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Prophylactic strategies for acute hemolysis secondary to plasma-incompatible platelet transfusions: correlation between qualitative hemolysin test and isohemagglutinin titration

*Cinthia Silvestre Landim, Francisco Carlos Almeida Gomes, Bernardete Martin Zeza, Alfredo Mendrone-Júnior, Carla Luana Dinardo**

Fundação Pró-Sangue, Hemocentro de São Paulo, São Paulo, SP, Brazil

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ABSTRACT

Objective: Brazilian legislation has recently suggested the use of the qualitative hemolysin test instead of isohemagglutinin titers as prophylaxis for acute hemolysis related to plasma-incompatible platelet transfusions. The efficacy of this test in preventing hemolytic reactions has never been evaluated while isohemagglutinin titers have been extensively studied. The main objective of this study was to evaluate the correlation between the results of these two tests. The impact of each type of prophylaxis on the platelet inventory management and the ability of the qualitative hemolysin test to prevent red cell sensitization after the transfusion of incompatible units were also studied.

Methods: A total of 246 donor blood samples were evaluated using both isohemagglutinin titers and the qualitative hemolysin test, and the results were statistically compared. Subsequently, 600 platelet units were tested using the hemolysin assay and the percentage of units unsuitable for transfusion was compared to historical data using isohemagglutinin titers (cut-off: 100). Moreover, ten patients who received units with minor ABO incompatibilities that were negative for hemolysis according to the qualitative hemolysin test were evaluated regarding the development of hemolysis and red cell sensitization (anti-A or anti-B).

Results: Isohemagglutinin titration and the results of qualitative hemolysin test did not correlate. The routine implementation of the qualitative hemolysin test significantly increased the percentage of platelet units found unsuitable for transfusions (15–65%; p-value <0.001). Furthermore the qualitative hemolysin test did not prevent red blood cell sensitization in a small exploratory analysis.

Conclusion: Qualitative hemolysin test results do not correlate to those of isohemagglutinin titers and its implementation as the prophylaxis of choice for hemolysis associated with plasma-incompatible platelet transfusions lacks clinical support of safety and significantly affects platelet inventory management.

* *Corresponding author at*: Hemocentro de São Paulo, Avenida Dr. Enéas de Carvalho Aguiar, 151, 1° Andar, Cerqueira César, 05403-000 São Paulo, SP, Brazil.
E-mail address: caludinardo@gmail.com (C.L. Dinardo).

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Introduction

The transfusion of non-ABO identical platelets may be associated with acute hemolysis, fever, recipient inflammation and a decreased response in the post-transfusion platelet count.[1] There are two types of ABO incompatibilities: (1) major, in which the recipient plasma is not compatible with the transfused platelets, a situation associated with a suboptimal response to the transfused product and (2) minor, in which the recipient is exposed to ABO-incompatible plasma when there is the risk of acute hemolytic transfusion reactions.[2] In the routine of any blood bank, the transfusion of platelets with minor ABO incompatibilities is not rare due to the shortage of platelet concentrates and the number of emergency platelet requests when no ABO typing is available.

The incidence of acute hemolysis due to plasma-incompatible platelet transfusions is low (approximately 50 in every 1,000,000 incompatible transfusions),[3] but the severity of the event justifies the application of prophylactic policies. The American Association of Blood Banks (AABB) standards state that the transfusion service shall have a policy concerning the transfusion of components containing significant amounts of incompatible ABO antibodies.[4] Titration of donor isohemagglutinins (anti-A and/or anti-B) followed by the transfusion of incompatible products with titers below 100 is the most studied prophylactic method reported in the literature.[1,5] In spite of the discussion over the safest isohemagglutinin titer, this strategy has already been evaluated in large studies which demonstrated its efficacy in preventing acute hemolysis after plasma-incompatible platelet transfusions.[5–7]

