Original Article

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Quick Response Code:

Website:
www.ajts.org

DOI:
10.4103/ajts.ajts_171_21

Application of blood group genotyping in complex cases of immunohematology

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Abstract:

BACKGROUND: Red blood cell (RBC) group systems are depicted by antigens on the surface of RBCs, which when transfused to a recipient that lacks them, can result in alloimmunization. Thus, transfusion of matched RBC components to the recipient is recommended, especially for the more immunogenic blood group antigens, such as Rh (E, e, C, and c), Kell, Kidd, Duffy, and MNS.

AIMS: The aim of this study was to perform the blood group genotyping from blood samples of 12 polytransfused patients whose phenotyping was inconclusive or incomplete.

METHODS: The amplicons were amplified by polymerase chain reaction–sequence-specific primers for the following alleles: RHCE (*RHCE * C, RHCE * c, RHCE * E,* and *RHCE * e*), KEL (*KEL * 01* and *KEL * 02*), FY (*FY * 01* and *FY * 02*), and KID (*JK * 01* and *JK * 02*), in addition to the GATA1-mutated gene (*FY * 02N.01*).

RESULTS: Discrepancies were found in the Rh (E) and Kidd systems, in addition to cases of Fyb antigen silencing attributed to the GATA mutation, which was present in all individuals with Fy (a-b-) phenotype. The technique also solved the inconclusive phenotyping caused by mixed-field agglutination.

CONCLUSION: The results show the contribution of blood group genotyping in complex immunohematology cases, optimizing the delivery of RBC components suitable for transfusion safety, and expanding the number of compatible donors for patients with the Fy (a-b) phenotype related to the FY (*02N.01*) allele.

Keywords:

Blood transfusion, genotyping, hematology, immunohematology, molecular biology, red blood cell antigens

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Submitted: 20-11-2021
Accepted: 29-05-2022
Published: 12-12-2022

Introduction

Blood groups are defined by genetically inherited substances present on the surface of red blood cells (RBCs), which can be detected by a specific alloantibody and may be present both in red cell lineages and in other cells of the body. Thus, the RBC blood group systems have antigens located on the surface of the membrane, these being carbohydrates, proteins, glycoproteins, or glycolipids.[1]

RBC antigens are important in transfusion medicine because the transfusion to a recipient that lacks such antigens can result in alloimmunization.[2] In addition, a patient exposed to such erythrocyte alloantigens during pregnancy or a transplant may also produce unexpected antibodies. These can cause acute and delayed transfusion reactions, as well as contribute to increased morbidity and mortality.[3] Other possible consequences of alloimmunization are hemolytic disease in the fetus and newborn, damage to transplanted tissues, and fewer matched donors for alloimmunized patients.[4]

How to cite this article: Musial LB, Prochaska CL, Moss MF, Cruz BR. Application of blood group genotyping in complex cases of immunohematology. Asian J Transfus Sci 2023;17:164-8.

To avoid such complications, transfusion of donor-matched RBCs with the recipient is recommended, especially when the more immunogenic blood group system antigens are involved in hemolytic transfusion reactions, such as the Rh (E, e, C, and c), Kell, Kidd, Duffy, and MNS systems,[4] thus avoiding the formation of alloantibodies and possible transfusion risks.

The phenotyping technique consists of hemagglutination reactions between antigens and RBC antibodies, in which the presence of such antigens is detected or not, thus being essential to maintain transfusion safety.[1] The technical regulation of hemotherapy procedures in Brazil defines the performance of ABO and Rh (D) phenotyping and search of irregular antibodies in the sample of erythrocyte components from donors and recipients. The Rh (C, c, E, and e), Kell (K, k), Duffy (Fya and Fyb), Kidd (Jka and Jkb), and MNS (S and s) phenotyping is also recommended for patients alloimmunized against RBC antigens or who are or may onset a chronic transfusion scheme, to help identify possible alloantibodies.[5] Therefore, phenotyping is also recommended for previously sensitized patients who already have alloantibodies, i.e., in patients with a positive antibody screening, and it is necessary to identify the suspected alloantibody.[6]

For the feasibility of the phenotyping technique, the recipient should not have been transfused for <3 months. In addition, samples from autoimmune hemolytic anemia recipients with a positive direct Coombs test or direct antiglobulin test may also interfere with the results.[7] In these complex cases, it is necessary to use ancillary techniques to determine the patients' blood groups, therefore, molecular biology techniques are applicable. The blood group genotyping can be used when conventional techniques have limitations, either due to weak or altered expression of antigens, mixed-field agglutination in polytransfused patients, lack of available commercial antisera, or in the search for donors with rare phenotypes on a large scale, seeking a better transfusion practice.[1,8,9]

Thus, the present study proposes the use of molecular biology as an auxiliary technique in cases where phenotyping was not a solution, solving phenotyping problems, adjusting transfusion procedures to improve patient safety, and possibly increasing the number of matched donors, by performing the genotyping of Rh, Kell, Kidd, and Duffy blood groups, and the search for the GATA1 mutation.

