, E, and Fy^a antigens and to low incidence antigens in the Rh, MNS, and Diego blood group systems (Hedde *et al.*, 1993; Moise, 2008, Koelewijn *et al.*, 2008). Before the introduction of anti-D immunoprophylaxis, HDFN due to anti-D affected approximately 1% of all newborns and was responsible for the death of 1 baby in every 2200 births in developed countries (Kumar & Reagan, 2005).

The prevalence of D-negativity varies in different ethnic groups with approximately 15% of Caucasians, 8% of Blacks and 1% of Asians being D-negative (Reid & Lomas-Francis, 2004). A retrospective study in Zimbabwe showed that 191 (0.85%) of 22,493 infants had HDFN; 25 (13.1%) and 163 (85.3%) of these having D- and ABO-HDFN respectively (Mandisodza *et al.*, 2008). However, in Zimbabwe only 3.3% of the population is D negative and anti-D prophylaxis is routinely available (Cakana & Ngwenya, 2000). In a recent study, the prevalence of RhD negative patients at Mbarara Regional Referral Hospital (MRRH) in Mbarara, Uganda, was found to be approximately 6.0% (Natukunda & Smit Sibinga, unpublished observations). No anti-D prophylaxis is provided at MRRH and other public hospitals in Uganda. The prevalence of maternal alloimmunization due to RhD and other RBC antigens in Ugandan women is not known. The aim of this study was to provide data on the frequency and nature of maternal RBC alloimmunization in pregnant women in South Western Uganda. The findings from this study might be of relevance in planning future management strategies for HDFN in Uganda.

STUDY DESIGN AND METHODS

Study participants

In a cross-sectional study, pregnant women attending the antenatal clinic and those in labour at Mbarara Regional Referral Hospital, Mbarara, Uganda, were consecutively enrolled between March 1st and May 31st, 2010. Informed consent was obtained from all participants. The demographic characteristics, obstetric and transfusion histories were recorded in a data collection form. Information regarding the ABO and RhD blood groups of the participants was retrieved from their antenatal cards whenever available. The study was approved by the research and ethics committees at Mbarara University of Science and Technology, Mbarara, Uganda.

Laboratory investigations

After consent, 4ml of whole blood was drawn from each participant and put in ethylenediaminetetraacetic acid (EDTA) tubes for laboratory investigations. Plasma samples were removed and stored frozen at -80°C, at the Epicentre Mbarara Research Base, until they were shipped to the Sanquin Blood Supply in Leiden, the Netherlands for immunohaematological studies. The samples were screened for the presence of RBC alloantibodies by use of a standard 3-cell panel of reagent group O RBCs. In the indirect antiglobulin test (IAT), a LISS-enhanced gel centrifugation technique (DiaMed ID, Micro Typing System, Cressier sur Morat, Switzerland) with polyspecific antihuman globulin (rabbit anti-IgG and monoclonal anti-C3d) was used. When the antibody screening was positive, antibody identification was performed by testing the plasma samples with commercial panels of reagent RBCs, of selected phenotypes, by similar or additional methods whenever needed. Participants were considered to be alloimmunized if antibodies to one or more RBC antigens could be identified.

Statistical methods

Statistical software packages (Excel 5.0, Microsoft, Redmond, WA; and Statistical Package for the Social Sciences 12.0, SPSS, Inc., Chicago, IL) were used for data management and analysis, respectively. For univariate analysis of possible associations between maternal RBC alloimmunization and age at the time of enrolment, gestational age, parity, history of blood transfusion, previous Caesarean deliveries, and a history of antepartum haemorrhage, the Chi-

square test or Fisher's exact test were used. Groups were assumed to differ significantly when the probability level was less than 0.05.

RESULTS

Patient data

We recruited a total of 2001 pregnant women at Mbarara Regional Referral Hospital during the 3-month study period. Of these, 717 (35.8%) were in labour and admitted to the maternity ward while the others (n=1284) were outpatients attending the antenatal clinic with a mean gestational age of 27.2 (median, 28; range, 10 - 42) weeks. The mean age at the time of enrolment was 25.1 (median, 24; range, 14 - 46) years. The mean parity was 2.6 (median, 2; range, 1-12) with 687 (34.3%) women being primigravidae. Of 1881 women typed for their RhD status, 67 (3.6%) were RhD negative.

Table 1: RBC alloimmunization and immunizing events among 2001 pregnant women in different age groups at Mbarara Regional Referral Hospital in Mbarara, Uganda

	Maternal age (years)			Total
	14 - 19	20 - 35	>35	
Number of women (n)	263	1634	104	2001
Parity (median, range)	1 (1-4)	2 (1-12)	6 (1-12)	2 (1-12)
History of immunizing event ¹	4 (1.5)	307 (18.8)	40 (38.5)	351 (17.5)
Alloimmunized women	10 (3.8)	30 (1.8)	5 (4.8)	45 (2.2)
Number of antibodies (n)	11	30	6	47
Women with panreactive or aspecific antibodies	3 (1.1)	4 (0.2)	2 (1.9)	9 (0.4)
Women with clinically significant antibodies	5 (1.9)	21 (1.3)	3 (2.9)	29 (1.4)
Clinically significant antibodies (n)	6	21	4	31
Anti-S	2	9	1	12
Anti-M	2	9	0	11
Anti-D	1	3	0	4
Anti-K, -Fy ^b , -Jk ^a or -Kp ^a ,	1	1	2	4

Data presented as number (%) unless stated otherwise. ¹ Caesarean delivery, antepartum haemorrhage, blood transfusion

In the obstetric history, 186 (9.3%) participants reported having delivered by Caesarean section and 14 (7.5%) of them had received a blood transfusion; 159 (7.9%) participants had experienced a prior antepartum haemorrhage (APH) of whom 59 (37.1%) had also been transfused. A history of blood transfusion for non-obstetric indications was recalled by 5 women. Overall, 78 participants (3.9%) gave a history of past exposure to blood transfusion.

Table 2: Demographic variables, transfusion history and obstetric characteristics of RBC immunized and non-immunized pregnant women at Mbarara Regional Referral Hospital in Mbarara, Uganda¹

Demographics	Immunized women (n=45)	Women with clinically relevant antibodies (n=29)	Non-immunized women (n=1956)
Age in years	24.9 (24; 17-37)	25.1 (24; 17-37)	25.1 (24; 14-46)
Gestational age ≤ 28 weeks	18 (2.4) ²	13 (1.8) ²	715 (36.6) ³
Parity:			
Primiparae	20 (2.9)	13 (1.9)	667 (34.1)
Para 2 - 5	23 (2.0)	15 (1.3)	1135 (58.0)
Para >5	2 (1.3)	1 (0.6)	154 (7.9)
History of sensitizing events	8 (2.3)	6 (1.7)	343 (17.5)
Multiple sensitizing events ⁴	1 (1.4)	0	72 (3.7)
No sensitizing events	37 (2.2)	23 (1.4)	1613 (82.5)

¹ Data are reported as mean (median; range) or number (percentage). ² Percentage per row calculated with total number of immunized and non-immunized women for: gestational age ≤ 28 weeks, parity, history of sensitizing events, multiple sensitizing events and no sensitizing events as the denominator; respectively.

³ Percentage per row calculated with total number of non-immunized women as the denominator.

⁴ Caesarean delivery and blood transfusion or antepartum haemorrhage and blood transfusion.

Maternal RBC alloimmunization

Forty five women (2.2%; 95% CI: 1.6-2.9) were found to be alloimmunized to RBC antigens; 20 (44.4%) of them being primigravidae. Only 1 (2.2%) of the alloimmunized women recalled a history of previous blood transfusion. The proportions of alloimmunized women and the antibodies relevant for HDFN in different age groups are shown in Table 1. Maternal age (<20 , 20-35 and >35 years), parity (primiparae, para 2-5 and para >5), past history of sensitizing events (Caesarean deliveries and APH with or without blood transfusions, blood transfusions for non-obstetric indications) and the gestational age (first, second or third trimester) at enrolment were

neither significantly associated with the overall rate of alloimmunization nor with the rate of formation of clinically significant alloantibodies ($p>0.2$ for all). Table 2 summarizes the demographic variables, transfusion history and obstetric characteristics of the 2001 pregnant women studied at Mbarara Regional Referral Hospital.

RBC antibody specificities

There were 38 RBC alloantibodies of known specificity produced by 36 of the alloimmunized women. The remaining 9 women possessed non-specific ($n=6$) or pan-reactive antibodies ($n=3$). The alloantibody specificities identified were: anti-S, 12; anti-M, 11; anti- Le^a , 6; anti-D, 4; and 1 each of anti-Fy^b, -K, -Jk^a, -Lu^a, and -Kp^a. These presented as antibody combinations of anti-M+S and anti-K+ Kp^a in two of the women (4.4%); the remaining of the identified alloantibodies were as single specificities.

DISCUSSION

In this cross-sectional study, 45 out of 2001 Ugandan pregnant women had RBC alloantibodies giving an overall maternal alloimmunization rate of 2.2% (95% CI: 1.6 - 2.9). Out of 47 alloantibodies detected, 31 antibodies (66.0%) in 29 women (1.4%) can be considered clinically relevant with reported potential to cause HDFN (Daniels, 2002). These included 4 anti-D, 12 anti-S, 11 anti-M, and 1 each of anti-K, -Fy^b, -Jk^a, and -Kp^a. A limitation of our study was the inability to determine the RBC antigens from newborns, to confirm the paternal alloantigen origin. We presume, however, that most of these alloantibodies were formed against paternally derived fetal RBC antigens since none of the alloimmunized women with clinically significant antibodies reported a history of prior blood transfusion, although anti-M is known for its occurrence as a natural antibody. The overall prevalence of maternal RBC alloimmunization is comparable with findings from a study in Zimbabwe by Cakana *et al.* (2000) in which 50 out of 3000 pregnant women (1.7%) had RBC antibodies. In this study, however, only seven women (0.2%) possessed antibodies clinically significant for HDFN (i.e. 4 anti-D, 2 anti-E, and 1 anti-Js^b). Recently, Belinga *et al.* (2009) reported a higher prevalence of maternal RBC alloimmunization in 15 of 225 (6.7%) Cameroonian women and of these, 9 women (4.0%) possessed clinically relevant RBC alloantibody specificities (i.e. anti-D).

Maternal age, parity, history of sensitizing events and gestational age were not significantly associated with the rate of alloimmunization. In a Dutch study, previous RBC transfusion was the most important risk factor for non-D alloimmunization during pregnancy (Koelewijn *et al.*, 2009). We previously showed that severe anaemia due to malaria was the indication for transfusion in 39% of Ugandan blood recipients and that 83% of them were young children (Natukunda *et al.*, 2010a). It is possible that not all the women in the current study could recall being transfused in early childhood, which may explain the absence of previous transfusion as a risk factor for maternal alloimmunization. Since parity increases with age, the clinically relevant RBC alloimmunization rate might also rise with maternal age. However, the alloimmunization frequency in our teenage pregnant women was comparable to the older women ($p=0.7$) and remarkably, primiparae showed the highest immunization rate. One could speculate that persistence of an antibody formed after an unrecognized transfusion during childhood explains antibody prevalence in the young women, while a high parity is responsible for antibody prevalence in the older women. Transfusion recipients in Uganda are more likely to become alloimmunized because of substandard pre-transfusion practices in some clinical and laboratory settings. Notably, we previously observed nulliparous sickle cell patients who produced anti-D post-transfusion despite a local transfusion policy of matching for the RhD antigen, suggesting RBC typing errors (Natukunda *et al.*, 2010b).

