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RESEARCH ARTICLE

Pre-transfusion compatibility tests in sickle cell disease

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Abstract

Introduction: Blood transfusion is a key component of sickle cell disease management. However, iterative transfusions of labile blood products for sickle cell patients increase the risk of anti-erythrocyte alloimmunization. It is usual to perform pre-transfusion compatibility tests in order to check ABO compatibility and to detect the possible existence of irregular antibodies in patients against the donor's antigens.

Methods: This is a prospective and descriptive study dealing with pre-transfusion compatibility tests over a 3-month period, from September 2018 to November 2018, at the Regional Blood Transfusion Centre (CRTS) of Analamanga Region, the capital of Madagascar.

Results: We included 33 out of 113 patients during the study period. Compatibility test was positive in 21.21%, of all tested samples. Major and minor compatibility tests were positive alone respectively in 6.25% and 12.50%. And both were positive in 3.12%. The self-test was positive in 3.03% with a positive direct antiglobulin test. Positive compatibility tests were more frequent in patients who received more than two transfusions. Compatible blood bag for the patient was found after testing 2 donor pockets in 27.27% of cases, and after testing 3 pockets in 3.03%.

Conclusion: Pretransfusion compatibility tests are essential and have to be performed systematically on each blood bag before any transfusion. They are used to identify the presence of alloantibodies and provide compatible blood products to patients.

Keywords: compatibility test, major/ minor tests, sickle cell disease, transfusion

Introduction

Sickle cell disease is a genetic pathology with autosomal recessive transmission. It is due to an abnormality in the hemoglobin structure leading to hemoglobin S formation (HbS) (1). It is the most common genetic disease in the world. According to the WHO, the estimated number of people affected by sickle cell disease is about 120 million, the 2.3% of the world population (2). Madagascar is classified among the areas with high sickle cell endemicity with an estimated prevalence of 10% throughout the country (3).

Therapeutic management of sickle cell disease is based on several strategies, including red blood cell transfusion, which is essential in the case of acute complications such as vaso-occlusive crisis, acute thoracic syndrome and cerebrovascular accident. The difference in blood groups according to the geographical origin of individuals but also in a population in another country is at the root of transfusion incompatibility problems. Patients with sickle cell disease may have multiple immuno-hematological characteristics: variant phenotypes (especially in the Rhesus and MNS systems), rare blood groups, allo- and autoimmunization due to the chronic inflammatory state (4).

Iterative transfusions of labile blood products expose sickle cell patients to anti-erythrocyte alloimmunization. The greater is the number of transfusions, the greater is the risk of alloimmunization. The occurrence of this alloimmunization can lead to transfusion impasse, post-transfusion hemolysis and a high frequency of autoantibodies. This results in the production of irregular agglutinins, a significant delay of time required to obtain compatible red blood cells, poor transfusion performance and an impact on the severity of the disease (5).

It is therefore essential to carry out compatibility tests before each transfusion in order to avoid the appearance of alloantibodies and to compromise the future transfusion of sickle cell disease patients. Pre-transfusion compatibility tests are used to check ABO compatibility as well as to detect possible irregular antibodies in the recipient against the donor's antigens. Our study aimed to describe the results of pre-transfusion compatibility tests in sickle cell patients in Antananarivo, and to highlight the possible presence of alloantibodies.

Materials and methods

A prospective and descriptive study of pre-transfusion compatibility tests between sickle cell patients and blood donor bags was performed. Selection was exhaustive for all sickle cell patients seen in the Center during the study period. We collected demographic data from the center register including age, gender, and sickle cell disease genotype as described by hemoglobin electrophoresis.

Blood samples from sickle cell patients requiring transfusion were collected from the reference center for the management of sickle cell disease.

Pre-transfusion compatibility tests were performed at the Analamanga Regional Blood Transfusion Centre. Both of them are located at the University Center Hospital Joseph Ravoahangy Andrianavalona, the biggest hospital in Antananarivo, the Capital of Madagascar. The study took place from September to November 2018. We included all requests for blood bags for sickle-cell patients, with a blood sample on EDTA tube and complete application forms. Sickle cell patients who required treatment other than transfusion, and/or incomplete application forms, and/or in the absence of a blood sample on EDTA tube were excluded. We performed pre-transfusion compatibility tests using the indirect antiglobulin test gel filtration technique (Antiglobulin test Anti-IgG, ref 004024, BIO-RAD). Three tests were performed: a major compatibility test, a minor compatibility test and a self-test for each sample. Incubation of the ID card for each test lasted for 15 minutes at 37°C in the water bath followed by centrifugation of the ID card in the ID centrifuge for 10 minutes before reading one or more reactions as recommended by the manufacturer guidelines. Data were collected and recorded on Microsoft Excel 2016 software. Statistical analysis was carried out using Epi Info software version 7.1.

