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High frequency of autoimmunization among transfusion-dependent Tunisian thalassaemia patients

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ABSTRACT

Background: Limited data are available on the frequency of RBC alloimmunization and autoimmunization in transfusion-dependant Tunisian β thalassaemia patients.
Materials and methods: We analyzed the clinical and transfusion records of 130 patients (57 females and 73 males; mean age 119 months; range 12–11 months) with β thalassaemia major and who had regular blood transfusions for periods ranging from 12 to 311 months.
Results: Of the 130 patients, ten (7.7%) developed RBC alloantibodies. The most common alloantibodies were directed against antigens in the Rh systems. Erythrocyte-autoantibodies as determined by a positive direct antiglobulin Coombs test, developed in 52(40%) patients with and without underlying RBC alloantibodies, thereby causing autoimmune haemolytic anaemia in eleven patients (21%).
Conclusions: Autoimmunization to erythrocyte antigens is a frequent complication in patients with β thalassaemia major. Several factors might have contributed to the high autoimmunization rate observed in this study, including non phenotypic blood exposure and alloantibody formation prior to positive Coombs test.

1. Introduction

Thalassaemia major is one of the most common hereditary diseases in Tunisia. Although its true incidence is unknown, it is estimated that 2.5% of Tunisian population harbour thalassaemic trait. Lifelong red blood cells (RBCs) transfusion remains the main treatment for severe homozygous β thalassaemia [1]. There are numerous risks and a considerable morbidity associated with chronic transfusion therapy [2]. Each unit of blood carries a small but definite risk of transmitting infections, including the human immunodeficiency virus, and hepatitis viruses [3]. In addition, long term erythrocyte transfusions inevitably lead to severe iron overload [4]. Finally, repeated blood exposure can induce alloimmunization to erythrocytes antigens, leading to difficulty in identifying compatible blood. Varying frequencies of alloantibody formation in transfused patients have been reported [5,6].

The formation of autoantibodies against RBCs has been documented in previous studies [7,8], and the majority of the previously published cases of RBC autoimmunisation reported strong association with RBC alloimmunization [2–9]. There are few reports of autoantibody formation after transfusion in the absence of demonstrable alloimmunization [10]. Autoantibodies are directed against the individual's own RBCs which can result in clinical haemolysis and difficulty in cross-matching blood. Patients with auto antibodies may have a higher transfusion rate and often require immunosuppressive drugs, splenectomy or alternative treatments.

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2. Background

The present study was designed to explore the prevalence of auto and allo antibody formation in transfusion dependant Tunisian thalassaemic patients, to evaluate factors influencing red cell autoimmunization and clinical significance of positive antiglobulin test and its transfusion's repercussion.

3. Materials and methods

3.1. Study population

One hundred and thirty transfusion dependant thalassaemic patients (57 females and 73 males; mean age 119 months; range 12–311 months) were included in the study. One hundred and eleven patients were previously exposed to non leucoreduced red blood cells (RBCs), and 19 were transfused since their first transfusion by filtrated red blood cells.

For each patient were identified: age, the presence of allo or autoimmunization, antibody specificity, age of antibody formation, blood exposure and spleen presence prior to antibody formation.

3.2. Methods

For each patient, we achieved systematically a serum antibody screening before each transfusion and a direct antiglobulin test (DAT) after three transfusions. DAT was performed with not washed patients RBCs, using the gel-filtration test: commercial ID-card "DC Screening I" DiaMed-ID Micro Typing System, consisting of monospecific antihuman globulin reagents, anti-IgG, anti-IgA, anti-IgM, anti-C_{3c} (rabbit), monoclonal antiC_{3d}, suspended in gel and a negative control. Antibody serum screening has been performed with a panel of a test RBC s prepared by NBTC using two different gel test methods: the indirect antiglobulin test with polyvalent antihuman antiglobulin (ID-card liss Coombs DiaMed-ID) and the enzymatic test with Bromeline treated RBCs (ID-card Nacl-enzyme DiaMed-ID).

We consider the DAT as positive only if positivity persists over 6 months. Occurrence of autoimmune haemolytic anaemia (AIHA) was based on a loss of haemoglobin exceeding 1 g/dl/week and a persistent positive DAT over 6 months without evident signs of hypersplenism.

3.3. Statistical analysis

Fisher's exact test was used to assess intergroup significance between categorical variables, and Student's *t*-test was used to determine differences between continuous variables. Logistic regression was performed to estimate odds ratio (OR) and 95% confidence interval (CI). Statistical analysis was carried out using software (SPSS version 11.5). A p value <0.05 was considered statistically significant.

4. Results

4.1. Development of erythrocytes alloantibodies

Alloantibodies were detected in 10 of 130 studied patients (7.7%). Eight patients developed alloantibodies before autoantibody formation. Identified alloantibodies belonged mainly to rhesus system (Rh) with two alloantibodies in two cases (Table 1).

4.2. Development of erythrocytes autoantibodies

Among 130 studied patients, 52 patients (40%) developed auto antibodies. In 44 cases a warm (IgG) autoantibody was identified by the direct anti globulin test. IgG with complement were identified in the other 8 cases. The mean time of autoantibody formation was 99 months of blood exposure with a range 7–220 months. Multiple factors influence autoimmunization in polytranfused β thalassaemia patients (Table 2).

Multivariate analysis of our data was illustrated in table 3 and showed that aged over 84 months, had a significantly increase risk of developing autoimmunization ($p = 0.001$).

4.3. Repercussion of erythrocyte autoantibody formation on transfusions' needs

Among the 52 studied patients with DAT, 11 patients developed haemolytic anaemia (21%). All nine patients needed a treatment with steroids for a mean period of 18 months. The only factor contributing to the increase of transfusion's needs in multivariate analysis, was alloimmunization prior to autoantibody formation ($p = 0.05$; odds ratio = 9.1).

5. Discussion

The development of erythrocytes autoantibodies after blood transfusions was first described over 60 years ago. Dameshek and Levine [12] reported a transfused patient who developed Rh alloantibodies followed by near fatal autohaemolysis. A similarly transfused patient with JKa alloantibody suffered from a fatal autohaemolytic crisis documented by radiolabelled chromium studies.

Lalezari et al. [13], described a woman who formed an erythrocyte autoantibody simultaneously with an anamnestic response of an Rh alloantibody, resulting in a severe haemolytic transfusion reaction.

To our knowledge, this is the first large prospective cohort reporting allo and autoimmunization in transfusion

Table 1
Number and specificity of alloantibodies in the 10 alloimmunized patients.

Alloantibody specificity	Patients number	%
AntiD	1	10
AntiE	3	30
AntiC	3	30
AntiK	1	10
Anti E + S	1	10
Anti D + C	1	10
Total	10	100