Anti-S alloantibodies were the most frequent RBC antibody specificity found. We have previously reported a high frequency of anti-S alloimmunization following blood transfusion in the Ugandan population as well (Natukunda *et al.*, 2010b; Natukunda *et al.*, 2010c). Therefore, anti-S may be considered for future studies in S-negative pregnant women to evaluate the associated incidence of (severe) HDFN. Anti-M was the second most frequent antibody. Due to the fact that low titer anti-M alloantibodies are rarely implicated in HDFN (Koelewijn *et al.*, 2008; Wikman *et al.*, 2007), and case reports suggest that HDFN is restricted to titers above 128, we carried out titrations for this specificity in 9 samples for which sufficient plasma was available. The titers were ≤ 32 in all the samples (data not shown). Therefore, the clinical significance of anti-M as a cause of HDFN in Ugandan women is not likely.

The frequency of anti-D immunization among RhD negative women was 6.0%. Of the four women with anti-D, three were multigravidae (gravida 2-6) while the fourth one was a primegravida at 26 weeks of gestation and she had no history of prior sensitizing events. One of

the multigravidae women with anti-D alloimmunization was in her sixth pregnancy and she also had a history of prior Caesarean delivery. The anti-D alloimmunization frequency herein reported is comparable to that in Caucasians before the introduction of Rh immune globulin (RhIG) prophylaxis (Woodrow & Donohue, 1968). Therefore, programs for prevention of maternal anti-D alloimmunization might be put in place in Uganda given the high immunogenicity of the D antigen. Although pregnant women are routinely tested for ABO and RhD blood groups during the antenatal booking visit, they are currently not screened for the presence of RBC alloantibodies. We recommend that all RhD negative pregnant women should be screened for alloanti-D. This will help to identify those RhD negative women who require anti-D prophylaxis, in particular within 72 hours postpartum when an RhD positive baby has been delivered. In the current study this policy might, in theory, have prevented anti-D in three out of the four cases. Challenges associated with implementation of this policy in Uganda include lack of constant availability of anti-D immunoglobulin, insufficient reagents for alloantibody screening, and absence of laboratory facilities for estimation of FMH – the Kleihauer-Betke acid elution technique (Kleihauer *et al.*, 1957; BCSH Guidelines, 1999) or flow cytometry (Nance *et al.*, 1989; Nelson *et al.*, 1998) – in order to determine the correct dosage for RhIG immunoprophylaxis. For those RhD-negative mothers who are found to be already alloimmunized, they should be followed up serologically and management strategies for safe delivery of the baby are required (Bowman, 1997), the lack of facilities and expertise for intrauterine transfusions notwithstanding. The affected neonate might then benefit from intensive phototherapy and exchange transfusions (Gottstein & Cooke, 2003).

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**COST-EFFECTIVENESS OF
INTRODUCING RED BLOOD CELL
ALLOANTIBODY SCREENING AS PART
OF PRE-TRANSFUSION TESTING
IN UGANDA**

Bernard Natukunda, Maarten Postma, Henk Schonewille, Anneke Brand and Marinus van Hulst. Cost-effectiveness of introducing red blood cell alloantibody screening as part of pre-transfusion testing in Uganda. *Manuscript in preparation.*

Abstract

Aims/Objectives: To determine the cost-effectiveness of introducing red blood cell (RBC) alloantibody screening as part of pre-transfusion serologic testing in Ugandan hospitals.

Background: Pre-transfusion immunohaematologic testing in Uganda is currently limited to ABO/D typing plus room temperature (RT) saline cross-matches with no screening for irregular RBC alloantibodies.

Materials and methods: Cost-effectiveness was evaluated from the health care providers' perspective. Costs of reagents, apparatus and drugs were estimated in 2011 US dollars. We compared a '*limited* testing scenario' covering 10,000 multiply transfused patients in 15 referral hospitals with a '*universal* testing scenario' involving all the 100,000 blood recipients in 65 district and referral hospitals countrywide per annum. Testing strategies included tube and gel techniques for RBC alloantibody screening and complete cross-matches.

Results: RBC alloantibody screening using the gel method in the '*limited* testing scenario' was the most expensive strategy costing US\$23.60 while the cheapest strategy was to perform complete cross-matches countrywide using the tube method at a cost of US\$8.57 per patient. Compared to *No Screening*, introduction of the *universal* TubeCC method was estimated to cost US\$174,675 and would theoretically prevent 5,490 haemolytic transfusion reactions (HTRs) annually. Complete cross-matches using the tube method dominated the other testing strategies (i.e. they were the most cost-effective option). Compared to this testing strategy, other options were more expensive with no effect on health gains.

Conclusion: Introduction of RBC alloantibody screening as part of pre-transfusion immunohaematologic testing in Uganda appears to be cost-effective and would contribute to improving blood transfusion safety.

Key words: cost-effectiveness, pre-transfusion testing, RBC alloantibody screening, Uganda

Introduction

The purpose of pre-transfusion testing is to select, for each patient, blood components that when transfused will have acceptable survival and will not cause clinically significant destruction of the recipient's red blood cells (RBCs). If performed properly, pre-transfusion tests will detect most clinically significant unexpected alloantibodies and ensure that the patient is issued the designated blood components that are ABO/D compatible. Pre-transfusion testing consists of a series of serologic tests plus clerical and history checks that take place within the larger process of RBC administration. Elements of pre-transfusion testing include obtaining a labelled patient blood sample, comparing identifying information on the blood request form with that on the blood sample, checking previous transfusion records and history, testing patient sample for ABO/D types, screening patient sample for unexpected RBC alloantibodies, identifying the alloantibodies detected and performing a cross-match (BCSH Guidelines, 1996; Shulman *et al.*, 2001).

In Ugandan hospitals, pre-transfusion testing is currently limited to ABO/D typing plus room temperature (RT) saline cross-matches without the addition of antihuman globulin (AHG) reagent. No screening for irregular alloantibodies is carried out putting immunized blood recipients at risk of haemolytic transfusion reactions (HTRs). Transfusion-induced RBC alloantibodies have been implicated in both acute and delayed HTRs (Cox *et al.*, 1988; Vichinsky, 2001). Immune mediated HTRs may result in severe sequelae including disseminated intravascular coagulation, renal failure, and death (Hillman, 1979; Capon & Goldfinger, 1995). RBC transfusions to immunized patients with clinically significant antibodies require the availability of compatible blood units lacking the antigens to which the antibodies are directed. Recent studies reported a 6.1% rate of RBC alloimmunization following blood transfusion among Ugandans with different diseases (Natukunda *et al.*, 2010a; Natukunda *et al.*, 2010b).

The authors recommended that there was a need to improve pre-transfusion testing in Uganda, including the introduction of RBC alloantibody screening, in order to prevent the occurrence of HTRs. However, introducing RBC alloantibody screening would increase the costs. Therefore, we carried out an economic evaluation on whether it would be cost-effective to roll out such a program in Uganda. Economic evaluation [which can be defined as the 'comparative analysis of alternative courses of action in terms of both their costs and consequences' (Drummond *et al.*,

2004)] is important because resources are scarce and choices must be made concerning their deployment. Cost-effectiveness analysis (CEA) guides us in minimizing the opportunity cost by allocating resources where more wealth will be created. The goal of CEA is to improve the population's health by using available resources in the most effective way (Pereira, 2000). There is a need to streamline pre-transfusion testing procedures in Uganda by screening for unexpected RBC alloantibodies, both in the interests of cost-effectiveness and patient safety.

Materials and Methods

Using the health care providers' perspective, we evaluated the cost-effectiveness of introducing pre-transfusion RBC alloantibody screening in Uganda for the following three scenarios:

1. Blood transfusion recipients with sickle cell disease (SCD), cancer and other multiply transfused (OMT) patients ('*limited* testing scenario')
2. All blood transfusion recipients in public hospitals ('*universal* testing scenario')
3. *No Screening* at all (the current scenario)

According to the Uganda Blood Transfusion Service (UBTS), there were 187,000 units of blood collected in 2009 in Uganda. Given a discard rate of 7.2% for blood (Kajja *et al.*, 2010), approximately 173,536 units were administered and the remaining 13,464 units were discarded. A recent study by Natukunda *et al.* (2010c), reported that the mean number of units of blood transfused per recipient per year at Mbarara Regional Referral Hospital was 1.7. The total number of patients transfused countrywide i.e. in 65 hospitals in one year (in the '*universal* testing scenario') was therefore estimated to be 100,000. In the above report from a regional referral hospital, 10.3% of all blood recipients in 2008 were cancer, SCD and OMT patients. Thus, the number of patients who would receive blood transfusions in 15 referral hospitals annually (in the '*limited* testing scenario') was estimated to be 10,000.

Testing strategies

To estimate the costs involved in RBC alloantibody screening, complete cross-matches and management of HTRs in the *limited* and *universal* testing scenarios, the following four screening strategies were evaluated:

- (i) *TubeSCREEN*: A tube method with normal saline and addition of AHG reagent followed by alloantibody identification in case of a positive antibody screen and an RT saline cross-match.

(ii) *TubeCC*: A tube method for a complete (indirect antiglobulin test, IAT) cross-match followed by antibody identification in case of a positive cross-match.

(iii) *GelSCREEN*: A manual LISS gel technique with *Cellbind* Screen® cards followed by alloantibody identification in case of a positive antibody screen and an RT saline cross-match.

(iv) *GelCC*: A manual LISS gel technique with *Cellbind* Screen® cards for a complete (IAT) cross-match followed by antibody identification in case of a positive cross-match.

We assumed that patients in either cohort received one unit of whole blood or an equivalent amount of RBC component. Therefore, alloantibody screening (using reagent RBCs) and cross-matching (using donor RBCs) of the patient's serum occurred only once. Testing procedures were as outlined (Appendix 1 and 2).

Risk for haemolytic transfusion reactions (HTRs)

In recent cross-sectional studies by Natukunda *et al.* (2010a; 2010b), the prevalence of RBC alloimmunization in transfused Ugandans with SCD and other diseases was reported to be 6.1% (95% confidence intervals: 3.0 – 10.0%). We assumed that an equivalent proportion of recipients (i.e. 610 patients in the 'limited testing scenario' and 6,100 recipients in the 'universal testing scenario') were at risk of developing HTRs on subsequent exposure to allogeneic blood transfusion, unless they were screened for RBC alloantibodies or complete cross-matches were performed during pre-transfusion testing. To quantify the beneficial effects of putting resources towards improved pre-transfusion testing in Uganda by introducing RBC alloantibody screening, costs for the management of an HTR were analyzed. It was assumed that 10% of the HTR cases prevented would be severe.

Costs

The costs of reagents, apparatus and drugs were quoted from the latest price catalogues of two leading Ugandan pharmaceutical distributors (*Joint Medical Store [JMS]* and *National Medical Stores [NMS]*, Kampala, Uganda) and *Cellbind* Screen®, Sanquin reagents, Amsterdam, the Netherlands (Appendix 1, 2 and 3). Current salary scales from the Ugandan Ministry of Health were used in the calculation of laboratory and clinical staff costs per hospital (2 laboratory technicians, 2 enrolled nurses and 1 medical doctor). Laboratory instruments (centrifuges, incubators, dispensers, working tables etc) were to be purchased for all the hospitals involved.

Also, the costs per HTR prevented were calculated and these included laboratory investigations and treatment (Appendix 3). All costs were calculated in 2011 US dollars and there was no discounting used for future costs because all expenses occurred within one year.

