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Blood group genotyping in multi-transfused patients

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Abstract

Aim. To assess the possibility of using blood group genotyping in recipients who received transfusions for 3 months.
Methods. The study included blood samples from 95 patients who received 3 or more erythrocyte transfusions within 3 months. The patients had the following diagnoses: multiple myeloma (n=7), beta thalassemia (n=4), non-Hodgkin's lymphomas (n=11), chronic myeloid leukemia (n=16), primary myelofibrosis (n=9), myelodysplastic syndrome (n=22), acute leukemia (n=21), aplastic anemia (n=5). Red blood cells phenotyping was performed in Diaclon Rh Subgroups+K Gel Cards. The Rh and Kell genotyping was performed by using RBC SSP-PCR kits — FluoGene vERYfy (Inno-train Diagnostics, Germany). The standard RHD/RHCE alleles, as well as polymorphisms associated with KEL1/KEL2 [T698C (Met198Thr)] of the KEL gene were genotyped.
Results. The concordance rate between serological and molecular genetic typing of RhCE and Kell blood groups for donors was 100%, while the patient´s results were discordant in 45.3% of cases. Discrepancies in antigens of the Rh system were registered in 41 patients: one antigen of the Rh system — in 30 patients, two — in 9 patients. Ten patients who had been previously phenotyped as RhCc were genotyped as *RHCE*CC*. 2 patients who had been previously phenotyped as Rhee were genotyped as *RHCE*EE*. In 2 patients, antigens D and C were not detected in the phenotype but were identified in the genotype. Discrepancies in antigen K were recorded in 2 patients, and the antigen was absent in the phenotype but was present in the genotype. The genotyping results were confirmed by serological typing at subsequent hospitalizations.
Conclusion. Blood group genotyping is a useful adjunct to traditional methods when serological typing is limited.
Keywords: RHCE and Kell blood group systems, blood group genotyping, hematological diseases, multi-transfused patients, donors.

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Background. Blood component therapy in patients with hemoblastosis and hematopoietic depression is often complicated by the presence of donor erythrocytes from a previous transfusion (transfusion chimerism) in the recipient's bloodstream, which prevents accurate phenotyping of blood groups if testing was not performed prior to transfusions. Transfusions of donor blood components carrying antigens absent in the recipient bloodstream increase the risk of alloimmunization. The formation of clinically significant alloantibodies increases the risk of post-transfusion hemolytic-type reactions during subsequent transfusions. The presence of alloantibodies in the recipient bloodstream further delays the search for compatible units of erythrocytes that do not carry the corresponding antigens. In addition, alloimmunization reduces the survival rate of transfused erythrocytes and increases the need for transfusions, thereby ultimately increasing the iron load on the body [1–7].

According to various sources, the level of alloimmunization in patients undergoing transfusions ranges from 1.0% to 3.2%, and can be up to 43% in patients with sickle-cell anemia. Antibodies against antigens of the Rh and Kell systems are the most common. Despite the fact that transfusion of blood components with phenotypically identical Rh and Kell, in addition to AB0 and RhD, significantly reduced the frequency of alloimmunization, this risk was not completely eliminated in the case of multiple transfusions [8–11].

Over the past decade, a number of researchers have demonstrated the advantages of blood group genotyping for such patients, since discrepancies are often found between phenotyping and genotyping [12–14].

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Aim. To assess the use of molecular genetic typing of erythrocyte genes in recipients who received transfusions for 3 months.

Materials and methods of research. The study included blood samples from 95 patients treated at the Russian Scientific Research Institute of Hematology and Transfusiology of the Federal Medical and Biological Agency (RSRIHT FMBA). All patients received three and/or more erythrocyte transfusions within three months. All participants signed an informed consent prior to participation in the study, and blood sampling.

The study was approved by the Local Ethical Committee of the RSRIHT FMBA (protocol No. 40 dated May 13, 2021).

Patients with β-thalassemia (n = 4), aplastic anemia (n = 5), multiple myeloma (n = 7), non-Hodgkin's lymphomas (n = 11), chronic myeloid leukemia (n = 16), primary myelofibrosis (n = 9), myelodysplastic syndrome (n = 22), and acute leukemia (n = 21) were registered. Phenotyping was performed by hemagglutination in DiaClon Rh-Subgroups+K gel cards in accordance with the manufacturer's instructions (BIO-RAD, Switzerland).

Genomic deoxyribonucleic acid (DNA) was obtained using a DNA-sorb-B reagent kit (Russia) from 9 mL of peripheral venous blood taken into polyethylene tubes containing 1 mL of 0.5M ethylenediaminetetraacetic acid. The DNA extraction process was performed according to the manufacturer's instructions. The concentration of DNA in each sample was determined by measuring the optical density at 260 nm and 280 nm. For polymerase chain reaction (PCR), 50–100 ng of DNA was used.

