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

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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.

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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

represented by the highest titer at which 1+ hemagglutination was observed.

Qualitative hemolysin test

QHT was performed according to a previously published technique.[10] As Brazilian law suggests performing the QHT after 15 min of incubation at 37 °C, this was the first method used in the current study. However, a 30% rate of inter-observer disagreement was detected. In the literature, the equivalent QHT technique uses 45 min of incubation at room temperature. This was also employed in this study without any inter-observer disagreement. Briefly, fresh type A1 and B non-commercial RBCs were used to prepare 3% suspensions for the tests. Fresh donor sera (100 μL) were added to tubes containing 50 μL of specific RBC suspensions (type A1 and/or B). After 45 min-incubation at room temperature, the presence or absence of hemolysis was macroscopically observed in the tubes. The test was considered negative in the absence of hemolysis. If there was hemolysis, the test was considered positive and classified as partial or total hemolysis. Macroscopic evaluation of all samples was performed by one technician and confirmed by a second to avoid any interpretation bias.

Results

Correlation between qualitative hemolysin test and isohemagglutinin titration results

Two hundred and forty-six donors were evaluated using both the QHL and IT techniques. On using the QHT, 61.38% of donors did not exhibit hemolysis and 38.62% exhibited either partial (17.07%) or total hemolysis (21.54%). By IT, 85.8% and 67.1% of platelet units were classified as low-titer considering cut-offs of 1:128 and 1:64, respectively (Table 1).

The median value of IT did not statistically differ between the groups with different QHT results, either when they were classified as absent, partial and total hemolysis (p-value = 0.919) or when they were classified as absence and presence of hemolysis (p-value = 0.733) (Figure 1). In fact, the median value of IT was 32 and the interquartile range was 48 in all analyzed groups.

Neither titers above 64 nor titers above 128 were correlated to the risk of hemolysis according to the QHT (p-value = 0.454 and 0.677, respectively) (Table 2). Logistic regression analysis demonstrated that the IT was unable to predict the QHT results (p-value = 0.702).

Table 1 – Isohemagglutinin titers and the qualitative hemolysin test results of the study sample.[a]

Qualitative hemolysin test	Frequency	Percent
Absence of hemolysis	151	61.38
Partial hemolysis	42	17.07
Total hemolysis	53	21.54
Presence of hemolysis	151	61.38
Absence of hemolysis	95	38.62
Isohemagglutinin titers (cut-off)	**Frequency**	**Percent**
<1:128	211	85.8
≥1:128	35	14.2
<1:64	165	67.1
≥1:64	81	32.9

[a] The isohemagglutinin titer test was performed in platelet units in one specific blood bank laboratory responsible for their distribution. Thus, the frequencies exhibited here are not the same as those of the overall platelet units of the institution, especially regarding the qualitative hemolysin test, due to necessity of providing units negative for hemolysis to patients.

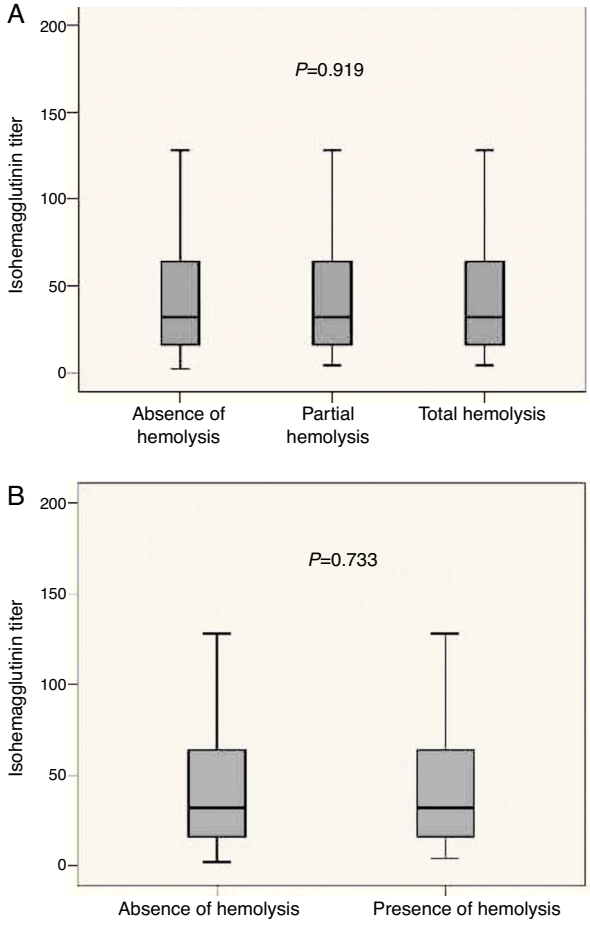

Figure 1 – Comparison of the median value of isohemagglutinin titers (IT) presented by the groups with different results in the qualitative hemolysin test (QHT). Groups did not statistically differ in terms of IT, in spite of their classification as absence of hemolysis, partial hemolysis and total hemolysis (p-value = 0.919) (A) or absence of hemolysis and presence of hemolysis (p-value = 0.733) (B). In fact, the median IT was 32 and the interquartile range was 48 for all analyzed groups.

