Extending the match to other Ethnicity & Disease, Advances in treatment such since Sickle cell calves, PhD; th alo Muniz Research Center, BRAZIL. The disease has multiple clinical implications. Prevalence of clinical complications such as leg ulcers, stroke, and others did not show differences between groups. In conclusion, alloimmunization did not significantly modify the clinical outcomes of SCD patients from Bahia, Brazil. (Ethn Dis. 2010;20:136–141)

Key Words: Sickle Cell Disease, Hemoglobinopathy, Alloimmunization, Red Cell Transfusion

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Alloimmunization and Clinical Profile of Sickle Cell Disease Patients from Salvador - Brazil

Sickle cell disease (SCD) is an important public health issue in Bahia, Brazil. Erythrocyte transfusions may reduce morbidity of SCD, however, they are associated with numerous risks. Among other risk categories, alloimmunization to red cell antigens may result from transfusions. The aim of this study was to compare the clinical profile of transfused adult SCD patients with and without alloantibodies. The study included 108 patients (105 homozygous SS and three with hemoglobinopathy SC), followed in the Outpatient Unit of the Hematology and Hemotherapy Center of Bahia. A retrospective review of clinical records of adult SCD patients who received at least three red blood cell transfusions from 2004 to 2007 was performed. Transfusion units were phenotypically matched for ABH-D and C,c,E,e, and K antigens. Alloimmunization developed in 56 patients (53 SS and three SC). The most prevalent alloantibodies were anti-E, anti-K, and anti-C (39.3%, 21.4%, and 16.1%, respectively). Age, sex and positive antiglobulin test displayed statistically significant differences such as leg ulcers, stroke, and others did not show differences between groups. In conclusion, alloimmunization did not significantly modify the clinical outcomes of SCD patients from Bahia, Brazil. (Ethn Dis. 2010;20:136–141)

The introduction of alloantibodies in SCD patients is considered an important public health issue by the Brazilian health authorities. Between the 16th and 19th centuries, more than 3,500,000 African slaves were brought to the country, mostly through Salvador, the capital of Bahia, which is the Brazilian state with the highest African ancestry. Since neonatal screening of abnormal globins began in the year 2000 in Bahia, the available data have shown that, out of every 650 children born alive in this Brazilian State, one has a hemoglobinopathy, mostly homozygous SS. The last three decades have witnessed a remarkable increase in SCD patient survival. Advances in treatment such as hydroxyurea, improved patient education and multidisciplinary teams in comprehensive centers have contributed to considerable improvements in morbidity and life span of these patients.

Processed red blood cell (RBC) transfusions play a prominent role in management of the disease; they may save lives in situations such as acute chest syndrome (ACS), stroke, splenic sequestration crisis, and others. Although blood transfusions can contribute to reducing morbidity and improving the quality of life in these patients, there still are risks. Among other risk categories, alloimmunization can result from transfusions and occurs in 5% to 50% of SCD patients. The development of antibodies against donor RBC antigens lacking in the transffusion recipient’s RBC can limit transfusion efficacy. It also may result in the development of transfusion reactions and occasional life-threatening events.

To avoid alloimmunization, it has been recommended to perform pretransfusional RBC matching to antigens of the Rh system (D,C,c,E,c) and Kell. Extending the match to other erythrocyte antigens can add an extra cost to transfusion, which may not be affordable for many blood banks. Furthermore, it is not known whether alloimmunization significantly affects the clinical outcomes in SCD.

The reported higher rate of alloimmunization in SCD patients is not completely understood. According to Vichinsky et al, one of the possible mechanisms underlying the development of alloantibodies in SCD patients is RBC antigen mismatch due to racial differences between blood donors and recipients. The Brazilian population is characterized by an ethnic mixture, mainly European (mostly Portuguese, but also Italian, German, Spanish, Polish, and others), native Amerindians and African slaves descendants, all heterogeneously scattered across the country. In Salvador, Bahia, 82.1% of people are predominantly of African
The main purpose of this study was to compare the clinical profile of transfused adult SCD patients who developed alloantibodies to transfused SCD patients who did not.

Blood transfusion in Brazil is accomplished through public and private institutions, which all must follow the directions of the Brazilian Health Authority on blood bank issues. The main purpose of this study was to compare the clinical profile of transfused adult SCD patients who developed alloantibodies to transfused SCD patients who did not.

