Titers of ABO antibodies in group O blood donors

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Background: *Plasma components of group O blood donations are rarely submitted to ABO antibody titrations even though it is well known that passively acquired antibodies may destroy the recipient`s own red cells and tissue grafts.*

Objective: *Thus, group O donations stratified by gender and age were randomly titrated to identify the best source of products for apheresis and exsanguinous transfusion.*

Methods: *Samples from 603 blood donors were tested by ABO antibody titration using the conventional tube technique at room temperature. ABO antibody levels higher than 64 were considered high. After correction for gender, statistical analyses were performed using the Fisher exact and Kruskal-Wallis tests.*

Results: *Most donors in the blood bank were male (65.7%). ABO antibody titers ranged from 1 to 2048. The estimations of prevalence for the titers were: anti-A,B < 128 = 86.9% and > 128 = 2.16%; Anti-A > 128 = 9.29% and anti-B > 128 = 4.81%. Low mean titers for both anti-A and anti-B antibodies were found in over 50-year-old men (p-value = 0.040). High anti-B antibody levels were found in young women (p-value = 0.002).*

Conclusion: *This study confirms that over 50-year-old O group men should be selected as blood donors in non-identical ABO transfusion situations. Also, titration of ABO antibodies in blood banks will increase safety in non-identical ABO transfusions.*

Keywords: *ABO Blood-Group System; Blood donors; Blood transfusion; Blood group antigens; Blood platelets/immunology; Agglutination tests/methods; Agglutinins; Antigen-antibody reactions*

Introduction

One century after the discovery of the ABO blood group system, most currently used techniques in blood banks are still based on the principle of interactions between antigen and antibody and subsequent agglutination of red blood cells. Tests that identify the antibody indicate the probable specificity of the antibody, but whether an antibody will destroy red blood cells bearing the corresponding antigen depends of various conditions. The concept of compatibility encompasses much more than crossmatching.[1]

The use of O blood group transfusions to patients of all groups has continued since the Second World War; nevertheless the transfusion of group O plasma to group A recipients sometimes causes severe red cell destruction. Acute hemolysis has been reported following transfusion of non-iso group single donor platelet (PLT) concentrates and may be more common than is appreciated. Other effects of incompatible plasma include hemoglobinemia, jaundice, progressive anemia, spontaneous agglutination, positive direct antiglobulin test and increased osmotic fragility of the patient's red cells.[2]

Levels of A and B antibodies appear to be influenced mainly by environmental factors and anti-A and anti-B molecules may be IgM, IgG or IgA. Some sera contain all three classes and non-stimulated individuals are predominantly IgM. Changes in the characteristics of anti-A or anti-B occur as a result of further immunization with pregnancy or by incompatible transfusions. They are serologically detectable through increases in titers, agglutinin avidity and hemolytic activity and have greater activity at 37ºC. Such "immune" sera are generally difficult to inhibit with saliva or with A or B substances. Sera from group O people contain two separable antibodies, anti-A and anti-B and a cross-reacting antibody called anti-A,B (mostly IgG).[3]

It is a challenge to understand how the role of ABO antibodies influences the fate of organ grafts after transplantation. Japanese groups have pre-transplant preparative regimens that include titration, immunosuppression and splenectomy associated to a therapeutic plasma exchange (TPE) program. However, there is only limited basic research within this area and a lack of standardized clinical protocols for TPE.[4] On the other hand, in transfusion medicine, from 10 to 40 percent of all PLTs transfusions in the United

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States are plasma-incompatible. Also, a small number of institutions prospectively screen group O single donor apheresis PTLs for the presence of high titer agglutinins.[5] Similarly, there is a shortage of identical organ and blood donors. Both situations need blood bank strategies to minimize the biological effects of ABO antibodies. Although, two major challenges in respect to standardized, prospective screening are absence of a recognized reference method and critical end titer that will reasonably differentiate safe from high-titer donors for both clinical settings.

According to Brazilian Legislation,[6] it is not mandatory to screen group O blood donors for ABO antibody titrations. The aim of this study was to evaluate agglutinin levels in blood donations.

Methods

Data collection

All blood donor data is confidential in Brazil. Titrations were performed for ABO antibodies in group O donations intended for exsanguinous transfusions in newborns and all group O apheresis donations. Samples of 603 intentional repeat donors were selected for pre-transfusion antibody titration over 9 months. The total number of blood donations from May 2008 until Jan 2009 was 10,967.