Evaluation of the efficacy of the qualitative hemolysin test in preventing red cell sensitization

Ten patients were evaluated regarding the presence of anti-A and anti-B within their RBC membranes after

Table 2 – Comparison between isohemagglutinin titers (IT) and qualitative hemolysin test results using the Chi-square test.

	Cut-off	Qualitative hemolysin test		Total	*p*-value
		Presence of hemolysis	Absence of hemolysis		
IT	<1:128	127	84	211	0.677
	≥1:128	24	11	35	
Total		151	95	246	
IT	<1:64	103	62	165	0.454
	≥1:64	48	33	81	
Total		151	95	246	

plasma-incompatible platelet transfusions with negative test results for hemolysis using the QHT. All the patients received less than 600 mL of incompatible plasma and there were no increases in the levels of DHL or IB in any patient after the transfusion. One patient developed a novel positive DAT after the transfusion and the eluate analysis confirmed anti-B specificity (IT 1:128) (Table 3). No signs or symptoms of transfusion reactions were detected.

Impact of the implementation of the qualitative hemolysin test on the blood bank routine

Six hundred platelet units (apheresis only) were studied and the percentage considered as unsuitable for transfusion due to minor ABO incompatibility was calculated. The percentage of group O platelet apheresis considered as unsuitable for transfusion using the QHT was 65% and, based on historical data, the percentage of group O platelet units considered as unsuitable for transfusion using IT (cut-off of 100) was 15% (*p*-value <0.01). This would significantly affect platelet supply in blood banks in the case of minor ABO incompatibility. As the test could not be automatized, QHT was performed manually and only using the sera of apheresis donors.

Discussion

The present study demonstrates that the results of IT, the gold-standard prophylaxis against the hemolysis associated with plasma-incompatible platelet transfusions, are not correlated to the results of the QHT, the recently proposed prophylactic strategy. Moreover in an exploratory analysis, the absence of hemolysis in QHT did not prevent RBC sensitization after transfusion and the implementation of this prophylaxis in the blood bank routine negatively affected platelet inventory management due to a significant increase in the number of units classified as unsuitable for transfusion.

IT has been the method of choice for the prophylaxis of hemolysis associated with plasma-incompatible platelet transfusions in most blood bank services, mainly due to reports of its safety in spite of the fact that the best cut-off level remains elusive.[5,6,11,12] The rationale underlying IT prophylaxis is that incompatible transfused antibodies are diluted within the recipients' organism due to the presence of A and/or B antigens in epithelial tissues and in plasmatic proteins, besides the RBC membrane. The higher the titer of isohemagglutinins, the greater the chances of RBC sensitization and passive hemolysis.[13] Other alternatives

Table 3 – Overall characterization of the patients including the outcome after plasma-incompatible platelet transfusions.

Case	Transfused platelet units					Patient			
	Type of transfused platelet	Transfused volume (mL)	% of incompatible platelet units (pool)	ABO/ RhD	QHT result	ABO/ RhD	Signs or symptoms of acute hemolysis	Post-transfusional DAT	Increase in LDH/IB
1	Apheresis	250		O+	AH	B+	No	Negative	No
2	Pool of random units	420	100	O−	AH	A−	No	Negative	No
3	Pool of random units	360	100	O+	AH	B+	No	Negative	No
4	Apheresis	250		O+	AH	AB+	No	Negative	No
5	Apheresis	250		O+	AH	B+	No	Positive 3+[a]	No
6	Apheresis	250		A+	AH	AB+	No	Negative	No
7	Pool of random units	300	100	O−	AH	B+	No	Negative	No
8	Apheresis	250		O+	AH	A+	No	Negative	No
9	Apheresis	250		O+	AH	A+	No	Negative	No
10	Apheresis	250		O+	AH	A+	No	Negative	No

AH: absence of hemolysis.

