Red blood cell alloimmunization in sickle cell disease patients in Uganda

Bernard Natukunda, Henk Schonewille, Christopher Ndugwa, and Anneke Brand

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 pretransfusion 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 immunohematologic techniques.

RESULTS: There were 428 patients (median age, 12 years; female/male ratio, 1.0) and they had received a median of 3 units in a median of three 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.

CONCLUSIONS: 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 immunohematologic testing in Uganda so that RBC alloimmunization and its consequences may be prevented.

Sickle cell disease (SCD) is the most common genetic disease in Uganda where approximately 5 million people (20% of the total population) have the sickle cell trait and approximately 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 (Hb) concentration and decreasing the percentage of sickle Hb 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% to 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

ABBREVIATION: SCD = sickle cell disease.

From the Department of Hematology and Transfusion Medicine, Faculty of Medicine, Mbarara University of Science and Technology, Mbarara, Uganda; the Department of Pediatrics and Child Health, Faculty of Medicine, Makerere University Medical School, Kampala, Uganda; and the Department of Research and Development, Sanquin Blood Supply, South West Region, and the Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands.

Address reprint requests to: Dr Bernard Natukunda, Department of Hematology and Transfusion Medicine, Faculty of Medicine, Mbarara University of Science and Technology, PO Box 1410, Mbarara, Uganda; e-mail: bn0012@yahoo.co.uk.

Received for publication May 25, 2009; revision received July 30, 2009; and accepted July 31, 2009.

doi: 10.1111/j.1537-2995.2009.02435.x

TRANSFUSION 2010;50:20-25.

Cooperative Study of Sickle Cell Disease in the United States, multiple antibodies were detected in more than 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 sex 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 posttransfusion 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 Sickle Cell Clinic at Mulago National Referral Hospital in Kampala, Uganda, were investigated. The study took place between February 1 and July 31, 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 two 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 that were not leukoreduced.

Data collection

Records at the Sickle Cell Clinic 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 immunohematologic studies. The plasma samples were screened for the presence of RBC alloantibodies by use of a standard three-cell panel of reagent group O RBCs. For the indirect antiglobulin test, a low-ionic-strength saline–enhanced gel centrifugation technique (DiaMed ID, Micro Typing System, DiaMed, Cressier sur Morat, Switzerland) with polyspecific anti-human globulin (rabbit anti-immunoglobulin [Ig]G 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 mini kit, Qiagen, Hilden, Germany) 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 coworkers.[26]

Statistical analysis

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 alloimmunization and age at the time of enrollment, age at first transfusion, sex, pregnancy history, number of transfusion episodes, number of units received, and the indication for transfusion, the chi-square test or Fisher's exact test was 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)

TABLE 1. Demographic and transfusion characteristics of SCD patients in Uganda*

Demographic	Alloimmunized patients	Nonimmunized patients	p Value
Patients	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 < 10 years	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 reported as number (%), percent, or median (range), unless otherwise specified.
† NS = p-value ≥ 0.1.

years. The patients were transfused with a total of 3366 (median, 3; range, 2-100) units of blood in 2463 (median, 3; range, 2-80) transfusion episodes. Twenty-six patients (6.1%; 95% confidence interval, 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 toward 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 from those in the nonimmunized group (Table 1).

RBC antibodies

The 26 alloimmunized patients produced a total of 30 RBC alloantibody specificities. This implies that after transfusion with 3366 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 five (16.6%) alloantibodies, four (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 five (20.8%) had multiple antibodies.

D genotyping

Seven of the alloimmunized patients possessed anti-D and their RHD genotyping revealed the following results:

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
NA*	Panreactive	2

* NA = not applicable.

one was D–, 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 D pseudogene. The D– individual was a 5-year-old girl who had been transfused four times in the previous 2 years while the patient with a probable 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– by serology. Most likely, they received D+ RBC transfusions despite the local transfusion policy of matching for the D antigen.

