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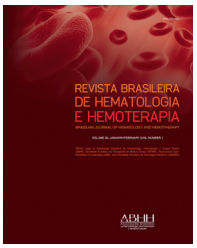

Original article

Dangerous universal donors: the reality of the Hemocentro in Belo Horizonte, Minas Gerais

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ABSTRACT

Background: The term dangerous universal blood donor refers to potential agglutination of the erythrocytes of non-O recipients due to plasma of an O blood group donor, which contains high titers of anti-A and/or anti-B hemagglutinins. Thus, prior titration of anti-A and anti-B hemagglutinins is recommended to prevent transfusion reactions.

Objective: The aim of this study was to estimate the frequency of dangerous universal donors in the blood bank of Belo Horizonte (Fundação Central de Imuno-Hematologia – Fundação Hemominas – Minas Gerais) by determining the titers of anti-A and anti-B hemagglutinins in O blood group donors.

Method: A total of 400 O blood group donors were randomly selected, from March 2014 to January 2015. The titers of anti-A and anti-B hemagglutinins (IgM and IgG classes) were obtained using the tube titration technique. Dangerous donors were those whose titers of anti-A or anti-B IgM were ≥128 and/or the titers of anti-A or anti-B IgG were ≥256. Donors were characterized according to gender, age and ethnicity. The hemagglutinins were characterized by specificity (anti-A and anti-B) and antibody class (IgG and IgM).

Results: Almost one-third (30.5%) of the O blood group donors were universal dangerous. The frequency among women was higher than that of men (p-value = 0.019; odds ratio: 1.66; 95% confidence interval: 1.08–2.56) and among young donors (18–29 years old) it was higher than for donors between 49 and 59 years old (p-value = 0.015; odds ratio: 3.05; 95% confidence interval: 1.22–7.69). There was no significant association between dangerous universal donors and ethnicity, agglutinin specificity or antibody class.

Conclusion: Especially platelet concentrates obtained by apheresis (that contain a substantial volume of plasma), coming from dangerous universal donors should be transfused in isogroup recipients whenever possible in order to prevent the occurrence of transfusion reactions.

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Introduction

Knowledge about the ABO system, from the first experiments of Karl Landsteiner established the beginning of scientific transfusion medicine.[1] Ottenberg gave a priceless contribution to start the practice of transfusions, when he demonstrated that the occurrence of transfusion reactions depend on, among other factors, the titers of hemagglutinins in donor plasma.[2]

It is known that anti-A and anti-B hemagglutinins are potent IgM and IgG antibodies that bind to antigens A and B on the surface of erythrocytes and may activate the complement cascade resulting in acute intravascular hemolysis.[3] The antigens of the ABO system are expressed in erythroid precursors from the fifth or sixth week of intrauterine life. The maximum expression of these antigens is obtained between two and four years old.[4]

The term dangerous universal blood donor was first described in 1923 in reference to the agglutination potential of erythrocytes of non-O recipients, due to plasma of O blood group donors that contains high titers of anti-A or anti-B hemagglutinins.[5] In the clinical practice, the term 'dangerous' refers to the *in vivo* hemolytic potential of high titers of hemagglutinins present in the plasma of O blood group donors. Thus, prior titration of anti-A and anti-B hemagglutinins is recommended to prevent transfusion reactions.

The aim of this study was to estimate the frequency of dangerous universal donors in the blood bank of Belo Horizonte (Fundação Centro de Hematologia e Hemoterapia de Minas Gerais – Hemominas) by determining titers of anti-A and anti-B hemagglutinins in O blood group donors and to propose measures to prevent iatrogenic complications.

Method

Study design

This study was approved by the local Ethics Committees (Fundação Hemominas and the Universidade Federal de Minas Gerais) and was conducted in the Immunohematology Center of the blood bank in Belo Horizonte.