Titration procedure

Diluted sera were incubated in glass tubes at room temperature with a 3% commercial suspension of red blood cells (A1 and B - Diacell, Diamed® Lagoa Santa, Minas Gerais, Brazil). Immediately, after the samples were centrifuged at 3,400 rpm for 15 seconds. Agglutination was considered positive if the red blood cells remained agglutinated after gentle shaking. The highest dilution causing agglutination was assumed to represent IgM antibody titers. By using this semi quantitative technique the anti-A and anti-B were measured and titers higher than 64 were considered high. In these cases, additional information was added on the product label and in the computer system. At the moment of compatibility testing, the system allows only recipient and donor ABO iso groups for the transfusion of packed red blood cells. When possible, our first choice is to stock up to three O blood group units with low titers for exsanguinous transfusion. It is not feasible to follow the same policy with PLT concentrates due to the short period of validity. So our computer system forbids the use of concentrates with high titers in non-identical transfusions.

Statistical analysis

Descriptive statistics were used to describe the age, gender and anti-A and/or anti-B titers in serum. The sample was corrected for gender and statistical analysis was performed using the Fisher exact and Kruskal-Wallis tests.

Results

Men were prevalent (7210 - 65.7% male donors) in the donor population of 10,967; correction was done to adjust the frequency distribution. In this period, the titration procedure corresponded to 5.5% of our blood typing routine. The estimates in prevalence for titer results were: anti-A,B < 128 = 86.9% and > 128 = 2.16%; Anti-A > 128 = 9.29% and anti-B > 128 = 4.81%.

Table 1 illustrates gender and age distribution for anti-A, anti-B and anti-A,B titer levels. Low mean titers were found in over 50-year-old men for anti-A in comparison with women of the same age (p-value = 0.040). On the other hand, high mean anti-B titers (p-value < 0.001) were observed in young women (19 - 29 years old). High anti-A,B titers (greater than 128) were observed in women of between 30 and 39 years old and those of more than 50 years old (p-value = 0.049). Anti-A,B titers below 32 were found in over 50-year-old men (p-value < 0.001). Interestingly, between 40 and 49 years old there is a different tendency in titer levels for both genders; the lowest mean anti-B titer is for over 30-year-old men and the highest frequency for anti-A > 128 is in over 30-year-old women.

Discussion

The true biological reason for evolution and phenotypic variations of the ABO antigens and related carbohydrate epitopes remains obscure. ABO antibody levels depend on the ethnic background and environmental factors. In Japan anti-A and anti-B titers decreased over 15 years (1986-2001) and titers of more than 100, as measured using the saline method, are rare. Similar to North Americans, the Japanese population eats more processed food than other Asiatic populations (Laotian and Thai populations).[7] Indeed, lifestyle changes of a group A PLT apheresis donor also explained a report of passively mediated hemolytic transfusion reactions in two different group B recipients.[8]

This study shows that our donor population has a predominance of low titers in the studied period even though 13% of titers above 100 were found in São Paulo city. Unfortunately, we do not have data of the location and ethnic background of our donor population, therefore, the reasons that young women have high Anti-B titer levels could not be investigated. We believe that the socioeconomic status and genetic background may explain the results.[9] A comparison between two cities in São Paulo state showed high titers for both men and women with an increase in levels in recent years.[10] The authors did not stratify for age and the technique used for titration was not mentioned. Our titration procedure did not include the determination of IgG titer levels, just IgM. The concern of ABO incompatibility in organ

Table 1 - Gender and age distribution for anti-A, anti-B and anti-A,B titer levels