Methods

Blood samples were obtained and phenotyped by the Hematology and Hemotherapy Center of Parana (HEMEPAR). The samples of complex cases in immunohematology were then sent for Rh, Kell, Kidd, and Duffy blood system genotyping, including the search for the GATA1 gene mutation. The participation of the individuals followed the rules recommended by the Ethics Committee of the Ponta Grossa State University (Approval Number: 4.960.246), State Health Department (Approval Number: 5.100.193), and HEMEPAR, as well as the Helsinki Declaration of 1975.

The study sampling was probabilistic, obtained for convenience, with consecutive selection. All the samples that presented phenotyping problems were included in the study. The only exclusion criteria were samples collected in heparin tubes.

The serological method for phenotyping was the hemagglutination gel test, performed using the Bio-Rad ID-cards: DiaClon Rh-Subgroups + C^w + K, ID-Antigen Profile II and ID-Antigen Profile III (Bio-Rad, Lagoa Santa – MG – Brazil).

Genomic DNAs were extracted from whole blood obtained by the collection of peripheral blood in a tube containing ethylenediaminetetraacetic acid, followed by its homogenization. The DNA extraction technique was performed using the "Biopur Kit Extraction Mini Spin Plus" (Mobius Life Sciences, Pinhais – PR- Brazil) column extraction kit, following the manufacturer's instructions.

After extraction, the genetic materials were amplified by polymerase chain reaction–sequence-specific primers (PCR-SSP) using Biocycler (Biosystems, Brazil) or Mastercycler nexus (Eppendorf SE, Germany) thermal cyclers. PCR-SSP was performed for the determination of the following antigens of the erythrocyte blood group systems: *RHCE* (*RHCE* * *C*, *RHCE* * c, *RHCE* * *E*, and *RHCE* * e), KEL (*KEL* * 01 and *KEL* * 02), FY (*FY* * 01 and *FY* * 02), and KID (*JK* * 01 and *JK* * 02) as previously described in the literature.[10] In addition to these antigens, the search for the *FY* * 02*N.01* allele referring to the mutation in the GATA1 gene was also performed using the same methodology.

The fragments of interest of the DNAs, previously amplified by PCR, were separated according to the number of nitrogenous base pairs using electrophoresis in 1% agarose gel stained with GelRED (Uniscience, Brazil).

Results

The samples used in the analyses were provided by HEMEPAR together with information about the pathology and general data of the patient, as well as

Table 1: Health data of patients seen at HEMEPAR whose samples were analyzed by genotyping

Patient	Diagnostic	Adversity
1	Sickle Cell Disease	*
2	Sickle Cell Disease	*
3	Hereditary spherocytosis	Anti-D
4	Thalassemia major	*
5	SCD	Anti-C and anti-D
6	Thalassemia major	Mixed field for Fy^a, Fy^b e S
7	Thalassemia major	Mixed field for Fy^a, Fy^b, N and Jk^b
8	SCD	*
9	SCD	Anti-C, anti-E, anti-Fy (3), anti-S, Auto-Ac, Inconclusive
10	Thalassemia major	*
11	SCD	*

*Lack of mixed field and negative antibody screen in the sample analyzed. Source: The authors. SCD=Sickle cell disease

Table 2: Comparison of the phenotyping data of the samples with the genotyping found for each patient in the RHCE, Kell, and Kidd blood group systems