Cost-effectiveness

The incremental cost-effectiveness ratio (ICER) was estimated for each cohort of recipients i.e. the additional costs of a screening strategy divided by the additional health gains (HTRs prevented) compared with the next least expensive strategy. Strategies that cost more and prevented less HTRs were excluded. Additionally, the relative cost-effectiveness to the current strategy was estimated by dividing the additional costs and HTRs prevented relative to *No Screening*.

Budget Impact

The budget impact for implementing the most cost-effective screening strategy, i.e. the net cost to the health care system in Uganda, was estimated for both the *limited* and *universal* cohorts.

Results

Base case analysis

The estimated costs of the four testing strategies and management of an HTR are shown in Table 1. Alloantibody screening for multiply transfused blood recipients in referral hospitals using the gel method (*limited* GelSCREEN) was the most expensive strategy costing US\$23.60 per patient.

Table 1. Costs of the screening strategies investigated and the management of a haemolytic transfusion reaction (HTR).

Cost item (per patient)	Cost per scenario (US\$)	
	<i>Limited</i>	<i>Universal</i>
TubeCC	8.75	8.57
TubeSCREEN	10.16	9.99
GelCC	20.50	18.98
GelSCREEN	23.60	22.07
Management of a patient with an HTR	111.92	111.92

On the other hand, the cheapest strategy was *universal* TubeCC at a cost of US\$8.57 per patient. The cost of management of an HTR was estimated at US\$111.92 per patient.

The annual cost of testing all blood recipients in Uganda would range from US\$857,405 to 2,207,363 for *universal* TubeCC and *universal* GelSCREEN, respectively (Table 2). In comparison, introduction of pre-transfusion testing in only multiply transfused patients in 15 regional referral hospitals would range from US\$87,509 to 235,969 annually for *limited* TubeCC and *limited* GelSCREEN, respectively. The net cost, hence the budget impact of introducing pre-transfusion immunohaematologic testing in Uganda, is shown in Table 2 column ΔC . The budget impact for all transfusion recipients per annum ranged from US\$174,675 to 1,524,633 for *universal* TubeCC and *universal* GelSCREEN, respectively; while that in the 'limited testing scenario' was estimated to be in the range of US\$19,236 to 167,696 for *limited* TubeCC and *limited* GelSCREEN, respectively.

Table 2. Base case analysis for the four strategies to improve pre-transfusion testing in Uganda using either the *limited* or *universal* scenario relative to *No Screening*.

Testing strategy	Costs (US\$)	HTRs	ΔC	ΔE	Cost-effectiveness ratio relative to 'No Screening'
<i>No Screening</i>	682,730*	0	-	-	-
<i>limited</i> GelSCREEN	850,425	610	167,696	610	275
<i>universal</i> GelSCREEN	2,207,363	6,100	1,524,633	6,100	250
<i>limited</i> GelCC	819,482	610	136,753	610	224
<i>universal</i> GelCC	1,897,932	6,100	1,215,202	6,100	199
<i>limited</i> TubeSCREEN	716,095	610	33,366	610	55
<i>universal</i> TubeSCREEN	1,016,385	6,100	333,656	6,100	55
<i>limited</i> TubeCC	701,966	610	19,236	610	32
<i>universal</i> TubeCC	857,405	6,100	174,675	6,100	29

HTRs, haemolytic transfusion reactions; ΔC = costs of the scenario relative to 'No Screening'; ΔE = number of HTRs prevented; * Equivalent to the cost of investigation and treatment of all HTRs occurring as a result of no alloantibody screening, in the current 'No screening' scenario

The costs and effects of the four testing strategies proposed in improving pre-transfusion testing in Uganda are shown in Table 2. When the cost-effectiveness ratios (CERs) relative to *No Screening* were calculated, it was found that those for *universal* TubeCC and *limited* TubeCC were the lowest and most favourable. The CER for *universal* TubeCC versus *No Screening* was US\$29 while that for *limited* TubeCC versus *No Screening* was US\$32 per HTR prevented (Figure 1). Therefore *limited* TubeCC was less cost effective than *universal* TubeCC and it was formally excluded by extended dominance. All other strategies showed higher costs and less or equal HTR prevention and were therefore dominated by *universal* TubeCC.

For Uganda, the per capita gross national income (GNI) in 2011 was US\$490. To achieve a one to three times the GNI per capita per disability-adjusted life year (DALY) averted threshold of cost-effectiveness for *universal* TubeCC, the DALYs prevented per HTR should be at least 0.06 and 0.02, respectively.

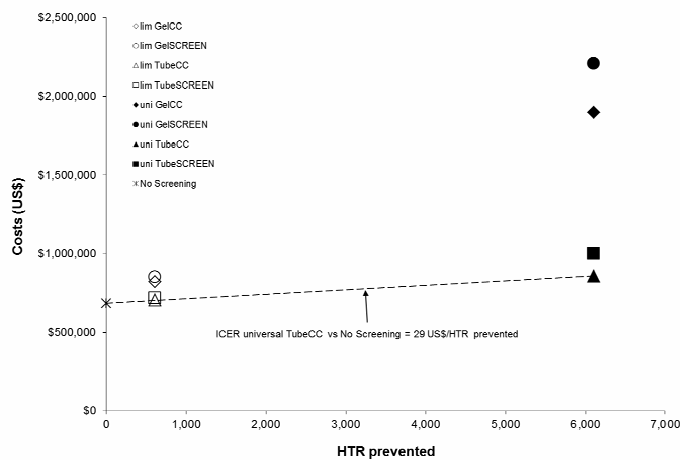


Figure 1. Total costs and HTRs prevented in the four screening strategies for the *limited* (open symbols) and *universal* (closed symbols) cohorts of transfused Ugandans. The CER of the *limited* TubeCC relative to *No Screening* was US\$32 per HTR prevented. Therefore, the *limited* TubeCC was formally excluded by extended dominance. HTR = haemolytic transfusion reaction; lim = *limited*; uni = *universal*.

Sensitivity analysis

Sensitivity analyses were performed to determine which parameters had substantial impact on costs and outcomes. The prevalence of RBC alloimmunization in transfused Ugandans was assigned a range of 3 – 10% according to the published 95% confidence intervals (Natukunda *et al.*, 2010a; Natukunda *et al.*, 2010b). The proportion of HTRs that became severe was assumed to range from 5 – 20%. Other parameters (i.e. costs of antibody tests, management of HTRs, personnel and equipment) were varied using lower and upper limits of 50% and 200% of their original values respectively. Univariate sensitivity analyses revealed that the cost-effectiveness of *universal* TubeCC relative to *No Screening* was very sensitive to the test costs and the prevalence of RBC alloimmunization (Figure 2). Doubling the test costs to US\$17.15 yielded a CER of US\$169 per HTR prevented, a 6-fold increase in the base case value. TubeCC remained the most cost-effective strategy of the four strategies investigated. Decreasing the test costs to 50% of the base case value (i.e. to US\$4.29 per patient) would achieve cost savings for *universal* TubeCC. The break-even point for cost savings was estimated at US\$6.83, test costs below this value would make TubeCC a cost saving intervention. At a 2-fold increase in the TubeCC and TubeSCREEN costs, TubeCC still remained the most cost-effective strategy. *Universal* TubeSCREEN would become most cost-effective at costs higher than US\$10.16 per patient for TubeCC. GelCC would be a dominant strategy if costs decreased from US\$18.98 to 6.83 per patient.

The prevalence of alloimmunization had a profound impact on cost-effectiveness. Using the lower limit of the 95% confidence interval yielded a 6-fold higher CER for *universal* TubeCC relative to *No Screening* (Figure 2). TubeCC remained the most cost-effective strategy and at an alloimmunization prevalence rate higher than 7.7%, it would become cost saving.

Halving the costs of HTR management would yield a 3-fold higher CER. *Universal* TubeCC screening would become cost saving at HTR management costs higher than US\$140.56. The severity of an HTR had a modest impact on cost-effectiveness. The sensitivity of the CERs to personnel and equipment costs was also very limited (Figure 2). The univariate sensitivity analyses for the *limited* cohort were not shown as they closely resembled those for the *universal* cohort.

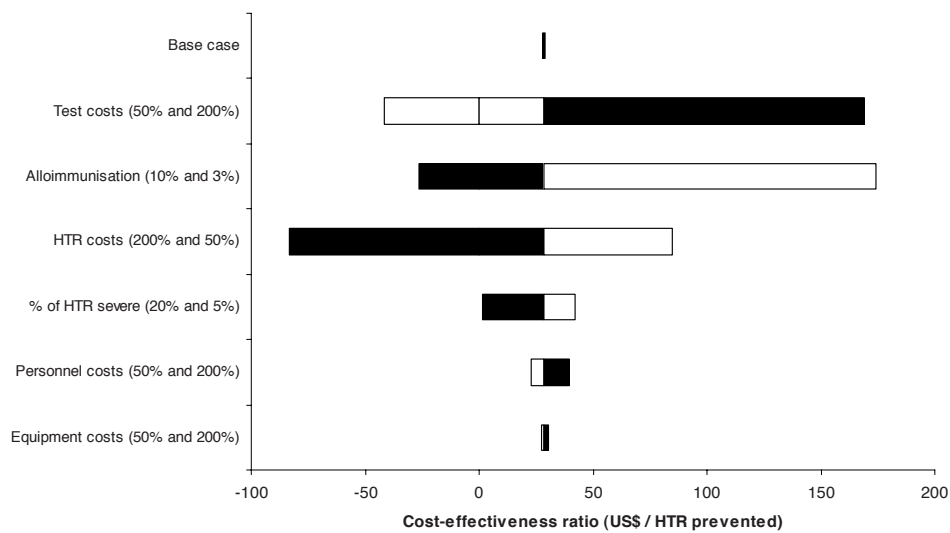


Figure 2. Tornado diagram showing the sensitivity of the cost-effectiveness ratio in the *universal* TubeCC screening strategy relative to *No Screening*. The solid line represents the base case cost-effectiveness ratio of US\$29 per HTR prevented. Open and solid bars depict an estimation using lower or upper limit, respectively. Cost-effectiveness ratios below 0 (dashed line) represent cost-saving sensitivity analyses.

Discussion

Using the health care providers’ perspective, we investigated the cost-effectiveness of introducing RBC alloantibody screening in Uganda and found that it would be cost-effective. We assessed the ‘*limited*’ and ‘*universal*’ testing scenarios using tube and gel tests for alloantibody screening and complete cross-matches. To our knowledge, this is the first ever CEA study on pre-transfusion testing in Uganda and Africa at large. The cheapest (*universal* TubeCC) and the most expensive (*limited* GelSCREEN) strategies ranged from US\$8.57 – 23.60 per patient, with a cost difference of US\$15.03. In general, gel techniques were more expensive than tube tests. Furthermore, TubeCC dominated all the other testing strategies (i.e. it was the most cost-effective option). Alloantibody screening was a more expensive option than complete cross-matches without an effect on health gains relative to *No Screening*. CERs for all the testing

strategies were in the range of US\$29 – 275 per HTR prevented. According to the WHO and World Bank thresholds for cost-effectiveness, *universal* TubeCC becomes a highly cost-effective intervention if DALYs per HTR prevented are higher than 0.06 and below 0.02 DALYs per HTR prevented, TubeCC does not become cost-effective.

To our knowledge, disability and quality of life weights for HTRs are not yet published. Our estimated DALYs per prevented range of 0.02 to 0.06 would correspond to 7 – 22 days of complete disability, or 14 – 44 days of 50% disability, comparable to severe chronic obstructive pulmonary disease, schizophrenia, and neurological sequelae of malaria (WHO, 2004).