Recently, the Brazilian legislation has suggested the use of the qualitative hemolysin test (QHT) instead of isohemagglutinin titers (IT) as prophylaxis for acute hemolysis secondary to plasma-incompatible platelet transfusions.[8] The rationale is to identify, within the incompatible sera, the presence of antibodies with the ability of causing red blood cell (RBC) lysis, thereby reducing the risk to blood recipients. The proposed test has three possible results: 'absence of hemolysis', 'partial hemolysis' and 'total hemolysis', with the 'partial' and 'total' hemolysis categories precluding transfusion. In spite of its biological plausibility, the efficacy of the QHT in preventing hemolytic reactions after the transfusion of products with minor ABO incompatibilities has never been evaluated in the literature, raising concerns about the safety of its use.

Thus, the main objective of this study was to evaluate the correlation between isohemagglutinin titers (gold-standard prophylaxis) and the qualitative hemolysin test (suggested prophylaxis). A secondary objective was to evaluate the impact of each type of prophylaxis on platelet inventory management and the presence of RBC sensitization by anti-A or anti-B antibodies after plasma-incompatible platelet transfusions tested negative for hemolysis (absence of hemolysis) by QHT.

Methods

Study design

This study was approved by the local Ethics Committee (Faculdade de Medicina da Universidade de São Paulo #797.385). In the first stage, samples obtained from type O platelet donors between January 9, 2014 and September 30, 2014 were evaluated using both the QHT and IT techniques. The QHT was performed in the immunohematology laboratory and the IT was measured in the laboratory responsible for the distribution of platelet units. All donor samples were collected using tubes without anti-coagulant and the QHT was performed within 6 h of collection. The IT was performed directly from the sera of platelet units. The results of QHT and IT were statistically compared using the Kruskal–Wallis test, Chi-square test and logistic regression. Statistical analysis was performed using the SPSS software (18th version) and a p-value less than 0.05 was considered significant.

In the second stage of the study, an exploratory sample of ten patients who received minor ABO-incompatible platelet transfusions were evaluated regarding the direct antiglobulin test (DAT), lactate dehydrogenase (LDH) and indirect bilirubin (IB) before and 1 h after the transfusion of platelet units with negative results for hemolysis using the QHT test. In the specific case of type O platelet units, only those presenting absence of hemolysis with both type A1 and B RBCs were included, irrespective of the recipients' ABO type. All the patients were transfused in a day-hospital regimen and patients were observed for 1 h after the end of transfusion for signs and symptoms of acute hemolysis: fever, dark urine, hypotension and lumbar pain. Increases of 15% in LDH or IB levels were considered evidence of hemolysis as this level exceeds the analytical variability of the laboratory for both tests.

The QHT was also performed in samples obtained from all platelet apheresis donors between June and September 2014. The percentage of units classified as unsuitable for transfusion using the QHT (partial or total hemolysis) was compared to historical data of units classified as unsuitable for transfusion using IT with a cut-off of 100 (Olympus PK 7200). These percentages were compared using the Chi-square test.

Acid elution

Acid elution was performed in cases of positive DAT using the DiaCidel® kit, according to manufacturer's instructions (Biorad®). Briefly, the RBCs of recipients were washed ten times with 0.9% saline solution and 1 mL of the elution solution was added to 1 mL of washed RBC. The mixture was centrifuged and buffer solution was added to the supernatant until it became blue. The eluate was then tested with commercial type A1 and B RBCs.

Isohemagglutinin titration technique

Anti-A and anti-B titration was performed in tubes according to the AABB Technical Manual (18th version).[9] Briefly, the serum of platelet units was sequentially diluted in sterile saline solution from 1:1 until 1:2048 giving a final volume in each case of 100 μL. The titers were added to properly identified tubes containing 50 μL of type A1 or B RBCs (Biorad®). After 15 min of incubation at room temperature the tubes were centrifuged at 3000 rpm for 20 s. The results were interpreted by macroscopically observing hemagglutination and classified as previously described.[9] The platelet isohemagglutinin titer was