Patient	Phenotype	Predicted genotype
1	ccee, kk, Jk(a+b-)	*RHCE*c/c, RHCE*e/e, KEL*02/KEL*02, JK*01/JK*02*
2	ccee, kk, Jk(a+b+)	*RHCE*c/c, RHCE*e/e, KEL*02/KEL*02, JK*01/JK*02*
3	ccee, kk, Jk(a+b-)	*RHCE*c/c, RHCE*e/e, KEL*02/KEL*02, JK*01/JK*02*
4	Ccee, kk, Jk (a+b-)	*RHCE*C/c, RHCE*e/e, KEL*02/KEL*02, JK*01/JK*02*
5	ccee, kk, Jk (a+b+)	*RHCE*c/c, RHCE*e/e, KEL*02/KEL*02, JK*01/JK*02*
6	Ccee, kk, Jk (a-b+)	*RHCE*C/c, RHCE*e/e, KEL*02/KEL*02, JK*01/JK*02*
7	CcEe, kk, Jk (a+b‡)	*RHCE*C/c, RHCE*e/e, KEL*02/KEL*02, JK*01/JK*02*
8	Ccee, kk, Jk (a+b-)	*RHCE*C/c, RHCE*e/e, KEL*02/KEL*02, JK*01/JK*01*
9	ccee, kk, Jk (a+b+)	*RHCE*c/c, RHCE*e/e, KEL*02/KEL*02, JK*01/JK*02*
10	Ccee, kk, Jk (a-b+)	*RHCE*C/c, RHCE*e/e, KEL*02/KEL*02, JK*02/JK*02*
11	ccEe, kk, Jk (a+b-)	*RHCE*c/c, RHCE*E/e, KEL*02/KEL*02, JK*01/JK*01*
12	†	*RHCE*C/c, RHCE*e/e, KEL*02/KEL*02, JK*01/JK*02*

†Data not informed, ‡Mixed field. Source: The authors

their phenotyping, whenever possible. Thus, from the 12 samples analyzed, 11 had information about data such as the pathology presented by the patient and/or about adversities encountered when performing the phenotyping in the laboratory. This information is important in the analysis and interpretation of some cases and, for better visualization, is presented in Table 1.

Analyzing Table 1, it can be seen that of the 11 patients who had information regarding the pathologies presented, six of the individuals had sickle cell disease (SCD), while other five had thalassemia major, and one of them had hereditary spherocytosis, both groups requiring frequent transfusions.

Another piece of data analyzed was the adversities encountered by the immunohematology laboratory. In this case, three of the 11 samples (27,3%) presented irregular antibodies, including against some of the most immunogenic blood group system antigens, such as RhCE, Duffy, and MNS. In addition, one of the samples showed autoantibodies and inconclusive results in the investigation of other antibodies, being even more important in these cases the transfusion of matched RBCs since the patient is already sensitized against certain RBC antigens. Still regarding the adversities found, samples six and seven (18,2%) showed a mixed-field population for some RBC antigens from the Duffy, Kidd, and MNS systems.

The genotyping performed allowed its comparison with the phenotyping of patients, demonstrating agreement and disagreement between the two techniques, and in the identification of the erythrocyte antigens. The results of the genotyping of the 12 samples, obtained through the proposed methodology, are shown in Table 2.

Analysis of the data from Table 2 shows that there were five discrepancies between phenotyping and genotyping of RhCE, Kell, and Kidd. Furthermore, the case of mixed field in the phenotyping of the Jk (b), antigen in sample seven was resolved, clarifying the correct blood typing of the patient, and providing it to HEMEPAR, making it possible to identify compatible donors and ensuring transfusion safety for this patient.

Besides these systems, the genotyping analysis of the Duffy blood group system was also performed, in which of all 12 samples analyzed, only 1 (8.33%) was positive for the *FY* 01* allele, while the other 11 samples (91.67%) had negative results for this allele. For the *FY* 02* allele, all 12 samples (100%) were positive but presented Fy (b-) phenotyping. For this reason, a PCR-SSP was performed to detect the *FY* 02N.01* allele, which refers to a mutation in the GATA1 gene in the promoter region of the *FY* 02* allele, which can silence this allele in the erythrocytes of homozygous individuals for this mutation (*FY* 02N.01/FY*02N.01*).[2] The results obtained in this analysis are shown in Table 3.

By analyzing Table 3, it can be seen that of the 12 samples, only 1 (8.33%) was positive for both antigens (*FY* 01/FY*02*), and 2 samples (16.67%) were positive only for the *FY* 02* antigens and did not present the GATA1 mutation. The other nine samples (75%) were also positive only for the *FY* 02* allele but were positive for the GATA1 mutation.

Table 3: Data from phenotyping and genotyping of the Duffy blood group system (*FY*01* and *FY*02*) and research of the mutation in GATA1 (*FY*02N.01*)

Patient	Phenotype	Predicted genotype
1	Fy(a-b-)	*FY*02N.01/FY*02N.01*
2	Fy(a-b-)	*FY*02N.01/FY*02N.01*
3	Fy(a+b+)	*FY*01/FY*02*
4	Fy (a-b-)	*FY*02N.01/FY*02N.01*
5	Fy (a-b-)	*FY*02N.01/FY*02N.01*
6	Fy‡	*FY*02N.01/FY*02N.01*
7	Fy‡	*FY*02/FY*02*
8	Fy (a-b-)	*FY*02N.01/FY*02N.01*
9	Fy (a-b-)	*FY*02N.01/FY*02N.01*
10	Fy (a-b-)	*FY*02N.01/FY*02N.01*
11	Fy (a-b-)	*FY*02N.01/FY*02N.01*
12	†	*FY*02/FY*02*

†Data not informed, ‡Mixed field. Source: The authors

Table 3 also shows the mixed-field population found in the phenotyping of samples six and seven for the Duffy system, a problem that was solved by applying blood genotyping as an analysis methodology. Since RBCs do not have a nucleus or genetic material, the RBC component transfused to the patient does not interfere in the molecular methodologies, which generate a result from the analysis of the genetic material of each individual.