PATIENTS AND METHODS

This is a cross-sectional study where medical records of SCD patients, referred to the Outpatient Unit of the Hematology and Hemotherapy Center from Salvador-Bahia, Brazil, were reviewed. This public health center provides care to about 2,000 SCD patients, adults and children, mostly SS. The patients came from Salvador and from smaller cities and villages of the countryside. Patients aged ≥18 years with confirmed SCD diagnosis who received at least three RBC transfusions from 2004 to 2007 were eligible for the study. We excluded patients under 18 years because, in our center, almost all under 18 transfused SCD patients received transfusions due to stroke; it was considered that this could constitute an important bias in our further analysis. The data were collected using standardized forms. In our institution, a mean of 270 RBC transfusions is given monthly to patients with several medical indications. Approximately 24% of these RBC transfusions are infused in SCD patients. In our center, before the year 2004, the RBC for transfusion were matched only with standard ABH-D. From 2004, a new transfusion policy for SCD patients was applied including screening for the Rh C,c,E,e and Kell antigens before the first transfusion. Further matching against other RBC antigens systems was not systematically done. The number of transfusions the patients received before being referred to our center was recorded based on information of patients and relatives, as well as on other medical reports, whenever available. Moreover, the transfusion records of other health centers were accessed in order to check the transfusion charts. Patient characteristics, clinical findings, number of transfusions, frequency and specificity of alloantibodies, laboratory data, and main clinical outcomes were all reviewed. Patients received either simple RBC transfusion or manual partial exchange-transfusion.

SPSS 15.0 Statistical Software was used for data management and analysis. The independent samples t-test was used to compare continuous variables with a Gaussian distribution, assuming equal variances. The chi-square tests were used for categorical variables, and Fisher’s exact test was applied whenever data sets contained fewer than five observations. All tests were two-tailed, and a P value < .05 was considered statistically significant. Before the data collection, this study was approved by the Bahia Foundation for Science Development Ethics Committee, protocol approval 47/2007.

RESULTS

One hundred eight patients (105 homozygous SS and three with hemoglobinopathy SC) from the transfusions’ registry of the outpatient unit were included in a sequence order. Fifty-six patients developed alloantibodies (53 SS and three 3 SC), and 52 (all SS) did not. Table 1 shows the main patients’ clinical and laboratory characteristics. The ages of the alloimmunized group (ALLO) ranged from 18 to 49 years, and 18 to 61 years in the non-alloimmunized group (NON-ALLO). Late SCD diagnosis occurred in both groups: aged 13.42 years ± 11.36 vs 13.10 years ± 10.91 (ALLO vs NON-ALLO, respectively). Alloantibodies were significantly more prevalent among females (P=.033). The number of transfusions was higher in ALLO SCD patients without statistical significance. In our center, only 10 patients were on the chronic transfusion regimen, seven ALLO and three NON-ALLO (P=.188). Forty-nine patients (26 ALLO, 23 NON-ALLO, 45.4% of the sample) received their first RBC transfusion before age 10 years, while 33 (17 ALLO, 16 NON-ALLO) were first transfused at ages 11 to 20 years (30.5%). These differences were not statistically significant (P=.74).

Nineteen out of 56 patients (33.9%) developed more than one antibody at one time (maximum of six alloantibodies in one patient), and in 10.2% of cases the antibody screening routine was not able to fully identify it. The alloantibodies identified are listed in Table 2 in order of frequency.