The sample calculation was made considering the number of O blood group donors in the blood bank in 2012 (34,647 donors), the prevalence of dangerous universal donors in similar studies conducted in Brazil (average approximately 10%) and a level of significance of 5%. This calculation indicated the need to analyze at least 400 samples of O blood group donors to estimate the frequency of dangerous universal donors.[6]

O blood group donors were selected randomly, according to the following inclusion criteria: absence of irregular antibody screening and negative hemoglobin S test results regardless of the RhD phenotype, ethnicity, age and gender. Exclusion criteria were O blood group donors that had any positive test results mentioned above and those with A, B or AB blood groups and subgroups. Samples obtained from O blood group donors were evaluated from March 2014 to January 2015.

Hemagglutinin titration technique

The titers of anti-A and anti-B hemagglutinins were performed using the tube technique, which is considered standard.[7]

The titration of anti-A and anti-B hemagglutinins (IgM class) was performed by serial dilutions of donor plasma collected in ethylenediaminetetraacetic acid (EDTA) using saline solution (from 1:1 until 1:1024). The last tube was kept for further dilutions if necessary.

Then, 5% suspensions of red blood cells (A_1 and B) were added, giving a final volume of 100 μL. The tubes were incubated for 15 min at room temperature and centrifuged for reading, in accordance with the laboratory's norms (1000 rpm for one minute). An agglutination reading was performed for each tube. The titer was defined as the inverse of the last dilution that produced an equivalent of 1+ agglutination. This is characterized by a slightly agglutinated blurred background as described in the Technical Manual of the American Association of Blood Bank (AABB).[8] When the titers of anti-A or anti-B hemagglutinins were ≥128, donors were considered to be in the dangerous universal group.[9]

For the titration of anti-A and anti-B hemagglutinins (IgG class), the donor plasma was treated with 0.01 M dithiothreitol (DTT – Sigma–Aldrich®) to destroy IgM class immunoglobulins, so that they would not interfere with the quantification of IgG class hemagglutinins. Then, serial dilutions were prepared with the treated plasma in saline solution [from 1:2 (DTT + plasma) until 1:1024]. The last tube was kept for further dilutions if necessary. Then, 5% suspensions of red blood cells (A_1 and B) were added, giving a final volume of 100 μL. The tubes were incubated for 15 min at 37 °C and the erythrocytes were washed three times with saline solution. Coombs monospecific IgG anti-serum (Lorne®) was added and the tubes were centrifuged for reading in accordance with the laboratory's norms (1000 rpm for one minute). An agglutination reading was performed for each tube. The titer was defined as the inverse of the last dilution that produced an equivalent of 1+ agglutination. This is characterized by a slightly agglutinated blurred background as described by the Technical Manual of the AABB.[8] When the titers of anti-A or anti-B hemagglutinins were ≥256, donors were considered to be in the dangerous universal group.[10]

Statistical analysis was performed using the GraphPad Prism (version 5.0) and Minitab (17th version) software. A calculation of the number of samples classified as dangerous was performed with the result expressed as a percentage of O blood group donors. The analyses of the association between hemagglutinin titers and gender, ethnicity and age used the chi-square test (χ^2). When the expected values were ≤5, Fisher's exact test was used to verify associations. *p*-Values ≤0.05 were considered significant.

Odds ratio (OR) and 95% confidence intervals (95% CI) were determined for the variables that showed any association with the hemagglutinin titers.

Results

Of the 400 samples from O blood group donors, 122 (30.5%) had anti-A and/or anti-B hemagglutinin titers greater than or equal

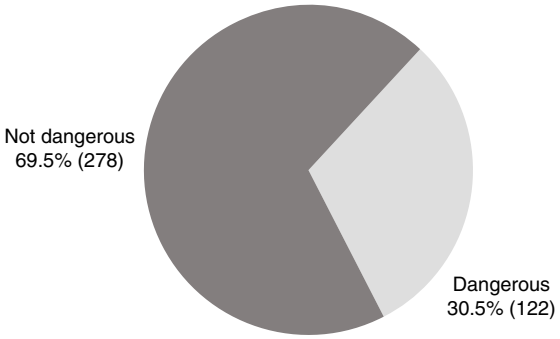

Figure 1 – Relative frequency of O blood group donors classified as dangerous and non-dangerous.

