



ISSN: 2230-9926

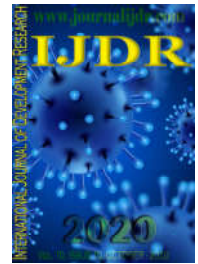
Available online at <http://www.journalijdr.com>

# IJDR

International Journal of Development Research

Vol. 10, Issue, 10, pp. 41228-41231, October, 2020

<https://doi.org/10.37118/ijdr.20026.10.2020>



RESEARCH ARTICLE

OPEN ACCESS

## IMMUNOPHENOTYPING IN A POPULATION OF BLOOD DONORS APPLIED TO TRANSFUSION MEDICINE

Linduarte Varela de Moraes<sup>1\*</sup>, Maria Zélia Fernandes<sup>2</sup>, Geraldo Barroso Cavalcante Jr.<sup>3</sup>

<sup>1</sup>Adjunct Professor of the discipline Diseases of Lymphohematopoietic System of UFRN Medicine course, MD  
<sup>2</sup>Associate Professor of the discipline Diseases of Lymphohematopoietic System of UFRN Medicine course, PhD  
<sup>3</sup>Associate Professor of UFRN Postgraduation Program in Health Sciences, PhD

### ARTICLE INFO

#### Article History:

Received 27<sup>th</sup> July, 2020  
Received in revised form  
29<sup>th</sup> August, 2020  
Accepted 06<sup>th</sup> September, 2020  
Published online 30<sup>th</sup> October, 2020

#### Key Words:

Blood donors, Immunophenotyping,  
Blood Group Antigens

#### \*Corresponding author:

Linduarte Varela de Moraes

### ABSTRACT

**Objective:** To determine the immunophenotyping of a population of blood donors, intended to build a database for transfusion medicine. Show the importance of erythrocyte immunophenotyping as prevention of alloimmunization. **Method:** A cross-sectional study was carried out on 11,664 blood donors for the ABH system typing and of these, 1255 blood donors were selected randomly for the determination of blood group antigens of the Rh system and Kell antigen. Blood centrifugation methods, centrifuge hemolysis tube test and indirect Coombs test were used for blood typing. The results obtained were compared by the Chi-square test. A level of statistical significance of  $p \leq 0.05$  was considered. **Results:** Antigenic frequencies for the ABH system found: the frequency found for the blood group O 48.8%, the frequency found for the blood group A 35.4%; the frequency found for the blood group B 10.6% and the frequency found for the blood group AB 3.2%. In the Rh-System the most frequent antigens found: e 94.5%, D 88.9%; c 80.6; C 56.4%; E 26.3%. For the Kell antigen, the frequency found was 6.7%. The most frequent phenotypes found were DCcEe 23.3%; ddcEe 18.1%, DCCe 16.7%; DcEe 11.0%; DCcEe 10.2% and DccEe 8.8%. The lowest frequency was found: DCcEE 0.64% and ddCcEe 0.08%. **Conclusion:** The antigenic and phenotypic frequencies found, shows the importance of immunophenotyping these blood group antigens in order to transfuse them, making them as compatible as possible, decreasing the risk of alloimmunization.

Copyright © 2020, Linduarte Varela de Moraes<sup>1</sup> et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Citation:** Linduarte Varela de Moraes, Maria Zélia Fernandes, Geraldo Barroso Cavalcante Jr. 2020. "Immunophenotyping in a population of blood donors applied to transfusion medicine", *International Journal of Development Research*, 10, (10), 41228-41231.

## INTRODUCTION

In this paper, the Brazilian people, the anthropologist Darcy Ribeiro presents another specific feature in the formation of our population: the existence of five great cultures that, although different from each other, would be complementary. Here they are: the creole Brazil (which extended from the coast of the cities of São Luís to Rio de Janeiro and was very influenced by Africa), the caboclo Brazil (Northeast, its hinterland, caatinga), the countryside Brazil (midwest, southeast, influence of the city of São Paulo in the country formation) and the southern Brazil (mamluks living in a very rich and fertile area, the pampas of the state of Rio Grande do Sul, with a strong European interference)<sup>1</sup>. Three ethnic types generated the Northeast population: white, black and indigenous. According to the autosomal study of 2011, conducted by the Brazilian geneticist Sérgio Pena, the European component is prevalent in the Northeast population, with African and indigenous contributions.