Multivariate and univariate sensitivity analyses revealed that the cost-effectiveness of improved pre-transfusion testing was sensitive to test costs, the prevalence of alloimmunization and HTR management costs, in descending order. Univariate sensitivity analysis showed that the cost of TubeCC had to rise substantially before it was to become less cost-effective than other screening strategies. Also, a modest reduction in test costs for *universal* TubeCC would yield a cost-saving strategy.

A limitation of our study was that the analysis was based on a key assumption that the number of patients who would develop HTRs was equivalent to those who possessed transfusion-induced RBC alloantibodies. Whereas the prevalence of RBC alloantibodies was reported at 6.1% in transfused Ugandans in cross-sectional studies (Natukunda *et al.*, 2010a; Natukunda *et al.*, 2010b), the actual alloimmunization frequency might even be higher since alloantibodies are known to disappear with time (Schonewille *et al.*, 2000) negating the assumption that we could have overestimated the number of HTRs. At a slightly higher alloantibody prevalence of 7.7% the TubeCC strategy showed cost savings in the sensitivity analysis. This may well reflect the case with SCD and OMT patients. On the other hand, in first-time transfusion recipients (e.g. obstetric patients) the prevalence of RBC alloantibodies may be lower and hence less favourable cost-effectiveness can be expected from improved pre-transfusion testing. Data on the occurrence of HTRs in transfused Ugandans are lacking and there are no prospective studies on the frequency of post-transfusion RBC alloimmunization. We relied on clinical experience in the base case analysis. Because of the underlying statistical distribution, it is likely that costs of HTR management were higher relative to the base case value. The actual frequency of HTRs and associated management costs in sub-Saharan Africa warrants further research.

These findings provide additional evidence to support our earlier recommendations (Natukunda *et al.*, 2010a; Natukunda *et al.*, 2010b) that there was a need to introduce RBC alloantibody screening in Uganda for multiply transfused patients and those with prior blood transfusion or pregnancy. Our current health economic evaluation shows that *universal* TubeCC should be implemented in Uganda to prevent immunohaemolytic complications in transfused patients. Gel technology, based on the principle of controlled centrifugation of RBCs through a dextran-acrylamide-gel, is reported to have some advantages over tube tests. The technique addresses issues of standardization and the RBC washing step before the AHG phase is entirely eliminated saving time and requiring less skill (Lapierre, 1990; Delaflor-Weiss & Chizhevsky, 2005). However, in this health economic evaluation, gel technology was not found to be a cost-effective strategy. The cost of GelCC testing should have to decrease by more than 64% to become more cost-effective than the other screening strategies investigated. Introduction of RBC alloantibody screening calls for improvements in standards of laboratory and clinical transfusion practice. Issues of documentation on operational procedures, guidelines, manuals, storage conditions, error reporting and quality assessment schemes need to be addressed. Laboratory staff should liaise with their clinical colleagues and re-design blood request forms to allow for a record of alloantibody screening results (historical and/or current). Records on RBC alloimmunization should be properly kept by the local hospital transfusion laboratory and copies thereof given to the patients so as to prevent future HTRs. Since transfusion laboratory technicians are already conversant with saline tests, only limited extra training will be needed for them to appreciate the additional AHG phase with the introduction of *universal* TubeCC.

In conclusion, introduction of RBC alloantibody screening in Uganda appears to be cost-effective and would contribute towards improvement in blood transfusion safety. Therefore policy makers and other stakeholders should consider the implementation of the above recommendations on improved pre-transfusion testing. The UBTS can play an important role by supplying the necessary laboratory reagents to hospitals alongside blood components and in the overall monitoring of the program.

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Appendix 1: *A tube method with normal saline and addition of AHG reagent*

The tube method would be performed in a 12 x 75 mm glass tube with two drops of plasma being transferred using a plastic pipette. One drop of 3 - 4% RBC suspension and 2 drops of saline solution would be incubated for 10 minutes at 37°C and test reactants would be washed 4 times before adding 2 drops of AHG reagent. Reactions would then be read macroscopically. Estimated costs calculated in performing tube tests are summarized in Table 3.

Appendix 2: *A manual LISS gel technique with Cellbind Screen® cards*

Fifty microlitres of each test cell reagent and 25 µL of patient's plasma would be transferred using an automatic pipette into the appropriate microtubes and the *Cellbind* cards would be incubated for 15 minutes at 37°C in a *Cellbind* incubator. *Cellbind* screen gel cards would then be centrifuged for 10 minutes in a *Cellbind* centrifuge and read macroscopically. In this technology, the washing of test reactants and RBC button re-suspension steps are eliminated. Estimated costs calculated in performing gel technique tests are summarized in Table 4.

Appendix 3: *Management of a haemolytic transfusion reaction (HTR)*

Whenever a HTR was suspected, the transfusion would be stopped immediately and the patient hydrated with Normal Saline. Vigorous supportive care (to the patient's airway, blood pressure, urine output, and heart rate) would be applied while clerical and serologic investigations and notification of the blood provider were being carried out. The HTR would then be managed according to the laboratory and clinical findings. Average costs estimated in the management of a HTR are summarized in Table 5.

Table 3. Costs of RBC alloantibody screening and identification tests, including RT and complete cross-matches, using the tube method (US\$1 = 2400 UG Shillings on 20.02.2011)

(a) Antibody screening					
	Total cost	Unit cost	Quantity per patient	Cost per patient (Sh)	US Dollars
100 Khan tubes	43,400	434	3	1,302	
100 Pasteur pipettes	16,000	160	3	480	
K7210 <i>Cellbind</i> P3, 3x10 mL	48.3		3 drops	725	
Coombs' AHG reagent	10,000		6 drops	300	
10820 Normal Saline 500 mL	789	0.0789	24 drops	1.9	
Subtotal				2,809	1.17
(b) Antibody identification					
	Total cost	Unit cost	Quantity per patient	Cost per patient (Sh)	US Dollars
100 Khan tubes	43,400	434	16	6,944	
100 Pasteur pipettes	16,000	160	16	2,560	
10820 Normal Saline 500 mL	789	0.0789	128 drops	10.1	
Coombs' AHG reagent	10,000		32 drops	1,600	
K7230 <i>Cellbind</i> D16, 16x3 mL	114.1 €		16 drops	6,270	
Subtotal				17,384	7.24
(c) RT Saline cross-match					
	Total cost	Unit cost	Quantity per patient	Cost per patient (Sh)	US Dollars
100 Khan tubes	43,400	434	1	434	0.18
100 Pasteur pipettes	16,000	160	2	320	0.13
Subtotal				754	0.31
(d) Complete cross-match					
	Total cost	Unit cost	Quantity per patient	Cost per patient (Sh)	US Dollars
100 Khan tubes	43400	434	1	434	0.18
100 Pasteur pipettes	16000	160	2	320	0.13
Coombs' AHG reagent	10000		2 drops	100	0.04
Subtotal				854	0.35
(e) Staff costs					
Laboratory technologists			<i>Time (min)</i>	<i>Cost/min</i>	<i>Labour (US\$)</i>
<i>TubeSCREEN</i>			60	0.0189	1.13
<i>TubeCC</i>			45	0.0189	0.85
(f) Equipment costs					
			<i>Tests per annum/hosp</i>	<i>Cost/annum</i>	<i>Cost/test US\$)</i>
<i>Limited scenario</i>			666.67	\$208.08	0.312
<i>Universal scenario</i>			1538.46	\$208.08	0.135
Overall total cost of Tube test per patient					(US\$)
<i>limited TubeSCREEN (a+b+c+e+f)</i>					10.16
<i>universal TubeSCREEN (a+b+c+e+f)</i>					9.99
<i>limited TubeCC (b+d+e+f)</i>					8.75
<i>universal TubeCC (b+d+e+f)</i>					8.57

Table 4. Costs of RBC alloantibody screening and identification tests, including RT and complete cross-matches, using the gel technique (US\$1 = 2,400 UG Shillings on 20.02.2011)

(a) Antibody screening					
		Total cost	Quantity per patient	Cost per patient	
K7000	Cellbind screen, 48x6 tests	174.7	3 microtubes	1.82	UG Sh 6,006 US\$ 2.5
K7210	Cellbind P3, 3x10 mL	48.3	150 uL	0.72	2,376 0.99
	Pipette tips, 500 in a pack		3 tips		122 0.05
Subtotal				8,504	3.54
(b) Antibody identification					
		Total cost	Quantity per patient	Cost per patient	
K7000	Cellbind screen, 48x6 tests	174.7	16 microtubes	9.7	UG Sh 32,010 US\$ 13.34
K7230	Cellbind ID16, 16x3 mL	114.1	800 uL	1.9	6,270 2.6
	Pipette tips, 500 in a pack		16 tips		652 0.27
Subtotal				38,932	16.21
(c) RT Saline cross-match					
		Total cost	Quantity per patient	Cost per patient	
100	Khan tubes	43,400	1	434	UG Sh 434 US\$ 0.18
100	Pasteur pipettes	16,000	2	320	320 0.13
Subtotal				754	0.31
(d) Complete cross-match					
		Total cost	Quantity per patient	Cost per patient	
K7000	Cellbind screen, 48x6 tests	174.7	1 microtube	0.6	UG Ss 1,980 US\$ 0.83
	Pipette tips, 500 in a pack		1 tip		40 0.02
Subtotal				2,020	0.85
(e) Staff costs					
Laboratory technologists			<i>Time(min)</i>	<i>Cost/min (US\$)</i>	<i>Labour (US\$)</i>
GelSCREEN			45	0.0189	0.85
GelCC			40	0.0189	0.75
(f) Equipment costs					
			<i>Tests per annum/hosp</i>	<i>Cost/annum</i>	<i>Cost/test (US\$)</i>
Limited scenario			666.67	1792.05	2.688
Universal scenario			1538.46	1792.05	1.165
Overall total cost of gel test per patient					(US\$)
limited GelSCREEN (a+b+c+e+f)					23.60
universal GelSCREEN (a+b+c+e+f)					22.07
limited GelCC (b+d+e+f)					20.50
universal GelCC (b+d+e+f)					18.98

Table 5. Estimated costs involved in the laboratory investigation and treatment per HTR prevented. Severe HTR was assumed to occur in 10% of all HTR cases.

Item	Cost (US\$)
Repeat blood group; cross-match; and DAT (tube method)	4.49
Repeat blood group; cross-match; and DAT (gel method)	5.32
Alloantibody screening (tube method)	1.20
Alloantibody screening (gel method)	3.54
Haematology and clinical chemistry tests	23.00
Drug treatment for a <i>mild</i> HTR	1.05
Drug treatment for a <i>severe</i> HTR	2.23
Additional blood component therapy	50.00
Dialysis for acute renal failure (with tubular necrosis)	316.60
Staff costs: lab technologists, nurses and a medical doctor – <i>mild</i> HTR; tube method	2.82
Staff costs: lab technologists, nurses and a medical doctor – <i>severe</i> HTR; tube method	3.96
Staff costs: lab technologists, nurses and a medical doctor – <i>mild</i> HTR; gel method	2.55
Staff costs: lab technologists, nurses and a medical doctor – <i>severe</i> HTR; gel method	3.69
Equipment costs per HTR; <i>limited</i> scenario (tube method)	0.31
Equipment costs per HTR; <i>universal</i> scenario (tube method)	0.14
Equipment costs per HTR; <i>limited</i> scenario (gel method)	2.69
Equipment costs per HTR; <i>universal</i> scenario (gel method)	1.16
Cost of each HTR management; <i>limited</i> scenario (tube method)	109.58
Cost of each HTR management; <i>universal</i> scenario (tube method)	109.56
Cost of each HTR management; <i>limited</i> scenario (gel method)	115.04
Cost of each HTR management; <i>universal</i> scenario (gel method)	113.51
Average cost of investigation and treatment of each HTR	111.92

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GENERAL DISCUSSION

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- 8.2 Maternal RBC alloimmunization
- 8.3 Post-transfusion RBC alloimmunization
- 8.4 Conclusion and recommendations

8.1 Blood transfusion in Uganda

In 1986, the International Community responded to the new and then little known HIV/AIDS epidemic by setting up, under the WHO, a global program on AIDS. At about the same time, the European Commission (EC) started providing technical and financial support to safe blood interventions in under-resourced countries (Gerard *et al.*, 1995). EC assistance enabled the government of Uganda to rejuvenate the Uganda Blood Transfusion Service (UBTS).