DISCUSSION

This is the first ever study carried out to determine the frequency and nature of RBC alloimmunization after 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 posttransfusion autoimmunization because we lacked autologous RBCs and direct antiglobulin tests 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 and colleagues[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 and colleagues[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 significant in the latter case (the calculated p values were 0.02 and 0.08, respectively). These findings are consistent with previous reports that 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 before 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 model.[27] 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, seven (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, five (71%) of the patients who produced anti-D were females within the age range of 5 to 19 years and with no history of pregnancy. Since some of these patients had been typed as D+ by serology, we decided to investigate the molecular bases underlying these observations. Using an *RHD*-specific multiplex PCR, D genotyping of the seven SCD patients who were D-alloimmunized revealed that one of them was D–, five had partial D (D^{Va} in four patients and a probable D^{IIIb} in the other), and the last one had a probable D pseudogene (D– 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+ by serology. We could not proceed to perform DNA sequencing for confirmation of the probable D 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 immunohematologic 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 crossmatch and to transfuse;[8,21] others develop autoantibodies in addition to being alloimmunized;[28,30] and five nulliparous females in this 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 immunologic complications of allogeneic blood transfusions, including RBC alloimmunization in SCD patients.

ACKNOWLEDGMENTS

The authors thank the staff of the Sickle Cell Clinic at Mulago National Referral Hospital in Kampala, Uganda, for the help in recruiting patients and taking of blood samples and the Joint Clinical Research Center in Kampala, Uganda, for the storage of samples during the period of the study. They also extend their appreciation to the Sanquin Blood Supply in Leiden and Dordrecht, the Netherlands, for assistance regarding sample shipment, laboratory space, and reagents.

CONFLICT OF INTEREST

The authors certify that they have no affiliation with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in this manuscript.