[a] Eluate: anti-B.

to this strategy are platelet washing, which is associated with decreased transfusion efficacy,[1] or the preparation of hyper-concentrated platelets re-suspended in an additive solution during storage, which is more time-consuming and expensive.[14]

It is important to stress that group O blood donors typically present higher isohemagglutinin titers, a threat to recipients in the case of incompatible platelet transfusions. Similarly, apheresis platelet units with higher isohemagglutinin titers are more dangerous to incompatible recipients than random platelet units, as their plasma content is higher and cannot be diluted before transfusion. Thus, performing IT in type O apheresis donors is a valid prophylactic strategy used by some blood banks to avoid hemolytic reactions following plasma-incompatible platelet transfusions. One exception to this rule is the use of random units for the transfusion of pediatric patients, a situation associated with risk of hemolysis.

In our service, approximately 2468 transfusions of platelet units are performed each month and, of those, 5% have minor ABO incompatibilities. Since the implementation of IT as hemolysis prophylaxis ten years ago (cut-off level of 100), no cases of acute hemolytic reactions have been reported. Considering a cut-off level of 128 (tube-method), 14.2% of our type O platelet units were considered as high-titer, irrespective of the ABO typing. This data is consistent with other reports in the literature, in which approximately 26.3% of type O platelet units exhibit isohemagglutinin titers greater than 256 (gel-method).[5,13] Since IT can be automatized, if a cut-off level of 100 is chosen, less than 15% of type O platelet units will be considered 'dangerous' in most transfusion services, making it easier to deal with shortages of platelets and avoiding the expiry of units.

QHT has recently been suggested as a possible substitute to IT as the prophylaxis of choice for plasma-incompatible platelet transfusions. There are two types of hemolysin test: QHT and the quantitative hemolysin test, which measures the hemolysin titer and was not in the scope of this study. QHT evaluates whether the anti-A or anti-B antibodies are capable, under the worst conditions, of causing RBC lysis. The objective of this test is different to that of IT, which evaluates the titer of anti-A or anti-B antibodies capable of causing RBC agglutination. As demonstrated by our results, a higher titer of isohemagglutinins does not foresee the presence of hemolysins within one sample, and vice versa.

Even though the rationale underlying this recently proposed prophylactic method was valid (detecting the presence of anti-A and anti-B antibodies capable of causing RBC lysis in donor sera), it completely lacked evidence of efficacy in the literature. The current study demonstrates that there is no correlation between the results of IT and those of QHT. Hence, some platelet units with negative QHT results for hemolysis (suitable for transfusion) exhibited high IT and others low IT, considering both the cut-offs of 128 and 64. As the safety of QHT had never been clinically evaluated, in contrast to that of IT, this proposal is a matter of concern.

The results of the exploratory analysis regarding the ability of QHT to prevent RBC sensitization showed that 10% of recipients who received units with minor ABO incompatibilities with negative QHT results for hemolysis presented a positive DAT after transfusion. Even though the study sample was small, due to ethical limitations, the percentage of RBC sensitization was higher than that reported in the literature for plasma-incompatible platelet transfusions (3.7%) with IT titers greater than 512 (gel-method).[13] As the object of prophylaxis in plasma-incompatible platelet transfusions is to prevent acute hemolysis, which is rare, a large cohort of patients transfused based on QHT results alone is necessary to prove the clinical efficacy.

The implementation of QHT caused some logistic problems to the blood bank routine. It is known from the literature that approximately 60% of isohemagglutinins are capable of causing RBC lysis and, as a consequence, the percentage of platelet units labeled as unsuitable for transfusion after the implementation of this methodology in our service was similar to other publications at 65%.[15] This negatively affected platelet inventory management and caused delays in the transfusion process. Moreover, an extra blood sample had to be collected from donors, due to the necessity of performing QHT using sera instead of plasma, which is the material commonly available in the immunohematology laboratory. By collecting this extra tube, the volume recommended by Brazilian law regarding the maximum authorized blood volume that can be collected for testing was exceeded.[8] Finally, as the QHT could not be automatized, it was performed manually by technicians, thereby increasing the chances of mistakes.

This study has some limitations. The most important concerns the number of recipients transfused with non-compatible platelet units based on QHT results, which was low. Our main objective was to correlate QHT and IT results, with the evaluation of RBC sensitization as secondary. The ideal study design to evaluate the efficacy of QHT in preventing hemolytic reactions should involve at least 5000 patients. However, considering the lack of correlation between QHT and IT, the absence of studies in the literature addressing the efficacy of QHT in preventing hemolytic reactions, problems related to platelet inventory management using the QHT routine and the proven efficacy of IT in preventing hemolytic complications, enrolling more patients to be transfused based only on QHT results would be against ethical principles.

Conclusions

QHT results do not correlate to IT and the implementation of this technique as the prophylaxis of choice against the hemolysis associated with plasma-incompatible platelet transfusions lacks clinical support of safety and would significantly affect platelet inventory management.

Conflicts of interest

The authors declare no conflicts of interest.

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