A positive direct antiglobulin test (DAT) was identified in 22 of 52 ALLO patients and in only one of 48 NON-ALLO (P=.0001). The autoantibodies were warm-reactive to IgG with a typical panagglutination pattern in eluate. All patients had documented alloantibodies before autoantibody formation except the aforementioned patient. Despite the fact that a positive DAT does not always have clinical relevance, a few patients developed clinically significant immune hemolysis and were treated with prednisone with favorable responses. The majority of patients received transfusions because of severe symptomatic anemia, leg ulcers and intractable vaso-occlusive painful crises. Other less common causes were
Table 1. Patients’ clinical and laboratory characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>n*</th>
<th>SCD alloimmunized (n=56)</th>
<th>SCD non-alloimmunized (n=52)</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>108</td>
<td>30.1 (±8.1)</td>
<td>34.3 (±12.4)</td>
<td>.041†</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>108</td>
<td>20/36</td>
<td>28/24</td>
<td>.081</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>105</td>
<td>7.2 (±1.6)</td>
<td>7.5 (±1.2)</td>
<td>.235</td>
</tr>
<tr>
<td>White blood cell count (×10^9/L)</td>
<td>104</td>
<td>12.6 (±3.8)</td>
<td>11.5 (±3.6)</td>
<td>.125</td>
</tr>
<tr>
<td>Absolute neutrophil count (×10^9/L)</td>
<td>97</td>
<td>7969.8 (±5.8)</td>
<td>6545.6 (±3.8)</td>
<td>.157</td>
</tr>
<tr>
<td>Platelet count (×10^9/L)</td>
<td>101</td>
<td>413.2 (±169)</td>
<td>385.9 (±123)</td>
<td>.319</td>
</tr>
<tr>
<td>Reticulocyte count (%)</td>
<td>84</td>
<td>7.0 (±5.3)</td>
<td>6.5 (±4.5)</td>
<td>.631</td>
</tr>
<tr>
<td>Fetal hemoglobin (%)</td>
<td>70</td>
<td>6.8 (±5)</td>
<td>8.3 (±5)</td>
<td>.234</td>
</tr>
<tr>
<td>Positive direct antiglobulin test</td>
<td>100</td>
<td>22/52</td>
<td>1/48</td>
<td>.0001‡</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>81</td>
<td>6.1 (±2.3)</td>
<td>6.2 (±2.6)</td>
<td>.999</td>
</tr>
<tr>
<td>Lactate dehydrogenase (U/L)</td>
<td>70</td>
<td>886.4 (±451.6)</td>
<td>1046.9 (±759.4)</td>
<td>.283</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>95</td>
<td>27.36 (±17.56)</td>
<td>36.56 (±39.73)</td>
<td>.141</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>98</td>
<td>.72 (±0.3)</td>
<td>.85 (±0.5)</td>
<td>.144</td>
</tr>
<tr>
<td>Leg ulcer</td>
<td>108</td>
<td>285/6</td>
<td>31/52</td>
<td>.340</td>
</tr>
<tr>
<td>Stroke</td>
<td>106</td>
<td>4/51</td>
<td>4/55</td>
<td>1.000</td>
</tr>
<tr>
<td>Renal disease</td>
<td>63</td>
<td>9/24</td>
<td>22/39</td>
<td>.145</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>55</td>
<td>8/37</td>
<td>14/18</td>
<td>.0001‡</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>51</td>
<td>4/14</td>
<td>24/37</td>
<td>.020†</td>
</tr>
<tr>
<td>Number of transfusions received</td>
<td>95</td>
<td>14.96 (±12.97)</td>
<td>10.55 (±9.32)</td>
<td>.039</td>
</tr>
</tbody>
</table>

* Number of patients with available data.
† P values comparing the continuous variables were obtained by independent-samples t-test;
P Values comparing categoric variables were obtained by chi-square test. All the tests were 2-tailed.
‡ Significant (P<.05).

complicated infections, pregnancy, priapism, renal dysfunction, cardiac complications, pulmonary hypertension, ASC, stroke and preparation for surgery. Whichever the indications of transfusion were, there was no significant difference between ALLO and NON-ALLO patients.

Progressive elevation of serum creatinine levels, microalbuminuria, macroalbuminuria as well as end-stage renal failure were identified in patients either ALLO or NON-ALLO. Cardiac abnormalities were cardiomegaly, heart murmurs, valvulopathies and arrhythmias. Rheumatic valvulopathy was identified as a co-morbidity among the patients as well. The retina was affected in 28 patients (4 ALLO), mostly with severe retinopathy. The most prevalent Rh system haplotypes were R1 (DCE), R0 (Dce) and R2 (DCE), in order of frequency (respectively, 37.9%, 24.2% and 15.2%).

**DISCUSSION**

The lack of neonatal screening (NS) explained why so many patients had a late SCD diagnosis. As stated before in this article, this diagnostic tool was available in Bahia, Brazil only from the year 2000. Neonatal screening can give an opportunity for patients to have access to precocious medical care, with periodic referrals to comprehensive evaluations. In Bahia, a state with almost 14 million people, there are only two SCD reference centers, both in Salvador, the capital. A better health supervision system must urgently be organized in Bahia State in order to provide SCD patients with timely and appropriate care.

Age has been correlated with the risk of alloimmunization in SCD. Rosse et al, in the large Cooperative Study of Sickle Cell Disease showed that children who received their first transfusion at age 10 years or older had a higher rate of alloimmunization compared to the ones whose first transfusion was before that age. In our sample, age at first transfusion was not significantly different between ALLO and NON-ALLO patients. Despite this, ALLO patients were younger than NON-ALLO, a statistically significant difference as shown in Table 1.