The major compatibility test consisted of observing the reaction between the donor's red blood cell and the recipient's plasma. We mixed 50 µl of the red cell suspension from the donor(s) with 25 µl of the patient's plasma to each microtube. The minor compatibility test consisted of testing for donor antibodies to recipient red blood cells. We mixed 50 µL of the patient's red blood cell suspension with 25 µl of donor plasma to each microtube. The self-test consists of highlighting the recipient's antibodies against its own red blood cells. For this purpose we mixed 50 µl of the patient's red cell suspension with 25 µl of the patient's plasma to each microtube. Compatibility tests and self-test were considered positive if the agglutinated red blood cells form a red line on the gel surface or if the agglutinates are dispersed in the gel. The tests were negative if the red blood cells are in compact pellet at the bottom of the microtube. A negative reaction indicates that there is no detectable antibody against the donor's erythrocyte antigens in the recipient. And a positive reaction indicates an incompatibility between the donor and the recipient, indicating the presence of antibodies against the donor's antigens in the recipient. In the case of a positive compatibility test, we rechecked the ABO and Rh D blood groups for both donor and patient and performed a self-test for autoantibodies. We looked for another potential donor and re-tested until a donor with a negative reaction to the patient was found.

In response to a positive self-test, we performed a direct antiglobulin test (ADD) to detect an auto-antibody. We distributed 50 µl of the patient's red cell suspension to the appropriate microtube. Then the ID card was centrifuged for 10 minutes in the ID-centrifuge followed by reading the reactions (Figure 1).

Results

During the period of study, 113 sickle cell patients came at the reference center for the management of sickle cell disease at the University Center Hospital Joseph Ravoahangy Andrianavalona. We included 33 of them (Figure 2). Female predominance was found with a sex ratio of 0.83. The average age was 18.60 years with extremes of 3 years and 81 years. The population <15 years of age was the most represented (60.6%). The SS genotype was the most represented with 31 cases or 93.94%. One case (3.03%) of the AS genotype and one case (3.03%) of the ASC genotype were registered. The main reason for the request for transfusion were transfusion exchange in 78.79% of cases (26/33), followed by severe anemia in 15.15% of cases (5/33) (Table 1.) Our study showed that 45.45% (15/33) of the patients had B RhD+ blood group and 39.39% (13/33) had O RhD+ blood group.

For 90.91% of patients, one blood pocket was requested per patient; 2 bags were requested for 2 patients (6.06%) and 3 bags were requested for one patient.

Compatibility tests were positive in 21.21% of all tests (7/33). There was a reaction between donor blood and recipient blood for all the 7 patients. The auto-test was positive for one patient (3.03%) for whom direct antiglobulin test was positive. Positivity for the 32 remain patients is described in Table 2. We found 4 cases (12.50%) positive on the minor compatibility test, 2 positive cases (6.25%) on the major compatibility test and one positive case (3.12%) on both tests. Positive

compatibility tests were more frequent in 42.85% of patients who received more than two transfusions. In 69.70%, a compatible blood bag was found after testing 1 donor bag. But in 27.27% of cases, 2 donor blood bags were tested before finding a compatible blood bag for the patient and 3 bags in 3.03%.

Discussion

Gender predominance in Antananarivo sickle cells patients differs depending on the type of study (6). Diagne *et al* in Senegal have found male predominance (7). In fact, the possible difference found in previous studies in different countries would be related to the demographic data of each country and the recruitment of the study population because the transmission of sickle cell disease is known to be not gender-related (8,9).