REFERENCES

1. Serjeant GR, Ndugwa CM. Sickle cell disease in Uganda: a time for action. East Afr Med J 2003;80:384-7.
2. Wayne AS, Kevy SV, Nathan DG. Transfusion management in sickle cell disease. Blood 1993;81:1109-23.
3. Blumberg N, Peth K, Ross K, Avila E. Immune response to chronic red blood cell transfusion. Vox Sang 1983;44:212-7.
4. Orlina AR, Unger PJ, Koshy M. Post-transfusion alloimmunization in patients with sickle cell disease. Am J Hematol 1978;5:101-6.
5. Coles SM, Klein HG, Holland PV. Alloimmunization in two multitransfused populations. Transfusion 1981;21:462-6.
6. Ambruso DR, Githens JH, Alcorn R, Dixon DJ, Brown LJ, Vaughn WM, Hays T. Experience with donors matched for minor blood group antigens in patients with sickle cell anemia who are receiving chronic transfusion therapy. Transfusion 1987;27:94-8.
7. Luban NL. Variability in rates of alloimmunization in different groups of children with sickle cell disease: effect of ethnic background. Am J Pediatr Hematol Oncol 1989;11: 314-9.
8. Rosse WF, Gallagher D, Kinney TR, Castro O, Dosik H, Moohr J, Wrang W, Levy PS. Transfusion and alloimmunization in sickle cell disease: the Cooperative Study of Sickle Cell Disease. Blood 1990;76:143-7.
9. Davies SC, McWilliam AC, Hewitt PE, Devenish A, Brozovic M. Red cell alloimmunization in sickle cell disease. Br J Haematol 1986;63:241-5.
10. Cox JV, Steanne E, Cunningham G, Frenkel EP. Risk of alloimmunization and delayed hemolytic transfusion reactions in patients with sickle cell disease. Arch Intern Med 1988;148:2485-9.
11. Sarnaik S, Schornack J, Lusher JM. The incidence of development of irregular red cell antibodies in patients with sickle cell anemia. Transfusion 1986;26:249-52.
12. Castro O, Sandler G, Houston-Yu P, Rana S. Predicting the effect of transfusing only phenotype-matched RBCs to patients with sickle cell disease: theoretical and practical implications. Transfusion 2002;42:684-90.
13. Moriera G, Bordin J, Kuroda A, Kerbauy J. Red cell alloimmunization in sickle cell disease: the influence of racial and antigenic pattern differences between donors and recipients in Brazil. Am J Hematol 1996;22:196-200.
14. Olujohungbe A, Hambleton I, Stephens L, Serjeant B, Serjeant G. Red cell antibodies in patients with homozygous sickle cell disease: a comparison of patients in Jamaica and the United Kingdom. Br J Haematol 2001;113:661-5.
15. Bashawri LA. Red cell alloimmunization in sickle cell anemia patients. East Med Health J 2007;13:1181-9.
16. Vichinsky EP. Current issues with blood transfusion in sickle cell disease. Semin Hematol 2001;38 Suppl: 14-22.
17. Tuck SM, Studd JW, White JM. Sickle cell disease in pregnancy complicated by anti-U antibody. Case report. Br J Obstet Gynaecol 1982;89:91-2.
18. Diamond JW, Brown FL Jr, Bitterman P, Klein HG, Davey RJ, Winslow RM. Delayed hemolytic transfusion reaction presenting as sickle-cell crisis. Ann Intern Med 1980;93: 231-4.
19. Schonewille H, Haak HL, van Zijl AM. RBC antibody persistence. Transfusion 2000;40:1127-31.
20. Tahhan HR, Holbrook CT, Braddy LR, Brewer LD, Christie JD. Antigen-matched donor blood in the transfusion management of patients with sickle cell disease. Transfusion 1994;34:562-9.
21. Vinchinsky EP, Earles A, Johnson RA, Hoag MS, Williams A, Lubin B. Alloimmunization in sickle cell anemia and transfusion of racially unmatched blood. N Engl J Med 1990; 322:1617-21.
22. Klein HG, Anstee DJ. Immunology of red cells. In: Klein HG, Anstee DJ, editors. Mollison's blood transfusion in clinical medicine. 11th ed. Oxford: Blackwell Publishing; 2005. p. 48-113.
23. Alarif L, Castro O, Ofosu M, Dunston G, Scott RB. HLA-B35 is associated with red cell alloimmunization in sickle cell disease. Clin Immunol Immunopathol 1986;38:178-83.
24. Bauer MP, Wiersum-Osselton J, Schipperus M, Vandenbroucke JP, Briët E. Clinical predictors of alloimmunization after red blood cell transfusion. Transfusion 2007;47:2066-71.
25. Benacerraf B, McDevitt HO. Histocompatibility-linked immune response genes. A new class of genes that controls the formation of specific immune responses has been identified. Science 1972;175:273-9.

26. Maaskant-van Wijk PA, Faas BH, de Ruijter JA, Overbeeke MA, von dem Borne AE, van Rhenen DJ, van der Schoot CE. Genotyping of RHD by multiplex polymerase chain reaction analysis of six different RHD-specific exons. Transfusion 1998;38:1015-21.
27. Zimring JC, Hendrickson JE. The role of inflammation in alloimmunization to antigens on transfused red blood cells. Curr Opin Hematol 2008;15:631-5.
28. Aygun B, Padmanabhan S, Paley C, Chandrasekaran V. Clinical significance of RBC alloantibodies and autoantibodies in sickle cell patients who received transfusions. Transfusion 2002;42:37-43.
29. Josephson CD, Su LL, Hillyer KL, Hillyer CD. Transfusion in the patient with sickle cell disease: a critical review of literature and transfusion guidelines. Trans Med Rev 2007;21: 118-33.
30. Ahrens N, Pruss A, Mayer B, Genth R, Kiesewetter H, Salama A. Association between alloantibody specificity and autoantibodies to red blood cells. Transfusion 2008; 48:20-4.