According to the WHO (2002), there are four key objectives for blood transfusion services to ensure blood safety:

- (1) Establishment of a well-organized, nationally-coordinated blood transfusion service that can provide adequate and timely supplies of safe blood for all patients in need.
- (2) Collection of blood only from voluntary non-remunerated donors (VNRD) from low-risk populations.
- (3) Testing of all donated blood for transfusion-transmissible infections (TTIs), blood grouping and compatibility testing.
- (4) The appropriate clinical use of blood, including the use of alternatives to transfusion (crystalloids and colloids) wherever possible, and the safe administration of blood and blood products.

A well-organized blood transfusion service, with quality systems in all stages of the transfusion process, is a prerequisite for the safe and effective use of blood and blood products. Several systems for provision of blood are currently operational in Africa. The hospital-based system consists of transfusion units attached to the main laboratories of a hospital, most of which use donor replacement schemes, or a centralized transfusion centre that usually has a system for voluntary, altruistic donors. Many African countries have a hybrid system that incorporates certain centralized functions such as transfusion guidelines and collection from voluntary donors into their hospital-based system (Bates *et al.*, 2007). In Uganda, a national blood policy was developed and there is a centralized system consisting of a national blood transfusion centre in Kampala, the capital, that operates the services for the whole country with seven regional blood banks and six collection centres. For a population of about 33 million people, approximately 203,000 units of blood were collected in 2011. Blood is collected from VNRD (who are mainly

students in secondary schools) and screened for TTIs at the regional centres before it is distributed to respective hospitals countrywide. Standard operating procedures (SOPs) for donor recruitment, donor selection and counselling have been introduced and blood bank staff trained on screening procedures for TTIs and on quality assurance.

Shortages of blood for transfusion usually occur in times of high demand (such as during malaria seasons and school holidays) and hospitals have to mobilize replacement donors locally. During such shortages, blood from replacement donors may be the only life-saving option in emergency cases with severe obstetric haemorrhage or severe malarial anaemia in children. Thus, there are both the centralized and hospital-based models of blood supply in Uganda. Actually, the hospital-based replacement system of blood supply would be the better suited alternative for Uganda. It has been shown - at least in three sub-Saharan countries studied - that blood collected from replacement/family donors and first time VNRD has a similar prevalence of viral infections and in both donor populations safety increment is provided by repeat donations and using the same quality control criteria for both donations (Allain, 2010a; 2010b). Although the WHO objectives for blood safety (2002) include VNRD donations only, this is not yet fulfilled in most African countries and still shortages of blood for emergencies occur. Considering that safety is not the issue, replacement donors could fill this gap. Obviously drawbacks of a fragmented hospital-based blood service need to be solved. However, the lack of equipment for blood processing, poor quality of testing kits and inadequate staff are some of the disadvantages of a hospital-based system. Cooperation with the centralized blood supply system e.g. through national validation by exchanging blood samples or even viral screening of donors at the regional blood service may safeguard the quality of viral testing countrywide. The lack of facilities for blood component separation is, however, widespread in the whole country. Like hospital-based blood banks, most regional blood centres are currently unable to produce blood components especially platelets and frozen fresh plasma. For the patients in need of the above component therapy (e.g. those with acquired coagulation disorders and/or malignancies), blood prescribers have to arrange special orders from the UBTS headquarters in Kampala or they decide to give fresh whole blood transfusions as an alternative. Currently, the cost of a unit of blood in Uganda is approximately 50 US dollars including the recruitment of low-risk donors, testing, blood grouping, processing, storage and transportation. The Uganda government, with assistance from development partners, meets these financial needs of the UBTS. Any hospital in the country can

access the blood supply free of charge, with no cost recovery arrangements in place. However, one may raise questions on whether such a centralized system (top-down approach) would be sustainable in the long term, in developing countries like Uganda, in the event that there was no more financial support from external funders. Most sub-Saharan African countries without external financial support have been found to pay 2 – 4 times for their VNRD blood more than replacement donor blood (Bates & Hassal, 2010). In Africa, it would be preferable to initially establish safe, efficient and cost-effective hospital-based blood banks with a local donor base and then build upwards to regional and national transfusion centres (bottom-up approach). This would retain both VNRD and replacement donors since only repeat donation provides added blood safety and should be promoted (Allain, 2010a).

Despite the substantial progress made in the area of blood safety in Uganda, less attention has been paid to overall transfusion safety. There is still a challenge in the appropriate clinical use of blood and the safe administration of blood and blood products (WHO strategic objective 4 outlined above). *Transfusion safety* can be distinguished from *blood safety*. Blood safety concerns the safety of the component: it is largely the responsibility of blood collectors and has been a primary focus of both regulators and standard-setting agencies in the blood industry. In contrast, transfusion safety focuses on the overall process which results in the delivery of transfusion therapies to patients. Transfusion safety includes blood safety but also includes additional critical steps that relate to the medical use of components and the outcome of the recipient. These latter steps occur largely within the hospital and include the medical decision to transfuse, the collection of pre-transfusion samples, laboratory practices and the bedside administration of blood components (Dzik, 2003). Assessment of the clinical transfusion practice at Mbarara Regional Referral Hospital in South Western Uganda (**Chapter 3**) showed that documentation of the transfusion process was inadequate, monitoring of blood recipients was poor, and there was no hospital transfusion committee. Similar challenges in transfusion practice have been reported elsewhere in Uganda (de Graaf *et al.*, 2009). Additionally, pre-transfusion RBC alloantibody screening tests were not being carried out in all hospitals in the whole country. Therefore, from both clinical and laboratory standpoints, the practice of immunohaematology and blood transfusion in Ugandan hospitals still needs a lot of improvement.

8.2 Maternal RBC alloimmunization

Haemolytic disease of the fetus and newborn occurs when maternal IgG antibodies cross the placenta to the fetal circulation causing RBC destruction with consequent anaemia, jaundice or hydrops fetalis. In different populations, all alloantibodies reactive by the IAT have been implicated in causing HDFN. However, alloimmunization to the RhD antigen is the commonest cause of severe HDFN. Although there is no cure, blood transfusion technology provides information necessary for the diagnosis, clinical management and prevention of HDFN. Such information is used to identify the specific fetomaternal incompatibility, to provide the safest possible blood for transfusion therapy, and to identify the candidates for RhIG immunoprophylaxis.

The intention of the study on maternal RBC alloimmunization was to provide denominator data on the extent of the problem in South Western Uganda and to make recommendations for the prevention of HDFN in the country. In Uganda, the prevalence of maternal alloimmunization due to RhD and other RBC antigens was hitherto unknown before the publication of our research findings. We observed RBC alloantibodies in 45 out of 2001 pregnant women i.e. an overall maternal alloimmunization rate of 2.2% (95% CI: 1.6 - 2.9). There were 31 clinically significant alloantibodies (**Chapter 6**) with reported potential to cause HDFN (Daniels, 2002) i.e. anti-D, 4; anti-S, 12; anti-M, 11 and 1 each of anti-K, -Fy^b, -Jk^a, and -Kp^a. Our key finding was a 6.0% frequency of anti-D immunization among RhD negative women who contributed 3.6% of the study population. This high RBC alloimmunization frequency is comparable to that reported in Caucasians before the introduction of RhIG prophylaxis (Woodrow & Donohue, 1968). Pregnant women in Uganda are routinely tested for ABO/RhD blood groups during the antenatal booking visit, but they are not screened for the presence of RBC alloantibodies. Thus, babies of immunized pregnant women are at an increased risk of HDFN. Since there is no anti-D prophylaxis provided in public hospitals, programs for prevention of maternal anti-D alloimmunization should be put in place in Uganda. We recommend that all RhD negative pregnant women should be screened for alloanti-D at the first prenatal visit. RhD negative women who are not yet alloimmunized require anti-D prophylaxis within 72 hours of delivering RhD positive babies. This will protect future RhD negative pregnancies from HDFN. Therefore, RhIG should be put on the Uganda National Essential Drugs list for supply to all public hospitals. Antenatal administration of RhIG at 28 weeks of gestation in the RhD-negative woman

with no evidence of anti-D alloimmunization will be hampered by the uncertainty of the fetal D phenotype. To determine the correct dosage of RhIG, Kleihauer's acid elution technique of differential staining is the most feasible and affordable method for estimation of transplacental FMH in the Ugandan setting. However, this method - whose principle depends on the number of RBCs containing fetal haemoglobin (HbF) - may overestimate the FMH since some adults may have hereditary persistence of HbF and the level of maternal HbF has been reported to rise above the upper limit of the normal in about 25% of pregnant women (Klein & Anstee, 2005). Other technologies such as the use of flow cytometry are not currently affordable for routine use in Uganda.

For the RhD-negative mothers who are already alloimmunized, assessment of the severity of HDFN will mainly consist of closely monitoring the antibody titers. This is because expertise for fetal Doppler ultrasonography and invasive techniques such as serial amniocentesis and fetal blood sampling is still lacking locally. Given that there are also no facilities for intrauterine transfusions, management options for affected pregnancies remain controlled early delivery and neonatal phototherapy with or without exchange transfusions. As a tool for predicting the severity of neonatal disease, tests for Hb, bilirubin and DAT should be carried out on cord blood in an RhD positive baby whose mother is alloimmunized. Besides pregnancy, RhD haemolytic disease can follow mismatched blood transfusions. We recently detected post-transfusion anti-D alloimmunization in nulliparous SCD patients (**Chapter 4**) presumably following pre-transfusion testing errors. Urbaniak & Robertson (1981) reported that about 90% of RhD negative persons will make anti-D if exposed to a sufficient dose of RhD positive RBCs by transfusion. To prevent transfusion-induced maternal RBC alloimmunization, we recommend the transfusion of RhD negative RBCs to RhD negative recipients particularly in young females and those in the child bearing age.