According to the study performed, the Northeast composition can be described as follows: 60.10% of European inheritance, 29.30% of African inheritance and 8.90% indigenous inheritance.<sup>2</sup> Due to such a big miscegenation represented by the several matrices of skin, hair and eyes colors, which also reflect in the phenotypic constitution of the frequency of different systems of blood groups of the Brazilian population, causes each region of our country to have a different erythrocyte antigenic heritage, according to the ethnicities that have colonized it. For this reason, special attention should be paid to the Immunophenotyping of the population of each region or state of Brazil. And that has generated interest of researching, in the great Natal, the frequencies of the most interesting blood groups in transfusion medicine. This research will enable the finding of phenotypes of low frequency, making them accessible in special situations such as of politransfused patients having erythrocyte antibodies, whether post-transfusion or post-pregnancy. The need of capturing rare blood donors to meet the transfusion demands of patients with antibodies against blood group antigens of high frequency

caused that in 1959 the American Association of Blood Banks (AABB) created a database of rare donors, followed by France in 1965<sup>3</sup>. This need of compatibility extends to the transplantation area, notably the heart and kidney transplantation. Such histocompatibility between the donor and the receptor is at first assessed by the mandatory compatibility between the ABO blood group antigens.<sup>4,5</sup> The transfusion therapy, in addition to comply with the technical standards of the regulatory agencies (ANVISA), is subject to the clarifications on the risks and consent of the patient. "At this moment, there is a problem in relation to the consent for performing the blood transfusion. Some religious confessions, such as the Jehovah's Witnesses, prohibit the transfusion practice. Such religious confessions have legal basis to their refuse to transfusion therapy on art 5, III, of the Federal Constitution (CF), which provides for the religious freedom of the Brazilian citizen"<sup>6</sup>.

## MATERIALS AND METHODS

In the period from January to December 2014, blood was collected from 11,664 donors considered able to donation in Hemonorte - Dalton Cunha Blood Center of Rio Grande do Norte State, complying with the Technical Standards of the Ministry of Health for Blood Collection, Processing and Transfusion, Components and Derivatives in Hemotherapy<sup>7</sup>. The collection was performed in a CPD plastic bag. Additionally, two bottles with approximately 10 ml, without anticoagulant, were collected for conducting the Immunohematological tests. The research was performed in the Hemonorte's Immunohematology Laboratory. To determine the blood groups the samples were tested for the Immunotyping of the main erythrocyte antigens of the Rh (D,C,c,E,e) and Kell (K1) systems through the agglutination methodology in gel test column (Diamed Ag, Morat, Switzerland)<sup>8</sup> using cards with polyclonal antibodies. A 5% red blood cell suspension in bromelain was prepared and incubated for 10 minutes at room temperature. 10 µl of the suspension was pipetted in the microtubes. Next, the cards were centrifugated at 1030 rpm for 10 minutes. The reaction reading was performed due to the agglutination standards in gel test. The tube technique,<sup>9</sup> was also used employing anti-A, anti-B, Anti-AB and anti-Rh antisera for direct classification in the red blood cells with Biotest/SA reagents and e A1 and B red blood cells (RevercelBiotest). For the antigens of Kell, D, C, c, E, e blood group, having performed the direct classification only, once they do not have natural antibodies to determine the reverse classification, which is a characteristic showed by the ABH blood group system only, making it unique in this aspect. The statistical analysis of the results obtained was conducted by the Chi-square test.<sup>10</sup> The research was submitted and approved by the Research Ethics Committee - REC - of the Onofre Lopes University Hospital - HUOL - of UFRN (Federal University of Rio Grande do Norte) CAAE (Certificate of Presentation for Ethics Assessment) under no. 43687315.0.0000.5292 and favorable opinion no. 1,051,865.

## RESULTS

Of the total donors examined, 10,370 (88.91%) were classified as Rh positive and 1,294 (11,09%), were classified as Rh negative. For the ABH system, the results found were the following: The "O" blood group contemplated 5,790 (49.63 %)

of donors; the "A" group 4,230 (36.27 %), followed by the "B" group with 1,258 (10.79 %) and lastly the AB group with 386 donors with a percentage of 3.30 %. (Table 1).

