Chapter 8

GENERAL DISCUSSION

Contents

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 replacemnet 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 (Schonewille *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 immunesuppressed recipients failing to become alloimmunized (Calvery *et al.,* 1991; Boctor *et al.,* 2003), our findings indicated that immunesuppressed 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
Immunesuppressed 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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