8.3 Post-transfusion RBC alloimmunization

We demonstrated the presence of unexpected alloantibodies in the plasma of 39 out of 642 transfusion recipients studied (428 of them having SCD) i.e. an overall RBC alloimmunization rate of 6.1% (95% CI: 3.0 - 10.0%). This frequency of RBC alloantibody formation was within the reported range, of 2 - 15%, from previous studies on RBC alloimmunization in chronically transfused patients with various haematologic and oncologic disorders (Blumberg *et al.*, 1984;

Heddle *et al.*, 1995; Hoeltge *et al.*, 1995; Seyfried *et al.*, 1999; Shonewille *et al.*, 1999). Although higher rates of RBC alloimmunization (ranging up to 76%) have been reported among transfused SCD patients (Moriera *et al.*, 1996; Olujohungbe *et al.*, 2001; Aygun *et al.*, 2002; Castro *et al.*, 2002; Ameen *et al.*, 2009), our findings (**Chapter 4**) showed that there was no difference in the anti-RBC alloimmune response between SCD and OMT Ugandan patients with different diseases. Both groups of blood transfusion recipients had an equal RBC alloimmunization frequency of 6.1%. Several factors have been put forward to explain the previously reported increased incidence of RBC alloimmunization in SCD patients such as an altered immune response, increased frequency of transfusions, the chronic inflammation associated with the disease itself, or lack of phenotypic compatibility between donors and recipients (Ambruso *et al.*, 1987; Caccese *et al.*, 1987; Cox *et al.*, 1988). However, the low rate of RBC alloantibody formation observed among Ugandan SCD patients suggests that some of the mechanisms above are less important than the probable high phenotypic compatibility between blood donors and SCD patients who were black Ugandans in both cases. This proposition is supported by findings from a Jamaican study in which a cohort of transfused SCD patients had a low RBC alloimmunization rate of 2.6% due to the high racial homogeneity among blood donors and the patients (Olujohungbe *et al.*, 2001). RBC alloimmunization rates in chronically transfused SCD patients also reportedly decreased from a historic 3% per unit to 0.5% per unit with the use of phenotypically matched units of RBCs in the Stroke Prevention Trial in the United States with C, E and Kell matching (Vichinsky *et al.*, 2001). However, the lower prevalence of antibodies in transfused SCD patients in Uganda may be due to the low transfusion load (a median 3 units of blood were transfused). Due to the shortcomings of a cross-sectional design, some RBC alloantibodies might have been missed since they have been reported to disappear with time (Shonewille *et al.*, 2000; Reverberi, 2008). The above findings show that extended phenotype matching for SCD patients may not be relevant in Uganda and Africa at large.

Additional recipient-related factors influence the possibility of a patient to mount an immune response to blood transfusion. Table 8.3.1 shows a comparison of demographic variables, transfusion characteristics and other recipient factors in all alloimmunized and non-immunized SCD and OMT recipients in our research project. Female gender and number and events of RBC exposure are major risk factors. Whereas, the recipient's immune status has been suggested to

influence the rate of RBC alloimmunization with immunosuppressed recipients failing to become alloimmunized (Calvery *et al.*, 1991; Boctor *et al.*, 2003), our findings indicated that immunosuppressed patients with HIV and cancer were not impaired and patients with inflammatory states did not have enhanced capacity to form anti-RBC alloantibodies (**Chapter 5**).

Table 8.3.1: Demographic variables, transfusion characteristics and recipient-related factors of all the alloimmunized and non-immunized SCD and OMT Ugandan patients combined*

	Alloimmunized (n=39)	Non-immunized (n=603)	p-value
Recipient age ≤ 10 years	11 (28.2)	213 (35.3)	0.31
Recipient age ≥ 40 years	5 (12.8)	72 (11.9)	0.80
Female-to-male ratio	1.8	1.0	0.10
Adult females (≥ 18 years)	12 (30.8)	133 (22.1)	0.23
History of pregnancy	7 (58.3)	88 (66.2)	0.64
>10 transfusion episodes	7 (17.9)	58 (9.6)	0.10
Mean units of blood transfused	13.8 (5.0; 2-65)	7.8 (4.0; 2-100)	0.052
Mean transfusion episodes	9.4 (4.0; 2-57)	5.6 (4.0; 2-80)	0.044
Recipients with malignancy	6 (15.4)	93 (15.4)	1.0
Immunosuppressed recipients (HIV infection; history of anti-cancer chemotherapy)	8 (20.5)	112 (18.6)	0.83
Recipients with inflammation (malaria; bacterial and viral infections, excluding HIV)	20 (51.3)	352 (58.4)	0.41

* Data are reported as number (%) or mean (median; range), unless otherwise specified.

Clinically significant RBC alloantibodies have been reported to occur about twice as often in women compared to men (Spielmann & Siedl, 1974; Raki *et al.*, 1999). Our findings on post-transfusion RBC alloimmunization indicated that a positive history of pregnancy (58.3%) of adult women was not associated with higher alloimmunization prevalence (66% in non-immunized recipients). Nevertheless female gender was associated with immunization (in a ratio of 1.8:1) suggesting another contribution from pregnancy, such as fetomaternal chimerism that can also be established after (unnoticed) abortions. Recent murine studies suggest that the inflammatory status of the recipient at the time of the transfusion may influence rates of RBC

alloantibody formation (Zimring & Hendrickson, 2008), an observation also not confirmed in our patient cohort. The number of transfusion episodes and units of blood transfused was significantly associated with the rate of alloimmunization. This is consistent with previous reports that have shown an association between RBC alloimmunization and increased number of donor exposures (Davies, *et al.*, 1986; Rosse *et al.*, 1990) [Table 8.3.1]. Thus, RBC alloimmunization remains a significant complication of allogeneic transfusions, especially in Uganda where blood products are not appropriately selected or fully cross-matched.

Thirty five (83.3%) of the 42 clinically significant alloantibodies detected were directed against antigens of the Rh and MNS blood group systems i.e. anti-C, 2; anti-C^w, 1; anti-D, 8; anti-E, 16; anti-M, 1; and anti-S, 7. Multiple alloantibody specificities were demonstrable in 6 (15.4%) of the 39 alloimmunized recipients. This was comparable to the frequency of multiply alloimmunized patients in other studies (Schonewille *et al.*, 1999). The occurrence of post-transfusion anti-D alloimmunization indicates the presence of laboratory typing errors or the transfusion of RhD positive units to antigen negative recipients regardless of the local transfusion policy. Noteworthy is the fact that five of the patients who produced anti-D alloantibodies were nulliparous females aged between 5 and 19 years and these were at risk of producing future babies with RhD haemolytic disease. This underscores the need for improved pre-transfusion testing including the use of quality typing antisera and screening for immune alloantibodies. Currently, pre-transfusion testing in Uganda does not involve the detection or monitoring of alloantibody formation and its clinical consequences. ABO and RhD grouping are followed by an abbreviated RT saline cross-match with no other compatibility testing performed.

8.4 Conclusion and recommendations

Alloimmunization to RBC antigens is usually only appreciated when the laboratory detects an antibody in the serum of the subject. Immunization, however, may exist and not be recognized because: the serum is not examined at the appropriate time following antigenic challenge; the antibody strength is below the threshold for detection; the target reagent cells do not possess the corresponding antigen; serological test conditions are not optimal; or there is a cellular, not humoral, immune response. While the rate of RBC alloimmunization reported herein lies within the expected ranges, our research project had some limitations. Due to the cross-sectional designs employed, a snapshot of transfused patients and pregnant women was studied and

therefore these data were by no means conclusive and likely underestimated the true immunization risks. Since we had to freeze and then ship the samples to Europe several months after collection, we could not use self RBCs as autocontrols and neither could we determine the co-existence of autoimmunization. However, the findings herein presented are quite significant and should form the bases for policy changes in Uganda on pre-transfusion and antenatal testing. In future, there is a need to design larger, multicentre, prospective studies to determine the actual nature and frequency of the immune response to RBC and other blood cell antigens vis-à-vis the prognoses of transfusion recipients and pregnant women in Uganda, especially in this era of universal leucocyte depletion of blood products and therapeutic use of novel immunosuppressive drug regimens. We highly recommend the introduction of RBC alloantibody screening during pre-transfusion testing in Uganda. Our research findings from the cost-effectiveness analysis study (**Chapter 7**), which showed that such a program would be cost-effective, are in support of this recommendation. Laboratory manuals for improved pre-transfusion testing and standard operating procedures for improved clinical transfusion and obstetric practice should be formulated and operationalized within Uganda and other African countries that currently do not use standard immunohaematologic techniques. Consequently, the morbidity and mortality related to RBC alloimmunization and the immunohaemolytic consequences thereof, including HTRs and HDFN, will be significantly prevented while reducing the associated financial burden in many African countries with restricted health budgets.

There is a need to introduce antenatal and pre-transfusion screening tests for detection of RBC alloantibodies in pregnant women and transfusion recipients in Uganda. We specifically recommend the introduction of the following testing strategies:

(a) For pregnant women during the antenatal booking visit:

- Carry out ABO and RhD grouping of the women;
- Screen for the presence of alloanti-D in the serum of all RhD negative women; and
- If the antibody screen is positive, monitor the strength of alloanti-D using serial titrations in the course of pregnancy.

(b) For those with a history of previous pregnancy or blood transfusion(s) or in SCD, cancer and OMT patients:

- Carry out ABO and RhD grouping of the donor and recipient;
- Screen for irregular RBC alloantibodies in the recipient's serum using tube or gel test methods (37°C IAT with a 3-cell panel);
- Identify all the detected alloantibodies; and
- Carry out an RT saline cross-match between the patient's serum and the selected donor RBCs.
- If a cell panel analysis cannot be performed a cross-match including AHG should be performed.

(c) For other blood transfusion recipients

- Carry out ABO and RhD grouping of the donor and recipient; and
- Carry out a complete cross-match between the patient's serum and the donor RBCs (37°C incubation of the patient's serum with donor RBCs and addition of AHG serum). In case the complete cross-matches are positive, antibody identification should be carried out so that antigen negative blood is given to the recipients.

In so doing, there will be a reduction in the risk of HDFN, RBC alloimmunization, AHTRs, DSTRs and DHTRs (the frequency of post-transfusion immune haemolytic reactions in Ugandans is currently unknown), and challenges associated with the lack of compatible blood for alloimmunized transfusion recipients will be solved.

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SUMMARY / SAMENVATTING

Summary

Red blood cell (RBC) alloantibodies are formed after exposure to foreign RBC antigens in cases of blood transfusion, transplantation or pregnancy. The reported RBC alloimmunization frequency in ABO- and D-matched transfusion recipients varies between less than 1 to more than 70 percent; and is dependent on antigen immunogenicity, duration of transfusion therapy, and genetic and environmental factors. Why an immune response is or is not mounted against a particular incompatible antigen is, however, unknown. Upon subsequent exposure to blood transfusions, more than 20 percent of alloimmunized patients form additional RBC antibodies. Multiple alloantibody specificities are seen in up to 70 percent of the recipients, depending on the transfusion frequency. RBC alloantibodies can cause haemolytic disease of the fetus and newborn or acute and delayed haemolytic transfusion reactions, leading to potentially serious clinical consequences.

Chapter 1

In this chapter, the phenomenon of post-transfusion and maternal RBC aloimmunization is briefly introduced. The fact that pre-transfusion RBC screening tests and complete cross-matches are not carried out in Ugandan public hospitals is mentioned. Also, the paucity of data on RBC alloantibody formation in transfused Ugandans is underlined. Finally, a number of research questions pertinent to the subject of RBC alloimmunization in Uganda are outlined.

Chapter 2

The relevant scientific literature on post-transfusion and maternal RBC alloimmunization is reviewed in Chapter 2. The chapter begins with a short history of post-transfusion and maternal RBC alloimmunization, following the discovery of the Rh blood group system by P. Levine and R. Stetson in 1939. Next, we briefly review the diversity of human blood groups together with their functional heterogeneity.

In the pathogenesis of post-transfusion RBC alloimmunization, pathways for immune recognition of alloantigens, the role of costimulatory molecules, and types 1 and 2 immune responses are highlighted. The nature of RBC alloantibodies - “naturally occurring” or “immune” - is also discussed. Naturally occurring antibodies are most often IgM while immune alloantibodies are usually of the IgG isotype. Antibodies are considered to be *clinically*

significant if they have caused HTRs, HDFN or unacceptably short survival of the transfused RBCs.