**Table 1. Frequency of ABO blood groups and Rh Factor in blood donors**

Blood Group	n	%	Rh positive factor		Rh negative factor	
			n	%	n	%
O	5,790	49.64	5,142	44.08	648	5.55
A	4,230	36.27	3771	32.33	459	3.94
B	1,258	10.79	1,122	9.62	136	1.17
AB	386	3.30	335	2.87	51	0.43

Source: Hemonorte - Blood Center of Rio Grande do Norte State.

The prevalences found for ABH system antigens report what the other papers with this same focus already found. The "O" blood group with greatest prevalence, followed by the A blood group, the B blood group and lastly the AB blood group, differing only in its incidences in the population studied. Relating the results found in this work, with the European ethnicity of greatest influence in the colonization of the state of Rio Grande do Norte, the Portuguese population, there is a predominance of the A blood type in relation to the O group regarding the prevalence, followed by the B blood group and the AB blood group according to the study performed by Duran<sup>11</sup>, in 230,233 blood donors in Portugal, finding the results: 46.6% ; 42.2% ; 7.6% and 3.4% , respectively. Of the samples collected for the research, 1,255 donors were selected to verify the frequency of antigens and phenotypes for the Rh system and Kell antigen. The results found can be seen in tables 2 and 3. The frequency of Kell antigen found refers to its presence in homozygosis and/or heterozygosis (K/K , K/k )

There are very few papers on the frequency of Kell system antigen in the Brazilian population. Such few papers do not show its high importance in transfusion medicine or its immunogenic power.

**Table 2. Frequency found in Kell antigens**

Antigen	n	%
K	84	6.7

Source: Hemonorte  
Total samples 1,255

Table 2 shows the frequency found in this work, of 6.7%, in blood donors. Costa<sup>12</sup> found in his thesis a frequency of 6% in blood donors; Boturão Neto<sup>13</sup> reports a frequency of 6.3% also in blood donors in his research. In terms of immunogenicity, Kell only loses for the D blood type antigen. When K- patients receive transfusion of a unit of K+ blood, the probability of forming anti-K antibodies may reach 10%<sup>14</sup>. The Kell system includes 34 antigens of which three antithetical antigen sets are the most important ones (K, k, Kp<sup>a</sup>, Kp<sup>b</sup>, Jsa and Jsb). Antibodies against antigens of the Kell system blood type, in addition to provoke the immune destruction of red blood cells in the HDFN (Hemolytic disease of the fetus and newborn), also caused immunosuppression of erythropoiesis, which can result in anemia that is severe, prolonged and without any apparent signs of hemolysis.<sup>15</sup> In hematological patients, those having aplastic anemia (AA), acute myeloid leukemia (AML), the prevalence of irregular antibodies is between 11 and 16%. The main erythrocyte systems submitted to phenotyping, intended to transfusion in patients having sickle cell anemia are the Rh (D, C, c, E, e antigens) and Kell (K antigen), due to be those having the

greatest frequencies of alloimmunization in patients having this type of anemia<sup>16,17</sup>. The antibodies most involved in late hemolytic transfusion reaction are directed against antigens of the Rh (34%), Kidd (30%), Duffy (14%) and Kell (13%) systems<sup>18</sup>.

**Table 3. Phenotype frequency of the RH system in blood donors of the city of Natal**

Phenotype	n	%	p value
DCcee	293	23.35	p < 0.01
Dccee	138	23.35	p < 0.01
DccEe	111	11.00	p > 0.05
DCcEe	128	8.84	p > 0.05
DCCEe	210	10.28	p < 0.01
DccEE	40	3.19	p > 0.05
DCCEE	12	0.96	p > 0.05
DCcEE	8	0.64	p > 0.05
ddccee	228	18.17	p < 0.01
ddCcee	35	2.79	p > 0.05
ddccEe	18	1.43	p > 0.05
ddCcEe	1	0.08	p > 0.05
Others	33	2.63	p > 0.05
Total	1255	100.00	