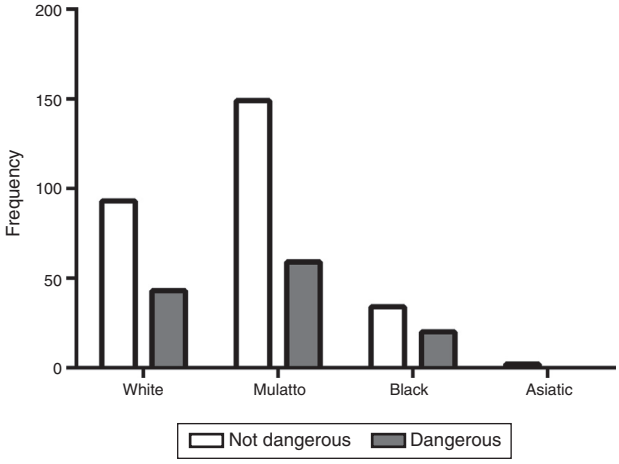

Figure 3 – Absolute frequencies of evaluated donors stratified by ethnicity and classified as dangerous or non-dangerous.

to the set cut-off points, regardless of the antibody class. In 278 samples (69.5%), the anti-A or anti-B hemagglutinin titers were lower than the cut-off point (Figure 1).

Of the 400 samples evaluated, 209 were from men (52.3%) and 191 were from women (47.7%). Of the men, 53 (25.3%) were classified as dangerous universal donors and 156 (74.7%) as non-dangerous. Of the women, 69 (36.1%) were classified as dangerous donors and 122 (63.9%) as non-dangerous. According to the chi-square test, the percentage of dangerous universal donors was significantly higher among women (p-value = 0.019) (Figure 2).

Regarding ethnicity, of the 400 donors included in the survey, 136 declared themselves as White (34%), 54 as Black (13.5%), 208 as Mulatto (52%) and two as Asiatic (0.5%). Of the 122 donors classified as dangerous, 43 (35.2%) declared themselves as White, 20 (16.4%) as Black and 59 (48.4%) as Mulatto. On applying the chi-square and Fisher's exact tests, there was no association between the ethnical background and being classified as a dangerous donor (Figure 3).

Regarding the age group, of the 400 donors included in this survey, 169 (42.2%) donors were between 18 and 28 years old, 116 (29.0%) were from 29 to 38 years old, 69 (17.3%) from 39 to 48 years old, 36 (9.0%) between 49 and 58 years old and 10 (2.5%) were from 59 to 69 years old. Of the 122 donors classified as dangerous, 64 (52.4%) belonged to the 18–28 year-old age group, 32 (26.2%) to the 29–38 year-old group, 18 (14.8%) to the 39–48 year-old group, six (5.0%) to the 49–58 year-old group and two (1.6%) to the 59–69 year-old group. On applying the chi-square test, there were no significant differences except in respect to the 18–28 year-old and 49–58 years-old groups (p-value = 0.015). For the 59–69 year-old group, statistical analysis used the Fisher's exact test and there were no significant differences compared to the other groups (Figure 4).

Regarding the specificity of hemagglutinins (Table 1), univariate analysis of the data revealed no significant association between being classified as a dangerous universal donor and higher anti-A or anti-B hemagglutinin titers (p-value = 0.7468).

In respect to the antibody class (Table 2), univariate analysis showed no significant association between being classified as a dangerous universal donor and higher IgM or IgG class antibody titers (p-value = 0.4846).