The frequency and nature of post-transfusion RBC alloimmunization in different populations of blood recipients, including SCD and OMT patients, is presented. A number of studies discussing RBC alloimmunization frequencies in SCD and OMT patients (ranging from 2.6 - 76% and 5 - 30% respectively) are also reviewed. Lastly, the literature on HDFN resulting from maternal RBC alloimmunization is presented.

Chapter 3

In this chapter, the clinical transfusion practice at Mbarara Regional Referral Hospital (MRRH) in South Western Uganda was assessed. Clinical data on the indications for blood transfusion, blood ordering practices and the post-transfusion complications related to RBC alloimmunization were collected. In 2008, there were 1674 blood recipients on all the five wards at MRRH and 58.4% of them were given whole blood transfusions. The mean number of units per recipient was 1.7 and the crossmatch-to-transfusion ratio was 1.3. The four most frequent indications for transfusion were malaria (38.8%), bleeding (27.1%) other infections (16.1%) and cancer (6.6%). Transfusion reactions were recorded for ten (0.6%) patients. Although no evidence of blood wastage was adduced, inadequacies were noted in the documentation of the transfusion process. We recommended the establishment of a hospital transfusion committee which would train staff, put in place local guidelines on the appropriate use of blood, design a standard 'blood transfusion form' for monitoring transfusions, and investigate consequences of RBC alloimmunization e.g. haemolytic transfusion reactions.

Chapter 4

The occurrence of RBC alloantibodies in 428 transfused SCD patients, who were mainly children (median age, 12 years), was investigated in a cross-sectional study. There were 26 patients with anti-RBC antibodies giving an alloimmunization rate of 6.1% (95% CI: 4.0% - 9.0%). Thirty RBC alloantibody specificities were found, with 20 (66.7%) and 5 (16.6%) belonging to the Rh and MNS blood group systems respectively. Anti-D alloimmunization was observed in five nulliparous females, irrespective of the local transfusion policy of matching for the RhD antigen. Alloanti-S antibodies contributed 80% of the detected specificities in the MNS

blood group system. Eleven of the alloantibodies (36.7%) presented as multiple antibody combinations. Of the immunized patients with specific antibodies, 19 (79.2%) produced only one antibody while 5 (20.8%) had multiple antibodies. The rate of RBC alloimmunization was significantly associated with the number of units of blood transfused (i.e. the number of donor exposures). However, the alloantibody prevalence was low compared to other studies in transfused SCD patients presumably because of a low transfusion load in this study (median number of units transfused = 3) and due to the racial homogeneity between blood donors and SCD patients in Uganda.

Chapter 5

To determine the prevalence and identify the specificities of RBC alloantibodies in transfused Ugandans with different diseases, we recruited 214 recipients at Mulago National Referral Hospital during a six-month study period. Of these, 113 (52.8%) were females and among them, 77 (68.1%) had a history of pregnancy. The patients had a mean age of 30.3 years and they had been transfused with a total of 1,869 units of blood in 1,285 transfusion episodes. Of the patients studied, 108 (50.6%) had malignant disorders i.e. haematologic, 64; and solid tumours, 44; while 62 (29%) had infectious diseases i.e. malaria, 34; AIDS, 24; and bacterial infections, 4. There were 13 patients (6.1%; 95% CI: 3.0 - 10.0%) found to possess RBC alloantibodies; 11 (84.6%) of them having experienced up to a maximum of 10 transfusion episodes. The alloimmunization rate of 6.1% in this study was similar to that observed in Caucasian blood recipients. The number of units of blood transfused and the number of transfusion episodes were significantly associated with the rate of alloimmunization. Eleven of the alloimmunized patients produced a total of 12 RBC alloantibodies of known specificity. The specificities of the alloantibodies identified were: anti-E, 6; anti-S, 3; and 1 each of anti-D, -K and -Le^a. In one patient (9.1%), two alloantibodies, anti-E plus anti-K, presented as a combination; the rest of the alloantibodies were as single specificities. Immunosuppressed patients with AIDS and cancer became alloimmunized. Patients with malaria were less likely to develop alloantibodies. Depending on the recipient's diagnosis, the introduction of pre-transfusion RBC alloantibody screening tests in Uganda was recommended.

Chapter 6

In this chapter, the prevalence of maternal alloimmunization was studied in 2001 Ugandan pregnant women in a three-month cross-sectional study. While 717 (35.8%) of the women were in labour and admitted to the maternity ward, the others (n=1284) were outpatients attending the antenatal clinic. The mean age at the time of enrolment was 25.1 years and the mean parity was 2.6. Out of the 1881 women typed for their RhD status, 67 (3.6%) were RhD negative. Overall, 78 participants (3.9%) gave a history of past exposure to blood transfusion. Forty five women (2.2%; 95% CI: 1.6 - 2.9) were found to be alloimmunized to RBC antigens; 20 (44.4%) of them being primigravidae. Only 1 (2.2%) of the alloimmunized women recalled a history of previous blood transfusion. There were 38 RBC alloantibodies of known specificity produced by 36 of the alloimmunized pregnant women i.e. anti-S, 12; anti-M, 11; anti- Le^a, 6; anti-D, 4; and one each of anti-Fy^b, -K, -Jk^a, -Lu^a, and -Kp^a. These presented as antibody combinations of anti-M+S and anti-K+ Kp^a in two of the women (4.4%); the remaining of the identified alloantibodies were as single specificities. To prevent HDFN, the introduction of RhIG prophylaxis in Ugandan public hospitals was recommended given the high rate of maternal anti-D alloimmunization herein reported and the high immunogenicity of the D antigen.

Chapter 7

A study to determine the cost-effectiveness of introducing RBC alloantibody screening as part of pre-transfusion testing in Uganda was carried out. Cost-effectiveness was evaluated from the health care providers' perspective. We compared a '*limited testing scenario*' covering 10,000 multiply transfused patients in 15 referral hospitals with a '*universal testing scenario*' involving all the 100,000 blood recipients in 65 district and referral hospitals countrywide per annum. Testing strategies included tube and gel techniques for RBC alloantibody screening and complete cross-matches. RBC alloantibody screening using the gel method in the '*limited testing scenario*' was the most expensive strategy costing US\$23.60 while the cheapest strategy was to perform complete cross-matches countrywide using the tube method at a cost of US\$8.57 per patient. Complete cross-matches using the tube method dominated the other testing strategies (i.e. they were the most cost-effective option). Therefore, introduction of RBC alloantibody screening as part of pre-transfusion immunohaematologic testing in Uganda appears to be cost-effective and would contribute to improving blood transfusion safety.

Chapter 8

A general discussion on the research findings reported in this thesis is presented in this chapter. In Uganda, blood transfusion services are centrally organized with regional blood centers across the country. Donated blood is tested for TTIs – including Syphilis, Hepatitis B and C, and HIV – and a documented quality system is in place. In hospitals, ABO/D typing and RT saline cross-matches are carried out before transfusion. However, RBC alloantibody screening is not performed during pre-transfusion and antenatal testing in the whole country. Therefore, alloimmunized blood recipients and babies of RhD negative mothers are at high risk of serious morbidity and mortality due to HTRs and HDFN respectively. Research findings in this thesis showed that one in every 16 transfused Ugandans and a similar number of RhD negative pregnant women possess clinically significant RBC alloantibodies in their plasma. Hence, we herein recommend the introduction of alloanti-D screening in all RhD negative pregnant women; and screening for the presence of irregular RBC alloantibodies in the plasma of potential blood recipients with a history of prior exposure to RBCs through transfusion(s) or pregnancy. Any detected alloantibodies will be identified and antigen negative blood transfused. Complete cross-matches (as opposed to the currently performed RT saline cross-matches) are also recommended for the rest of blood transfusion recipients. In so doing, the new measures will improve the health of Ugandans by reducing the risk of RBC immunohaemolytic complications including alloimmunization, acute and delayed HTRs, and HDFN.

Samenvatting

Rode bloedcel (RBC) antistoffen worden gevormd na contact met vreemde RBC antigenen na bloedtransfusie, transplantatie of zwangerschap. De frequentie van RBC alloimmunisatie, na transfusie van ABO en D gematchte transfusies, varieert tussen minder dan 1 tot meer dan 70 procent en is afhankelijk van de antigeen immunogeniciteit, duur van de transfusie therapie en genetische en omgevingsfactoren. Waarom een immuun reactie wel of niet wordt opgewekt tegen een zeker antigeen is vooralsnog grotendeels onbekend. Patiënten die eenmaal antistoffen hebben gevormd hebben een meer dan 20 procent hogere kans op de vorming van meerdere antistoffen na volgende transfusies. Afhankelijk van de transfusie frequentie kunnen meerdere antistof specificiteiten worden gevonden in 70 procent van de patiënten. RBC alloantistoffen kunnen hemolytische ziekte van de foetus en pasgeborene (HZFP) veroorzaken en verantwoordelijk zijn voor acute en uitgestelde hemolytische transfusiereacties (HTR), beiden met potentieel ernstige klinische consequenties.

Hoofdstuk 1

In dit hoofdstuk zijn post-transfusie en maternale RBC alloimmunisatie kort ingeleid. Het feit dat pre-transfusie RBC antistof screenings testen en complete kruisproeven niet worden uitgevoerd in Ugandese publieke ziekenhuizen wordt benoemd. Tevens wordt benadrukt dat er nauwelijks gegevens zijn over alloantistof vorming in Ugandese transfusie ontvangers. Dit heeft geleid tot een aantal relevante onderzoeksvragen met betrekking tot RBC alloimmunisatie in Uganda.

Hoofdstuk 2

Relevante wetenschappelijke literatuur over post-transfusie en maternale RBC alloimmunisatie wordt besproken in hoofdstuk 2. Het hoofdstuk begint met post-transfusie en maternale RBC alloimmunisatie in historisch perspectief, gevolgd door de ontdekking van het Rhesus bloedgroep systeem door P. Levine en R. Stetson in 1939. Vervolgens is een korte beschrijving gegeven van de humane bloedgroep diversiteit en hun functionele heterogeniteit.

Met betrekking tot de pathogenese van post-transfusie RBC alloimmunisatie worden pathways voor immuun herkenning van alloantigenen, de rol van costimulatoire moleculen en type 1 en 2 immuun reacties besproken. De oorsprong van RBC alloantistoffen – “natuurlijk voorkomend”

of “immuun” – wordt besproken. Natuurlijk voorkomende antistoffen zijn veelal IgM terwijl immuun antistoffen meestal van het IgG type zijn. Antistoffen worden als klinisch relevant geduid als zij HTR, HZFP of onacceptabel korte overleving van getransfundeerde RBC kunnen veroorzaken. Vervolgens worden aanbevolen immunohematologische pre-transfusie procedures besproken. De frequentie en oorsprong van post-transfusie RBC alloimmunisatie in verschillende transfusie ontvangers zoals sikkelcel (SCD) patiënten en andere ziekten (OMT) waarbij frequent getransfundeerd wordt gepresenteerd. Een aantal studies over RBC alloimmunisatie frequentie in SCD en OMT, met frequenties tussen 2,6-76% en 5-30% respectievelijk, worden besproken. Als laatste wordt de literatuur over HZFP veroorzaakt door maternale RBC alloimmunisatie besproken.