Source: Hemonorte - Blood Center of Rio Grande do Norte State

In the frequencies found, for the Rh blood group antigens, among the Rh-positive samples, the DCcee phenotype showed greater prevalence (23.3%), followed by the Dccee phenotype, "which is considered one of the black ethnicity phenotype"<sup>19</sup>, with a prevalence of 11.0%. Between the blanks, the ddccee phenotype the percentage is 39.0%<sup>20</sup>. In a study made by Silva<sup>21</sup>, in blood donors of the regional Blood Centers of Crato, state of Ceará, and Picos, state of Piauí, a prevalence of this phenotype of 15.6% was found. Data of the AABB (American Association of Blood Banks), found a prevalence of the Dccee phenotype of 22.9% between the North American afro-descendants and 3.0% between Caucasians. In the Rh-negative samples analyzed in this study, the ddccee phenotype showed the greatest prevalence (18.17%).

## DISCUSSION

In this paper, the phenotypic frequency for the blood group systems ABO and Rh, among blood donors, is in accordance with the values already found in other papers published in Brazil. In spite of the Portuguese colonists have a greater prevalence of the blood group "A", according to the papers published by Duran et al, a greater prevalence of the blood group "O" was found. A hypothesis for such result may be due to the crossover of Europeans with Brazilian Indians, who are predominantly of the blood group "O", as well as the Amerindians. Among the phenotypes found in the Rh system, the frequency of the phenotype Dccee called attention. According to Novaretti, this phenotype is considered one of the markers of individuals with black ethnicity, although mulattos predominantly form the Brazilian population.

The results found in this paper are highly relevant to employ planning policies for capitation of donors and thus have an appropriate supply of the transfusion therapy demands in Greater Natal. Knowing the phenotypic frequencies of the main blood group systems in blood donors may help to calculate the probability of capitation of negative units for a given phenotype, which is intended to blood receptors having antibodies resulted from alloimmunization and phenotypes of low frequency among blood donors.

## Conclusions

The results obtained in this assay allowed to know the prevalence of extremely important blood group antigens, as well as verifying that due to our big miscegenation, each Brazilian region or state have characteristics that are specific for the study in question, enabling the creation of a database with the genotyping of the donors in order to meet the demands of patients needing transfusion, notably those who have chronic diseases. Such experiments will initiate the building of a database with phenotyped donors representing the prevalence in each state or region, facilitating the search for donors with rare blood phenotypes. The objective of this study is to show the importance of the Immunophenotyping in the transfusion therapy, especially in patients having chronic diseases depending systematically of blood transfusions. With such initiatives, the tendency is to reduce the transfusion reactions, improve the quality of life of such patients, in addition to prove, not only by the color of the skin, hair or eyes, but also of the blood groups, that we are a unique people in terms of miscegenation.

**Acknowledgments:** Thanks to the team of the immunohematology laboratory of Hemonorte – Blood Center of Rio Grande do Norte State.

## Authorship contributions

ABO bloodgroup – Maria Zélia Fernandes research  
ABO and Rh bloodgroup – Linduarte Varela de Moraes research  
Statistical – Geraldo Barroso Cavalcante Jr research.

**Disclosure of conflicts of interest:** None

## REFERENCES

- Blumberg N, Peck K, Ross K, Avila E. Immune response to chronic red blood cell transfusion. *Vox Sang*[Internet]. 1983 [cited 2018 Jan 23];44(4):212-17. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/6601881>
- Brasil. Ministério da Saúde. Portaria no.1.376, 19 de novembro de 1993. Normas técnicas para Coleta, Processamento e Transfusão de Sangue, Componentes e Hemoderivados. Diário Oficial [República Federativa do Brasil] dez 1993; 131(229):18405-15.
- Castilho L. Fundamentos de Imuno-hematologia. Rio de Janeiro: Atheneu; 2015.
- Costa, DC. Investigação do Polimorfismo de genes de grupos sanguíneos em doadores voluntários de sangue e em pacientes com hemopatias no estado de Santa Catarina [Tese de doutorado]. Santa Catarina: Universidade Federal de Santa Catarina, Programa de Pós-graduação em Farmácia; 2016.
- DIAMED. Available from: <http://www.diamed.com.br/Cmi/Pagina.aspx?379>.
- Duran JÁ, Chanbert T, Rodrigues F, Pestana D. Distribuição dos grupos sanguíneos na população portuguesa. *Revista ABO* [Internet]. 2007 [cited 2018 Jan 23]; 29:5-17. Available from: [http://ipst.pt/files/IPST/INFORMACAO\\_DOCUMENTACAO/ABO\\_29\\_2007\\_pa\\_g\\_5a17.pdf](http://ipst.pt/files/IPST/INFORMACAO_DOCUMENTACAO/ABO_29_2007_pa_g_5a17.pdf).
- Fiorelli AI, Oliveira JLL, Stolf NAG. Transplante cardíaco. *Rev Med*[Internet]. 2009 [cited 2018 Jan 23]; 88(3):123-37.