Gender	Female				Male				p-value
Age (years)	19 - 29	30 - 39	40 - 49	more than 50	19 - 29	30 - 39	40 - 49	more than 50	
Donors (%)	81 (13.5 %)	38 (6.4 %)	40 (6.6 %)	40 (6.6 %)	100 (16.8%)	135 (22.6 %)	107 (18.0 %)	56 (9.4 %)	
Anti-A									
Median	32	32	16	32	32	32	16	16	
Mean (SD)	38.2 (36.83)	52.7 (56.55)	30.8 (28.48)	90.4 (198.49)*	55.9 (194.16)	56.1 (127.12)	59.8 (210.21)	28.3 (37.54)*	0.008
Min; max frequency (%)	8 : 256	8 : 256	4 : 128	4 : 1024	4 : 2048	4 : 1024	2 : 2048	4 : 256	
Anti-A < 128	96.3 %	78.9 %	97.4 %	82.5 %	89.1 %	88.9 %	90.7 %	94.7 %	
Anti-A ≥ 128	3.7 %	21.1 %*	2.6 %	17.5 %*	10.9 %	11.1 %	9.3 %	5.3 %	0.027
Anti-A < 32	42.5%	42.1%	60.0%	37.5%	48.0%	45.9%	56.5%	58.9%	
Anti-A ≥ 32	57.5%	57.9%	40.0%	62.5%	52.0%	54.1%	43.5%	41.1%	0.126
Anti-A < 16	8.6%*	2.6%*	27.5%	20.0%	17.8%	19.3%	26.2%	31.6%*	0.002
Anti-A ≥ 16	91.4%	97.4%	72.5%	80.0%	82.2%	80.7%	73.8%	68.4%	
Anti-B									
Median	32	16	16	16	32	8	16	8	
Mean (SD)	52.6 (74.89)*	28.4 (26.30)	20.1 (13.17)	27.9 (26.43)	39.4 (59.90)	26.2 (46.43)*	37.1 (185.15)	18.6 (33.69)*	< 0.001
Min : max frequency (%)	8 : 512	8 : 128	4 : 64	4 : 128	4 : 512	2 : 512	2 : 2048	2 : 256	
Anti-B < 128	85.2 %	97.4 %	100.0 %	97.4 %	94.0 %	97.0 %	96.3 %	98.2 %	
Anti-B ≥ 128	14.8 %*	2.6 %	0.0 %	2.6 %	6.0 %	3.0 %	3.7 %	1.8 %	0.002
Anti-B < 32	39.5%*	57.9%	62.5%	56.4%	48.0%	68.7%	73.8%	82.5%*	< 0.001
Anti-B ≥ 32	60.5%*	42.1%	37.5%	43.6%	52.0%	31.3%	26.2%*	17.5%*	
Anti-B < 16	11.3%*	23.7%	33.3%	23.1%	23.0%	39.3%	43.5%	62.5%*	< 0.001
Anti-B ≥ 16	88.8%*	76.3%	66.7%	76.9%	77.0%	60.7%	56.5%	37.5%*	
Anti-A,B frequency (%)									
Anti-A,B < 128	83.8 %	78.9 %	97.4 %	80.0 %	87.0 %	87.4 %	90.7 %	94.7 %	
Anti-A,B ≥ 128	16.3 %	21.1 %*	2.6 %	20.0 %*	13.0 %	12.6 %	9.3 %	5.3 %	0.04 9
Anti-A,B < 32	22.5%*	34.2%	46.2%	33.3%	32.7%	41%	47.7%	57.9%*	< 0.001
Anti-A,B ≥ 32	77.5%*	65.8%	53.8%	66.7%	67.3%	59%	52.3%	42.1%*	
Anti-A,B < 16	1.3%*	2.6%	12.8%	17.5%	7.0%	13.4%	14.8%	28.6%*	< 0.001
Anti-A,B ≥ 16	98.8%	97.4%	87.2%	82.5%	93.0%	86.6%	85.2%	71.4%	

Statistical significant = p-value < 0.050; SD = standard deviation

transplantation in some countries such as Sweden[11] and Japan,[12] encouraged the development of more sensitive titration techniques (gel hemagglutination and flow cytometry, respectively). Therefore, this current study, using a simple technique, shows the need of further studies to compare the exact measurement of ABO antibody levels.

This is not the first report on ABO antibody distribution in sera from healthy individuals of different age categories. Rieben et al. showed that donor to donor variation did not correlated exactly to age-related changes for all measured parameters. The authors determined isotypes and IgG subclasses of ABO antibodies from sera of 235 Swiss blood donors by enzyme-linked immunosorbent assay (ELISA). Indeed, they studied titers of up to 40 and found considerable variations between antibody levels by ELISA in different sera with the same agglutination titer. Although the relationship between ABO antibody levels and age of donors based solely on agglutination was first observed in 1929,[13] this is the first study that stratifies Brazilian blood donors by age.

A further complexity is that the tests of centers differ in almost every detail, including the use of donor and/or patient plasma or serum, the medium used or dilution, incubation time, centrifugation time and speed, use of polyclonal vs. monoclonal secondary step antibody for indirect agglutination.[9] All these details may affect titer results for transfusions and organ transplants. In a recent review of

ABO matched PLT transfusions, the authors concluded that transfusion medicine services should consider measures to increase ABO-identical PLT transfusions and physicians should be aware of potential adverse outcomes when transfusing non-identical ABO PLTs. Also supplying ABO-identical PLTs to all patients has significant resource implications given that there is currently a limited supply of these products and supply may not match the demand for these products.[14] With the abundance of Group O donors, methods to increase the safety of Group O products should be developed. European strategies have defined a safe level of isohemagglutinins for their donors and a cut-off determination to label products when the titer is high and thereby restrict its use.[15]

The challenge is for us to re-examine our practices and policies to ensure successful transplantation and transfusion and when necessary use non-identical ABO blood. The greatest challenge is to convince the authorities do establish, at a national level, a safe level of ABO antibodies in group O blood transfusions.

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