Hoofdstuk 3

Voor dit hoofdstuk werd de klinische transfusie praktijk in het Mbarara Regional Referral Hospital (MRRH) in Zuid-West Uganda onderzocht. Klinische gegevens met betrekking tot transfusie indicaties, bloed bestelling en post-transfusie complicaties gerelateerd aan RBC alloimmunisatie werden verzameld. In 2008 waren er 1674 transfusie ontvangers op de 5 afdelingen van het MRRH en 58,4% van hen ontvingen volbloed transfusies. Het gemiddelde aantal eenheden per transfusie ontvanger was 1,7 en de kruisproef-transfusie ratio was 1,3. De vier meest voorkomende indicaties voor transfusie waren malaria (38,8%), bloeding (27,1%), andere infecties (16,1%) en maligniteit (6,6%). Transfusie reacties werden gemeld bij 10 (0,6%) patiënten. Ondanks dat er geen harde bewijzen waren voor bloed verspilling, waren er wel hiaten in de dokumentatie van de transfusie keten. Voorgesteld wordt om een ziekenhuis transfusie commissie in te stellen, die vervolgens verantwoordelijk is voor opleiding van personeel, implementatie van lokale procedures met betrekking tot juist gebruik, ontwikkeling van een standaard ‘transfusie aanvraag formulier’ om transfusies te monitoren en onderzoek te doen naar de consequenties van RBC alloimmunisatie zoals hemolytische transfusie reacties.

Hoofdstuk 4

Het vóórkomen van RBC allantistoffen in 428 getransfundeerde SCD patiënten, voornamelijk kinderen (mediane leeftijd 12 jaar), werd onderzocht in een cross-sectionele studie. Er waren 26 patiënten met anti-RBC antistoffen resulterend in een alloimmunisatie frequentie van 6,1% (95%

BI 4,0-9,0%). Er werden 30 antistof specificiteiten gevonden, waarvan 20 (66,7%) en 5 (16,6%) behorend tot de respectievelijk Rhesus en MNS bloedgroep systemen. Anti-D alloimmunisatie werd gevonden bij 5 vrouwen die nooit zwanger waren geweest, ondanks het lokale transfusie beleid om D compatibel te transfunderen. Alloanti-S antistoffen waren verantwoordelijk voor 80% van de antistoffen gericht tegen antigenen in het MNS bloedgroep systeem. Elf antistoffen (36,7%) werden gevonden in combinatie met andere antistoffen. Van de patiënten bij wie de antistof specificiteiten bepaald konden worden hadden 19 (79,2%) patiënten één antistof en 5 (20,8%) patiënten meerdere antistoffen. De antistof frequentie was geassocieerd met het aantal transfusies (donor exposities) dat patiënten hadden ontvangen. De antistof frequentie was laag vergeleken met andere studies in getransfundeerde SCD patiënten, mogelijk doordat het aantal getransfundeerde eenheden in onze studie laag (mediaan 3 eenheden) was en er raciale homogeniteit bestond tussen de bloed donoren en SCD patiënten in Uganda.

Hoofdstuk 5

Om de prevalentie en specificiteit van RBC alloantistoffen in getransfundeerde Ugandezen met verschillende ziekten te onderzoeken werden 214 transfusie ontvangers gedurende een periode van 6 maanden in het Mulago National Referral Hospital onderzocht. Van deze patiënten waren 113 (52,8%) vrouwen en 77 (68,1%) waren zwanger geweest. De patiënten hadden een gemiddelde leeftijd van 30,3 jaar en in totaal 1869 eenheden bloed ontvangen gedurende 1285 transfusie episoden. Van de bestudeerde patiënten hadden 108 (50,6%) een maligne ziekte, waarvan 64 haematologisch en 44 solide tumoren, 62 patiënten een infectieuze aandoening, waarvan 34 malaria, 24 AIDS en 4 bacterieel en de overige 44 patiënten hadden gastrointestinale, nier en hartziekten (29), trauma (10) diabetes mellitus (3) en brandwonden (2). Dertien patiënten (6,1%; 95% BI: 3,0-10,0%) hadden RBC antistoffen en 11 (84,6%) van hen hadden tot maximaal 10 transfusie episoden meegemaakt. De alloimmunisatie frequentie van 6,1% was vergelijkbaar met Kaukasische transfusie ontvangers. Het aantal toegediende eenheden en transfusie episoden was significant geassocieerd met de immunisatie frequentie. De elf geïmmuniseerde patiënten hadden 12 antistof specificiteiten t.w. 6x anti-E, 3x anti-S, 1x anti-D, -K en -Le^a. Bij één patiënt (9,1%) waren twee antistoffen aantoonbaar met anti-E en anti-K specificiteit, de andere patiënten hadden één antistof specificiteit. Ook patiënten met immunosuppressie t.g.v. AIDS en kanker hadden antistoffen gevormd, terwijl patiënten met

malaria minder vaak antistoffen hadden. Afhankelijk van de diagnose van de ontvanger wordt introductie van pre-transfusie antistof screening in Uganda aanbevolen.

Hoofdstuk 6

In dit hoofdstuk is de prevalentie van maternale alloimmunisatie in een cross-sectioneel onderzoek bij 2001 Ugandese zwangeren gedurende een periode van 3 maanden bestudeerd. Bij 717 (35,8%) vrouwen werd het onderzoek ten tijde van de bevalling verricht terwijl zij opgenomen waren op de ‘maternity’ afdeling en bij de resterende vrouwen (n=1284) tijdens bezoek aan de antenatale kliniek. De gemiddelde leeftijd was 25,1 jaar en de gemiddelde pariteit 2,6 keer. Van de 1881 vrouwen bij wie de D bloedgroep bepaald kon worden waren 67 (3,6%) D negatief. Een transfusie in het verleden werd opgegeven door 78 vrouwen. Antistoffen tegen RBC antigenen waren aantoonbaar bij 45 vrouwen (2,2%; 95% BI: 1,6-2,9%), waarvan bij 20 (44,4%) vrouwen tijdens hun eerste zwangerschap. Een transfusie in het verleden werd maar opgegeven door één (2,2%) vrouw met antistoffen. Bij 36 vrouwen konden in totaal 38 antistoffen gespecificeerd worden, t.w. 12x anti-S, 11x anti-M, 6x anti-Le^a, 4x anti-D en 1x anti-Fy^b, -K, -Jk^a, -Lu^a en -Kp^a. Bij twee (4,4%) vrouwen waren multiple antistoffen aanwezig, t.w. anti-M + -S en anti-K + -Kp^a en de anderen hadden één antistof specificiteit. Om HZFP te voorkomen wordt de introductie van RhIg profylaxe aanbevolen, gegeven de hoge frequentie van maternale anti-D immunisatie gevonden in dit onderzoek en de hoge immunogeniciteit van het D antigeen.

Hoofdstuk 7

Een onderzoek werd gedaan naar de kosten-effectiviteit van introductie van RBC antistof screening als onderdeel van pre-transfusie onderzoek in Uganda. De kosten-effectiviteit werd geëvalueerd vanuit het ‘health care providers’ perspectief. Een ‘*beperkt test scenario*’ uitgaande van 10000 multiple getransfundeerde patiënten in 15 referentie ziekenhuizen werd vergeleken met een ‘*universeel test scenario*’, waarbij uitgegaan werd van alle 100000 transfusie ontvangers in 65 district en referentie ziekenhuizen in heel Uganda jaarlijks. De testen voor antistof screening en complete kruisproeven bestonden uit de ‘buisjes-methode’ en de ‘gel-techniek’. Antistof screening met de gel-techniek in het *beperkt test scenario* bleek de meest dure (US\$ 23,60) en volledige kruisproef met de buisjes-methode en het *universele test scenario* de minst

dure (US\$ 8,57) strategie. De complete kruisproef met de buisjes-methode was het meest kosten-effectief en domineerde over alle andere strategieën. Introductie van RBC antistof screening als onderdeel van pre-transfusie immunohematologisch onderzoek blijkt kosten-effectief en draagt bij aan verbetering van de bloed transfusie veiligheid in Uganda.

Hoofdstuk 8

In dit hoofdstuk wordt een algemene discussie gegeven van de bevindingen van het onderzoek voor dit proefschrift. In Uganda zijn bloed transfusie diensten centraal georganiseerd met regionale centra verdeeld over het hele land. Gedoneerd bloed wordt getest op via bloed overdraagbare ziekten (TTIs), - syphilis, hepatitis B en C en HIV- en een gedocumenteerd kwaliteitssysteem is aanwezig. In de ziekenhuizen wordt voor iedere transfusie de ABO en D bloedgroep bepaald en een zout-kruisproef gedaan. Onderzoek naar de aanwezigheid van RBC antistoffen voor transfusie en bij zwangerschap wordt niet uitgevoerd in Uganda. Hierdoor lopen ontvangers van bloed en babies van D-negatieve moeders een hoog risico op ernstige morbiditeit en mortaliteit ten gevolge van respectievelijk HTRs en HZFP. De bevindingen van het onderzoek beschreven in dit proefschrift laten zien dat één op elke 16 getransfundeerde Ugandezen en een vergelijkbaar aantal D-negatieve zwangere vrouwen klinisch relevante RBC antistoffen heeft. Wij bevelen de introductie aan van alloanti-D screening bij D negatieve zwangere vrouwen en screening op irregulaire RBC antistoffen in het plasma van potentiële transfusie ontvangers met een voorgeschiedenis van blootstelling aan RBCs door transfusie of zwangerschap. Bij een positieve antistof screening wordt de specificiteit bepaald en antigeen compatibel bloed getransfundeerd. Complete kruisproeven (in tegenstelling tot de huidige zout-kruisproef bij kamertemperatuur) worden ook aanbevolen voor alle andere ontvangers van bloed. Deze nieuwe maatregelen zullen de gezondheid van Ugandezen bevorderen door vermindering van het risico op RBC immunohematologische complicaties zoals alloimmunisatie, acute en vertraagde HTRs en HZFP.

CURRICULUM VITAE

Bernard Natukunda was born in Mbarara, Uganda, on 31st December 1968. In 1976, he went to St. Peter's Rubindi Boys' Primary School and then to St. Joseph's Vocational School in Mbarara where he completed his secondary education in 1989. Thereafter, he joined Makerere University Medical School from where he graduated with a Bachelor of Medicine and Bachelor of Surgery (MB.ChB) degree in 1995. He completed his one-year internship training (major disciplines: *Internal Medicine* and *Obstetrics & Gynaecology*) at St. Francis' Hospital Nsambya, Kampala, before working as a medical officer at St. Joseph's Hospital Kitovu, Masaka. In 1998, he was employed as an Assistant Lecturer in the Department of Pathology at Mbarara University of Science and Technology (MUST). Two years later, he left for further training at the University of Bristol, England, from where he graduated with a first class MSc degree in 2001. He has since been a lecturer of Immunology, Haematology and Transfusion Medicine at MUST. He is a member of the Faculty Research and Ethics Committee (FREC) and he is also the Chairman of the Hospital Transfusion Committee (HTC) at Mbarara Regional Referral Hospital (MRRH). He is fully registered with the Uganda Medical and Dental Practitioners Council (UMDPC) and is a member of the Uganda Medical Association (UMA). He is also a member of the Africa Society for Blood Transfusion (AfSBT) and the International Society of Blood Transfusion (ISBT). His research interests include: immunologic complications of blood transfusion; RBC alloantibody screening in pre-transfusion and antenatal settings in Uganda; and improving in-hospital blood transfusion safety in Africa. Bernard is happily married to Dorcus and they have a family of six children including a pair of twin sons.

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