The average age was 18.60 years and 60.60% were under 15 years of age. Sickle cell disease mainly affects children and young people. The low rate of sickle cell disease after the age of 60 in our study could be explained by the premature death of sickle cell patients due to the complications of sickle cell disease but also to the association with other age-related diseases. Complications of sickle cell disease occurs frequently in the majority of homozygous sickle cell patients, leading to a higher frequency of hospitalization. In this study, 31 out of 33 cases (93.94%) were homozygous with SS genotype, 1 patient (3.03%) was heterozygous with AS genotype and 1 case (3.03%) was classified as sickle cell SC. Homozygous sickle cell patients are the most concerned by complications (6). They regularly have scheduled transfusion to avoid major complications. This can explain the predominance of homozygous sickle cell cases versus heterozygous sickle cell cases in our study as well as Suell et *al'* study, conducted in Texas (10). During a sickle cell crisis, transfusion can be done in three ways for a specific purpose (11): a simple transfusion to correct hypovolemia and anoxia, a transfusion exchange that can be performed on an emergency basis (12). This exchange is carried out in order to avoid hyperviscosity by keeping the hemoglobin S level below 30%. Long-term transfusion is used as a preventive measure or during frequent complications (priapism, neurological accident), in order to obtain a hemoglobin A level equal to or higher than 50% (13).

The rates of positive major and minor compatibility test that we found reflected the transfers of units of blood incompatible with the presence of antibodies against the donor's red blood cells in the recipient. Only the antiglobulin test was performed in this study due to the absence of other new techniques. The techniques used for these tests can influence the test results.

The rate of positive compatibility test that we found is consistent with the result found by Novaretti *et al* in Brazil (14). A self-test is required because compatibility tests can only be interpreted in the event of a negative self-test. A positive compatibility test indicates reaction between the blood of recipient and the donor, so the blood bag cannot be delivered.

The major compatibility test, mandatory on each blood bag, and the minor compatibility test are not performed if the research of irregular agglutinin is performed on the donor. This latter is not performed at the Regional Center of Analamanga, reason why we simultaneously performed the major and minor compatibility tests. The major compatibility test is part of the pre-donation biological controls.

It is mandatory to do this test before any transfusion of red blood cells. This offers the possibility of checking the absence in the recipient of antibodies recognizing antigens present on the donor red blood cells. One way to make the transfusion safe is then to do a nominal distribution of the red blood cell. But

it does not exempt in any way the patient from ultimate control in bed. Its validity is 72 hours after the sampling date.

The minor compatibility test is mainly used for the transfusion of fresh frozen plasma. But although our patients are all transfused into red blood cells, we can still find donor antibodies in the blood bags because during whole blood separation, a reduced amount of plasma must be added to the red blood cells to provide the proteins and nutrients for the survival and storage of red blood cells. The minor compatibility test should be performed in the absence of alloantibodies in donors, especially for those with a history of transfusion, pregnancy, abortion or miscarriage due to the risk of possible immunization with the development of erythrocyte antibodies (15). The request for alloantibody testing is not systematic in Malagasy daily practice and especially in terms of blood transfusion. The introduction of minor compatibility testing in current practice is beneficial for patients to avoid possible transfusion-related accidents. Indeed, the donor's antibodies may recognize some of the antigens present on the surface of the patient's red blood cells.

Post-transfusion reactions are less important, but sometimes they can be responsible for hemolysis with adverse effects in the patient such as anuria, shock, renal failure or transfusion inefficiency. According to the High Council of Health (HCC), all units from the same donor should be recalled to perform an alloantibody screening with an identification panel to investigate antibody specificity and perform the donor phenotype (15).

The pre-transfusion compatibility test is very important because it makes possible to highlight agglutination reactions caused by antibodies not detected by alloantibody screening. They are directed against antigens not expressed by the test cells, but possibly expressed by the red blood cells selected for transfusion, especially if "intra-ethnic" transfusion is performed (16).

In our study, the self-test was positive in 3.03% and negative in 96.97%. Baglo T *et al* in Benin had found a positive self-test much lower than ours: 0.97% (17). The sickle cell patients most often receive blind transfusion, which could cause this reaction. Anti-erythrocyte alloimmunization promotes the production of anti-erythrocyte autoantibodies resulting in complex alloantibody screening, poor transfusion performance and the possibility of autoimmune hemolytic anemia (18).

Preliminary alloimmunization plays a role due to cross specificity and stimulation of self-reactive T clones by cytokines produced during alloimmunization (IL4) (18).