Discussion

After analyzing the results obtained and comparing them to each patient's data, it is possible to understand the benefits of genotyping, both about solving technical problems of phenotyping and changing the transfusion management to be taken based on such results.

First, it is interesting to note the pathologies identified among the patients analyzed, being the thalassemia major, in which blood transfusion is one of the pillars of treatment,[11] and the SCD, in which chronic blood transfusions are being increasingly used due to increased oxygen-carrying capacity and consequent reduction of tissue hypoxia in these patients.[12] Thus, these are patients who have a history of transfusions and, possibly, will continue to rely on transfusion schemes in their treatment throughout their lives, being the correct phenotyping very important to avoid cases of alloimmunization.

The genotyping showed to be resolutive in the mixed-field cases observed in samples six and seven, a problem that probably occurred due to the recent transfusion, since there is still the presence of donor's RBCs in the recipient's blood circulation, thus occurring the manifestation of two RBC populations (one positive and one negative) for the same antigen, preventing the correct phenotyping of the sample through hemagglutination methods. The mixed-field population does not interfere in the genotyping, because in this technique, the patient's leukocyte genetic material is used for the analysis, free of interference from the RBCs since they do not have genetic material.

The cases of discrepancy between phenotyping and genotyping were observed in samples one, three, four, six, and seven. For samples one, three, and four in which the discrepancy occurred in the Jkb and Jka antigens, respectively, the phenotyping was negative, while the genotyping demonstrated the presence of the alleles and, consequently, the antigens. This discrepancy occurred due to the possibility of an internal mutation in the *JK* * 02 and *JK* * 01 alleles, leading to a weak expression of the Jkb and Jka antigens on the RBC surface.[13] In these cases, the patients probably had no harm to their health, since the negative phenotype would be considered in the selection of compatible blood for transfusion.

The discrepancy observed in sample seven suggests that the patient received a recent transfusion of RBCs containing the "E" antigen erroneously, and the absence of the *RHCE* * *E* allele and of the "E" antigen expression by the recipient was demonstrated by genotyping. In this case, it is necessary to adjust the transfusion management, avoiding a new transfusion of RBCs containing this antigen, so that the patient may have been alloimmunized, being susceptible to future hemolytic transfusion reaction, and other complications of sensitization.

Regarding the Duffy blood group system, the homozygosity for the GATA1 mutation explains the "discrepancy" observed between phenotyping and genotyping, since the mutation silences the expression of the *FY* * 02 antigen on the RBCs, presenting a negative result in the phenotyping of such antigen, being known as "silent RBC."[2] This result clarifies that despite phenotyping Fy (b-), the nine patients listed have the *FY* * 02 allele, which is expressed in other tissues. Therefore, the supply of packed RBCs containing the Fyb antigen does not result in alloimmunization, since these individuals also express the antigen in their bodies. Such information, besides providing the correct blood typing of these patients, leads to a potential increase in the number of compatible donors, since, from the notification about the *FY* * 02*N.01/FY*02N.01* genotype, the transfusion agency can provide RBCs containing the Fyb antigen, thus expanding the spectrum of donors suitable for transfusion safety, as already described in the literature.[14]

The limitation of the study was the lack of phenotyping and transfusion data for one sample.

Conclusion

The results obtained show the contribution of blood group genotyping as an ancillary technique, especially in complex immunohematology cases, solving inconclusive, and/or incomplete phenotyping, thus indicating the best conduct to be taken and optimizing the release of RBCs suitable for transfusion safety. In addition, the genotyping helped in the expansion of matched donors for patients with Fy (a-b-) phenotype related to the *FY*02N.01* allele, who can receive RBCs containing the Fyb antigen as well.

Acknowledgment

We give special thanks to HEMEPAR for their collaboration and partnership in this work. We also thank the Master in Biological Sciences Larissa Glugoski for her support in the analyses.

Financial support and sponsorship

Ponta Grossa State University.

Conflicts of interest

There are no conflicts of interest.

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