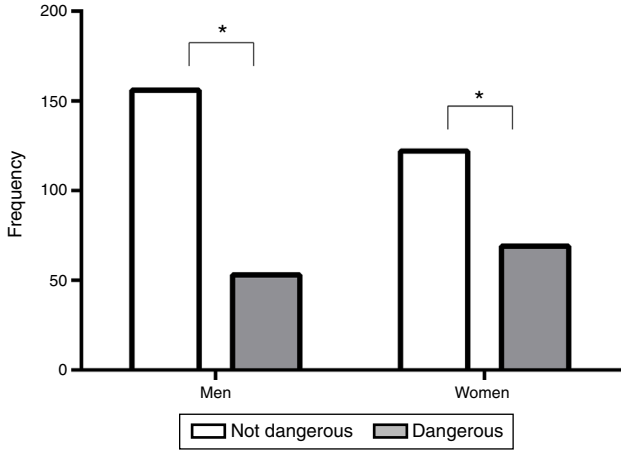

Figure 2 – Absolute frequencies of donors stratified by gender and classified as dangerous or non-dangerous. *Significant difference between groups (p-value <0.05).

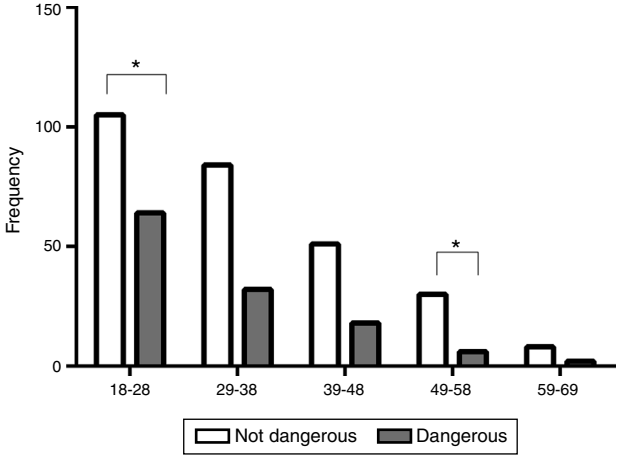

Figure 4 – Absolute frequency of donors stratified by age and classified as dangerous or non-dangerous. *Significant difference between groups (p-value <0.05).

Table 1 – Distribution of anti-A and anti-B agglutinins between dangerous and non-dangerous donors.

Agglutinin specificity	Dangerous donor?[a]		Total titrations	*p*-Value
	Yes	No		
Anti-A	88	712	800	
Anti-B	84	716	800	0.7468
Total titrations	172	1428	1600	

Four titrations were performed for each sample: anti-A IgM, anti-B IgM, anti-A IgG and anti-B IgG.
[a] 1:128 dilutions was the cut-off point for IgM antibodies and 1:256 dilutions for IgG antibodies.

Table 2 – Distribution of IgM and IgG antibody classes between dangerous and non-dangerous donors.

Antibody classes	Dangerous donor?[a]		Total titrations	*p*-Value
	Yes	No		
IgM	76	724	800	
IgG	68	732	800	0.4846
Total titrations	144	1456	1600	

Four titrations were performed for each sample: anti-A IgM, anti-B IgM, anti-A IgG and anti-B IgG.
[a] 1:128 dilutions was the cut-off point for IgM antibodies and 1:256 dilutions for IgG antibodies.

Discussion

Reports of hemolytic reactions after the transfusion of blood products due to dangerous universal donors are found in the literature.[11] The risk is directly associated to the hemagglutinin titer and residual volume of plasma present in the transfused blood components.[12] It is especially worrying in platelet transfusion, knowing that the platelet concentrates have a considerable amount of plasma, in particular those obtained by apheresis.[11]

In Brazil, hemagglutinin titration is not mandatory in blood banks. According to Ordinance 158 05/02/2016, the plasma contained in platelet concentrates will be ABO compatible with the recipient's red blood cells. If this is not possible, it is recommended that the plasma volume of the blood component and the presence of clinically relevant anti-A and anti-B hemagglutinins (hemolysin) is evaluated when the transfusion of non-isogroup platelet concentrates is necessary.[13] However, according to a recent study by Landim et al.,[12] there is no correlation between hemolysin and the risk of clinical hemolysis or hemagglutinin titers reinforcing the importance of analyzing hemagglutinins, which is considered the gold standard prophylaxis against hemolysis related to plasma-incompatible platelet transfusions.

