Chapter 9

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.