According to Vichinsky et al, the risk of allosensitization increases with the number of transfusions. This could not be demonstrated in our study. Anti-E, anti-K, and anti-C were the most prevalent alloantibodies identified among our patients. Similar data have been reported in the literature. Vichinsky et al found allo-anti-E in 24% of their SCD sample, while in our patients it prevailed in 39.3%. In Vichinsky’s study, allo-anti-K occurred in 26% of patients (compared to 21.4% in ours), and anti-C was identified in 16% (16.1% in our sample). In our center, antibody screening is performed right before transfusion and is not rechecked periodically unless a new transfusion is needed. The cost of antigen matching is high, and checking it from time to time, even if more transfusions are not needed for some time, may not prevent hyperhemolysis, the most severe clinical complication in this setting. In our patients, there were no registered instances of hyperhemolysis syndrome, and only a few, with or without alloantibodies, developed transfusion reactions, most of the febrile
**Table 2. Alloantibodies identified in 56 SCD patients**

<table>
<thead>
<tr>
<th>Alloantibody specificity*</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-E</td>
<td>25</td>
<td>39.3</td>
</tr>
<tr>
<td>Anti-K</td>
<td>12</td>
<td>21.4</td>
</tr>
<tr>
<td>Anti-C</td>
<td>9</td>
<td>16.1</td>
</tr>
<tr>
<td>Anti-Fya</td>
<td>3</td>
<td>5.3</td>
</tr>
<tr>
<td>Anti-Lea</td>
<td>3</td>
<td>5.3</td>
</tr>
<tr>
<td>Anti-Leb</td>
<td>3</td>
<td>5.3</td>
</tr>
<tr>
<td>Anti-e</td>
<td>2</td>
<td>3.6</td>
</tr>
<tr>
<td>Anti-D</td>
<td>2</td>
<td>3.6</td>
</tr>
<tr>
<td>Anti-M</td>
<td>2</td>
<td>3.6</td>
</tr>
<tr>
<td>Anti-c</td>
<td>2</td>
<td>3.6</td>
</tr>
<tr>
<td>Anti-Fyb</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>Anti-jkb</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>Anti-S</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>Anti-VS</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>Anti-Lu</td>
<td>1</td>
<td>1.8</td>
</tr>
</tbody>
</table>

* Alloantibodies were identified either single or in combination with other alloantibodies.
  One patient developed six antibodies against red cell antigens at one time.

non-hemolytic type. The use of leukocyte-removing filters promoted a decrease in this complication among our patients. A higher prevalence of alloimmunization has been described in females. Aygun et al found no difference in the antibody rates between sexes in SCD pediatric patients. As opposed to the prevalence in children, the same authors described higher allosensitization rates in adult females. Other authors reported an incidence of alloantibodies formation to be 1.5 times more likely for a given transfusion in SCD children and adult females than in adult males. Women would have more antigen exposure due to pregnancy and delivery, possibly explaining the higher rates of alloimmunization among SCD females. In our patients, 64.3% of the alloimmunized patients were female, a statistically significant difference (P = .033).

Our findings showed higher levels of autoantibody production among ALLO patients. The pathogenesis of autoimmunization following transfusions is not well understood. The development of erythrocyte autoantibodies was reported by Castellino et al studying transfused children with SCD, identified in 7.6% of cases. Aygun et al, studying immunization to RBC antigens in pediatric and adult SCD patients, reported autoimmunization in 8% of children and 9.7% of adults. In the SCD setting, binding of alloantibodies to the transfused erythrocytes could lead to conformational changes in antigenic epitopes, which would stimulate production of autoantibodies. Another possible explanation would be that formation of autoantibodies in SCD patients could reflect a global dysfunction of the immune system due to precocious autosplenectomy secondary to repetitive infarctions. Genetic determination may be involved in autoantibody formation, as stated by Ofosu et al in their study of the major histocompatibility complex in SCD patients.

In our sample the number of patients receiving RBC transfusions because of chronic leg ulcers (duration >6 months) was a real concern. Wanko and Telen, in their paper addressing transfusion management in sickle cell disease, considered the use of RBC transfusion in SCD leg ulcers as controversial, while Serjeant et al stressed the fact that there are no data to support transfusion therapy for SCD leg ulcers. In another study where 225 SS subjects were examined, the strongest predictors of chronic ulceration were high serum LDH, venous incompetence and low social economic status. In Salvador, Brazil, chronic leg ulcers were one of the main reasons why patients looked for medical assistance. Genetic mechanisms may be involved in the predisposition to leg ulcers. Ofosu, Castro and Alarif, determining HLA-A,B,C and DR types in patients with sickle-cell anemia who had chronic leg ulcers or a history of leg ulcers and in SS controls without leg ulcers, reported that the relative risk for development of leg ulcers in patients who had both HLA-B35 and Cw4 was 17 times greater than in patients without these antigens or who had only one antigen.