Positive compatibility tests were more frequent in patients who received more than two transfusions during our study: at 42.85%. Limited by the size of our study sample, we were unable to establish significant links between the positivity of compatibility tests and the number of transfusions received (p>1). Our results were comparable to the one found by Baglo T *et al* with 56% positive accounting tests in patients transfused with more than 2 bags (17). The risk of immunization increases with the number and rate of stimulation. Transfusion alloimmunization is "all-round" and extends many blood group systems.

Development of alloantibodies may compromise the transfusion future of sickle cell patients. Hence the importance of carrying out pre-transfusion compatibility tests for optimal transfusion safety in sickle cell patients. The test is valid for 72 hours after the date of sampling (18). But it does not in any way exempt the patient from ultimate control in bed.

Auto-antibodies can decrease transfusion performance if they also sensitize transfused red blood cells because they may have a greater affinity for transfused red blood cells, as the patient's red blood cells are already

saturated. The combination of autoantibodies and alloantibodies is very common. It is difficult to detect alloantibodies hidden by autoantibodies.

We highlighted the presence of alloantibodies in sickle cell patients (19).

Nonetheless, it has had its limitations: the research for and identification of irregular antibodies in sickle cell disease, in the case of a positive compatibility test, as well as the extended phenotyping of sickle cell disease and donor pockets could not be carried out (20). Elution and identification of the autoantibody, in the event of a positive self-test, as well as self-adsorption or treatment with Dithiothreitol (DTT) to search for an alloantibody hidden by the autoantibody, could not also be performed.

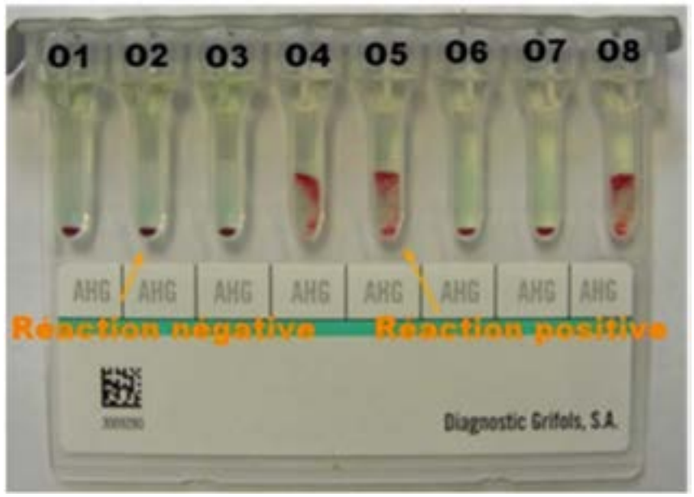

Figure 1: Positive and negative aspects of gel card during compatibility tests
Source: Authors 2018, Antananarivo

Figure 2: Recruitment process of study subjects

Table 1: Population characteristics

Parameters	Number (n=33)	%
Gender		
Male	15	45.45
Female	18	55.45
Age (years)		
<15	20	60.60
15-30	8	24.24
30-45	2	6.06
45-60	1	3.03
60-75	1	3.03
>75	1	3.03
Genetic status		
SS	31	93.94
AS	1	3.03
SC	1	3.03
Number of transfusions		
1	9	28.6
2	9	28.6
>2	15	42.8

Table 2: Distribution of compatibility test results

N=32*	Major CT + N=3	Major CT – N=29
Minor CT + N=5	01 (3, 12%)	04 (12, 50%)
Minor CT – N=27	02 (6, 25%)	25 (78, 12%)

** One case (01) among 33 patients had positive autotest on antiglobulin test*

CT: Compatibility Test

Conclusion

Iterative transfusions of labile blood products expose sickle cell patients to anti-erythrocyte alloimmunization and the vital prognosis can be initiated. The presence of alloimmunization can lead to a significant delay in the time required to obtain compatible red blood cells, to a difficulty in supply and even to a real transfusion impasse. Pre-transfusion compatibility tests are an essential test before each transfusion. The major compatibility test must be performed for all applications for red blood cells and the minor compatibility test for all applications for fresh frozen plasma to avoid post-transfusion accidents and alloimmunization in sickle cell patients that could compromise the transfusion and obstetrical future.

Conflicts of interest

No conflicts of interest.

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