- Godfrey GJ, Lockwood W, Kong M, Bertolone S, Ray A. Antibody development in pediatric cell patients undergoing erythrocytapheresis. *Pediatr Blood Cancer* [Internet]. 2010 [cited 2018 Jan 23]; 55(6):1134-37. Available from: <http://dx.doi.org/10.1002/xbc.22647>
- Harmening DM. *Técnicas Modernas em Banco de Sangue e Transfusão*. 4. ed. Rio de Janeiro: Revinter; 2006.
- Mourant AE. Establishment of an International Panel of Blood Donors of Rare Types. *VoxSanguinis*[Internet]. 1965[cited 2018 Jan 23]; 10: 129–132. Available from: <http://onlinelibrary.wiley.com/doi/10.1111/j.1423-0410.1965.tb04330.x/full>
- Neto, EB. *Estudo Imunohematológico molecular do Sistema de Grupo Sanguíneo Kell em indivíduos brasileiros* [Tese de doutorado]. São Paulo: Universidade Federal de São Paulo, Escola Paulista de Medicina, Programa de Pós-graduação em Hematologia; 2008.
- Nunes, HF. *Responsabilidade civil e a transfusão de sangue* [dissertação]. São Paulo: Faculdade de Medicina da Universidade de São Paulo, Programa de Ciências Médicas; 2010.
- Oliveira AG. *Bioestatística decodificada: bioestatística, epidemiologia e investigação*. 2. ed. Lisboa: Lidel; 2014.
- Pena SDJ, Pietro GD, Fuchshuber-Moraes M, Genro JP, Hutz MH, Kehdy FSG et al. The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. *PLoS ONE* [Internet]. 2011 [cited 2018 Jan 23]; 6(2):1-9. Available from: <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0017063>.
- Pestana JOMA. *Transplante renal* [Tipo de mídia]. São Luís: Universidade Federal do Maranhão (UNA-SUS), 2014. Available from: [file:///C:/Users/manuelatiburcio.huol/Downloads/livro\\_unidade\\_4\\_mod6.pdf](file:///C:/Users/manuelatiburcio.huol/Downloads/livro_unidade_4_mod6.pdf).
- Pinto PCA, Braga JAP, Santos AMN. Fatores de risco para aloimunização em pacientes com anemia falciforme. *Rev. Assoc. Med. Bras.* [Internet]. 2011 [cited 2018 Jan 23]; 57(6):668-73. Available from: [http://www.scielo.br/scielo.php?script=sci\\_arttext&pid=S0104-42302011000600014&lng=en](http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0104-42302011000600014&lng=en). <http://dx.doi.org/10.1590/S0104-42302011000600014>.
- Ribeiro D. *O povo brasileiro: a formação e o sentido do Brasil*. 2. ed. São Paulo: Companhia das Letras; 1995.
- Sanchez-Mazas AS, Langsganey A. Common genetic pools between human populations. *Hum Genet* [Internet]. 1988 [cited 2018 Jan 23]; 78(2):161-6. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/3422217>.
- Silva JM. *Fenotipagem eritrocitária em doadores de sangue no HEMOPI (Teresina – Picos) – PI e no HEMOCE (Crato) - CE* [dissertação]. Fortaleza: Universidade Federal do Ceará, Faculdade de Medicina; 2016.
- Walker RH. *Technical Manual*. 11. ed. Bethesda, Maryland: American Association of Blood Banks; 1993.
- Zago MA, Falcão RP, Pasquini R. *Tratado de Hematologia*. 1th ed. São Paulo: Atheneu; 2013.

\*\*\*\*\*