In our patients, cerebrovascular accidents were identified in the same proportion among patients with and without alloimmunization. The cumulative evidence favoring chronic transfusion, either for primary prevention of stroke or recurrence of a previous episode, led us to change the transfusion policy in our center in the year 2004, as stated before in this article. Hoppe et al emphasized that clarifying the genetic basis for stroke in SCD would help identify high-risk patients for cerebrovascular accidents. That would allow preventive interventions such as chronic transfusion or bone marrow transplantation, while avoiding the use of these therapies in those patients who are at a reduced risk.

Progressive renal dysfunction was expected to occur in a significant proportion of our patients, as our sample was constituted of adult patients. Guasch et al, studying adult SCD patients, showed that, at age 40 years, 40% of patients with SS disease had macroalbuminuria.

It is well-known in the context of SCD that early stages of ocular complications may be asymptomatic, in spite of the risk of severe visual consequences as the disease progresses. In our center there were ALLO and NON-ALLO patients who had the first SCD diagnosis while investigating complaints of
ALLOIMMUNIZATION IN SICKLE CELL DISEASE - Zanette et al

visual deficit. This illustrates how late diagnosis can also be cause of remarkable morbidity in SCD.

Echocardiogram, recognized as a very useful exam to be done in SCD patients, could not be performed in all of our patients. That was probably the reason why we found only a few cases of pulmonary hypertension (PH), one case among ALLO and two among NON-ALLO patients. According to Ohene-Frempong and Steinberg cardiac examinations in SCD patients are rarely normal.33 Sachdev et al suggested that 18% of all adults with SCD have diastolic dysfunction and that 11% have both pulmonary hypertension and left ventricle diastolic dysfunction.34 Additionally, cardiac abnormalities in SCD may result from myocardial iron overload, due to blood transfusions, or from ischemic damage secondary to repetitive vasoocclusive episodes.33,35

Recently, Campbell-Lee suggested that recipient inflammatory status could be a risk factor for alloantibody development.36 SCD is recognized as an inflammatory status, where the adhesive interactions involving red cells and damaged endothelium have a central role in the pathophysiology of the disease.37—39 Hendrickson et al, in a murine model of alloimmunization, found recipient inflammation significantly increased the development of alloantibodies.40 Therefore allo sensitization in SCD may be related to the number of transfusions received, the genetic predisposition, and inflammation.

Our study has weaknesses such as retrospective design and a small number of patients. Moreover, a possible underestimation of total number of blood transfusions cannot be ruled out. Nevertheless, our study addresses a relevant issue. In spite of a better current management of SCD occurring in the United States and Europe, in Bahia-Brazil, many SCD patients still do not have access to proper diagnosis and treatment. As life expectancy of SCD improves more blood transfusions are expected to be needed and allo sensitization is an important problem to be addressed. A better understanding of the alloimmunization mechanisms and of their impact on SCD clinical outcomes will help in the identification of patients at risk. It will also help in developing strategies to avoid the clinical complications of allo sensitization.

CONCLUSIONS

Although alloimmunization is a potentially serious complication of blood transfusions, we did not find relevant clinical differences between SCD patients who developed alloantibodies and those who did not. As SCD patient lifespan improves, further studies in biochemical and genetic markers, and clinical and transfusion issues, such as alloimmunization, will prevent morbidity in these patients. Finally, we stress the urgent need for local measures to be developed to facilitate the access of SCD patients to proper diagnosis and treatment.

Although alloimmunization is a potentially serious complication of blood transfusions, we did not find relevant clinical differences between SCD patients who developed alloantibodies and those who did not.

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**AUTHOR CONTRIBUTIONS**

*Design, Concept of Study:* Dias Zanette, Santos Bahia, Vasconcelos Nogueira, Freitas Brandão, Neves de Azevedo, Marcos Arruda

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*Manuscript Draft:* Dias Zanette, Vilasboas Schettini, Magalhães Aguiar

*Statistical Expertise:* Dias Zanette, Souza Gonçalves, Vasconcelos Nogueira

*Acquisition of Funding:* Dias Zanette

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