It is worth noting that it is difficult to define dangerous universal donors with the titers because of disagreements regarding titration techniques and the delineation of critical limits. Many studies use a cut-off point of 100 to classify a donor as dangerous using the microplate titration technique.[14–18] It should be noted that standardization in the reading of agglutination intensities using this technique is more complex compared to the tube technique in respect to the titer definition of the AABB Technical Manual. The titer is given as the highest dilution that produces a macroscopic agglutination of one cross (1+).[8]

This study revealed that 30.5% of O blood group donors at the blood bank in Belo Horizonte were classified as dangerous universal donors. This result is similar to that obtained in an Italian study (27.7%) carried out in 1977, which investigated samples from 504 donors and performed the titrations using the tube technique.[19]

In contrast, a Thai study found a frequency of 75.7% of dangerous universal donors due to anti-A IgM antibodies and 80.0% due to anti-B IgM antibodies, while the frequencies of high-risk donors due to anti-A IgG and anti-B IgG antibodies were 93.0% and 95.3%, respectively. The researchers linked the high frequency of dangerous donors to environmental factors, differences in the composition of the intestinal microbiota, presence of intestinal parasites, outcomes of vaccination or exposure to other antigens. They also noted that the number of female donors had grown in recent years, which can justify the proportion of high-risk donors, as pregnancy is a factor that contributes to higher anti-A and anti-B hemagglutinins.[20] Note that the cut-off point adopted in this study was 64, that is less than that in most other studies, which explains, in part, the high number of dangerous donors.[12]

Two studies involving African populations that aimed to determine the hemolytic activity of anti-A and anti-B hemagglutinins in O blood group donors, by investigating hemolysin reactivity at 37 °C revealed that in Nigeria the frequency of hemolytic activity was 23.2%.[21] In Zimbabwe, more than 60% of plasma from donors had hemolytic activity and high titers of IgG class antibodies (≥64). The researchers correlated these findings to the characteristics of the population, such as the high incidence of perinatal hemolytic disease and history of previous transfusions.[22]

Another African study aimed to compare the hemolytic activity of anti-A and anti-B in two racial groups, Black and White Zimbabweans, living under similar conditions. Serum from Black subjects showed greater hemolytic activity of anti-A and anti-B hemagglutinins, which shows that the ethnic background may be an important factor in the hemolytic activity of ABO antibodies.[23] However, statistical analysis of the present study revealed no significant correlation between

ethnicity and being classified as a dangerous universal donor, despite the great ethnic complexity of the Brazilian population.

In fact, high titers of hemagglutinins in plasma from O blood group women can be explained, in part, by gestations of fetuses with non-identical ABO blood groups. This may explain the higher frequency of women classified as dangerous universal donors in this study. There is evidence that perinatal hemolytic disease due to ABO incompatibility is severe in the first pregnancy; probably the second incompatible fetus will also be affected by the disease, which reinforces the theory that high titers of anti-A and anti-B remain in women for a long time.[4,19] At the blood bank in Belo Horizonte, the chance of a woman being classified as a dangerous universal donor was 1.66 times higher than men [odds ratio (OR) = 1.66; 95% CI = 1.08–2.56].

The high percentage of people who declared themselves as mulatto reflects the great heterogeneity of the Brazilian population. Brazil is considered to have one of the most heterogeneous populations in the world resulting from the mixing of people from different continents. In addition, the vast territory and the displacement of different population groups in different regions of the country has led to a considerable phylogeographical heterogeneity.[24] The statistical analysis revealed no significant correlation between ethnicity and being classified as a dangerous universal donor, which probably reflects the complex ethnic categorization of the population.

Regarding the age group, the data of this study show a significant association between young donors (18–28 years old) and high titers of hemagglutinins compared to older donors (49–58 years old). It is known that, in general, the production of anti-A and anti-B hemagglutinins starts between the third and sixth months of life. The titers of these antibodies reach peaks between the ages of five and ten years. After that, a progressive decline in the hemagglutinins is observed as the individual ages.[4] At the blood bank in Belo Horizonte, the chance of a young donor (18–28 years old) being classified as a dangerous universal donor was 3.05 times greater than 49–58 year-old individuals (OR = 3.05; 95% CI = 1.22–7.69).

This study did not find any significant association between the specificity of hemagglutinins and being classified as a dangerous universal donor. However, it is known that anti-A hemagglutinin titers tend to be higher in the plasma of O blood group individuals than anti-B hemagglutinin titers.[4,25]

Moreover, there was no significant association between the antibody class and being classified as a dangerous universal donor. In general, the goal of automated titration of anti-A and anti-B hemagglutinins is just to search for IgM antibodies because there is a significant association between high levels of anti-A and anti-B IgM and hemolysis *in vitro* and not for IgG antibodies. Thus, the titration of anti-A and anti-B IgM antibodies is recommended to test for incompatible platelet transfusions, especially related to female apheresis donors.[20]

In Brazil, few studies have investigated the frequencies of dangerous universal donors in blood banks. In Botucatu, São Paulo, 12.8% of O blood group donors were classified as dangerous; 58.4% due to anti-A IgM antibodies, 14.2% due to anti-B IgM antibodies and 27.2% due to both.[16]

In São José dos Campos, São Paulo, a study that included 6210 samples from O blood group donors revealed a frequency of 13.6% of dangerous universal donors due to anti-A and anti-B IgM antibodies. However, the influence of each hemagglutinin was not distinguished and IgG antibodies were not evaluated.[15]

Another study involving 4447 samples of O blood group donors found frequencies of 1.2% of donors classified as dangerous universal donors in Itapeva and 5.3% in Ourinhos both in São Paulo, without distinguishing the influence of each hemagglutinin and without assessing IgG antibodies.[18]

In Guarapuava, Paraná, the frequency of dangerous universal donors was 7.3% (44.4% had high titers of anti-A IgM antibodies, 35.6% of Anti-B IgM antibodies and 20% of both). IgG antibodies were not evaluated.[17]

All these studies have common characteristics: they used the microplate titration technique after diluting the samples in saline, adopted a titer of over 100 to classify donors as dangerous and only evaluated the IgM antibodies, which may explain the low frequency of donors classified as dangerous compared to the current study. However, it is important to remember that it is technically difficult to grade the intensity of hemagglutination using microplates.

Recent Brazilian studies employed the tube titration technique.[12,26,27] In São Paulo, França et al.[26] evaluated 603 frequent O blood group donors by apheresis or whose blood components would be transfused in newborns; only 13% of donors presented high titers of hemagglutinins (cut-off point >64). The IgG antibodies were not evaluated, which may explain the low frequency of dangerous universal donors. Vargas et al.,[27] in Porto Alegre, Rio Grande do Sul, evaluated 610 O blood group donors who were donating platelets by apheresis and found a frequency of 50.7% of donors with high titers of anti-A antibodies and 41.5% of anti-B antibodies. The methodology used in this study was the tube technique diluted 1:100 in saline. The results were similar to the current study, but the IgG antibodies were not evaluated.

Some methodological differences that may influence the results obtained in studies conducted in Brazil can be mentioned. These differences include performing serial dilutions of samples or just diluting 1:100 in saline, the time of incubation before reading, aspects related to the calibration of equipment (centrifuges and pipettes) and the subjectivity of readings. On remembering the size of Brazil, it is worth noting that differences in the characteristics of the population might justify the differences in frequencies of dangerous universal donors in different studies.

Conclusion

In conclusion, platelet concentrates from dangerous universal donors, particularly those obtained by apheresis, must be identified and when possible transfused in isogroup recipients in order to prevent the occurrence of transfusion reactions. The results of this study aroused the interest of Fundação Hemominas in respect to dangerous universal donors, resulting in the beginning of the standardization of automated titrations of anti-A and anti-B hemagglutinins that is eventually expected to be routine. This, undoubtedly, is an important

step toward making transfusion therapy safer for patients who benefit from blood components from O blood group donors.

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Conflicts of interest

The authors declare no conflicts of interest.

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