Rh, Kell genotyping was performed using SSP-PCR RBC—FluoGene vERYfy kits (Inno-train Diagnostics, Germany). The kits consisted of PCR plates with wells containing pre-placed and dried reaction mixtures containing allele-specific primers, internal control primers, and nucleotides. The master mix was prepared from 10X PCR buffer, DNA solution, Taq polymerase, and distilled water. Amplification parameters were used according to the manufacturer's instructions. Amplification products were evaluated using FluoVista software. Molecular genetic typing of the standard *RHD/RHCE* alleles and the C^W allele, as well as polymorphisms associated with KEL1/KEL2 [T698C (Met198Thr)] of the *KEL* gene, was performed.

Results and discussion: Comparison of the results of serological and molecular genetic typing of RhCE and Kell blood groups showed that among donors of blood and its components, the results coincided by 100% (10/10), while discrepancies were noted in the studied blood samples of patients previously examined at the institution (Table 1).

Table 1. Results of serological and molecular genetic typing of blood groups of patients who received 3 and/or more erythrocyte transfusions within 3 months

Patients examined, *n*	Correspondence of phenotype to genotype, *n* (%)	Inconsistency of phenotype to genotype, *n* (%)
95	52 (54.7%)	43 (45.3%)

Thus, discrepancies in the antigens of the Rh and Kell systems were registered in 43 patients (Table 2).

Discrepancy in one antigen of the Rh system was detected in 30 patients, and in two antigens was detected in 9 patients. The results of the examination of 39 patients revealed transfusion chimerism, namely the presence of antigens C, c, E, e in the phenotype and the absence in the genotype, which indicates transfusions of blood components that are not identical in antigens of the Rh system.

The high discrepancy incidence of antigen c, which is highly immunogenic, is noteworthy. Ten patients, whose phenotype was determined as RhCc, were genotyped as *RHCE*CC*, which necessitated the search for c-negative units of erythrocytes. In such patients, the probability of alloimmunization was high.

Similarly, two patients who were phenotyped as Rhee had the *RHCE*EE* genotype, and required a search for e-negative erythrocyte units. In two patients, antigens D and C were not detected in the phenotype, but were identified in the genotype. These patients had chronic myeloid leukemia and myelodysplastic syndrome, and the absence of these antigens in the phenotype indicated a loss of antigen expression by erythrocytes. In one patient, the loss of expression was short-term and the erythrocytes gained expression after therapy. In the other patient, who was previously monitored, the loss of expression was associated with the development of terminal relapse leading to death.

Discrepancies in the K antigen were recorded in two patients, and the antigen was absent in the phenotype, but was present in the genotype. The resulting discrepancies were associated with high transfusion activity (18 and 22 doses of erythrocyte suspension, respectively) in patients who received transfusions of erythrocyte components void of the Kell system K antigen.

All subsequent transfusions of blood components were received by patients considering the genotype of the Rh system. Hemolytic reactions and complications were not recorded. The results of molecular genetic typing were confirmed by serological testing at subsequent hospitalizations.

Table 2. Discrepancies in the results of serological and molecular genetic typing of RhCE and Kell blood groups in patients

Antigen system	Antigen for which discrepancy was detected	Number of patients with antigen discrepancy, *n* (%)	Phenotype, (*n*)	Genotype, (*n*)
Rh	C	11 (11.5)	Cx*cEe (7)	*ccEe* (7)
			Cx*cee (1)	*ccee* (1)
			Cx*cEE (3)	*ccEE* (3)
	E	9 (9.5)	CcEx*e (5)	*Ccee* (5)
			CCEx*e (4)	*CCee* (4)
	c	9 (9.5)	Ccx*ee (4)	*CCee* (4)
			Ccx*Ee (5)	*CCEe* (5)
	cE	4 (4.2)	Ccx*Ex*e (4)	*CCee* (4)
	e	3 (3.2)	CcEex* (1)	*CcEE* (1)
			ccEex* (2)	*ccEE* (2)
	Ce	2 (2.1)	Cx*cEex* (2)	*ccEE* (2)
	CE	1 (1.1)	Cx*cEx*e (1)	*ccee* (1)
	DC	2 (2.1)	ccdee (2)	*CcDee* (2)
Kell	K	2 (2.1)	CcEeK– (2)	*CcEeK*+ (2)

Note: x (chimera)—dual population of erythrocytes.

Our data showed a high agreement of results between the two research methods for blood donors (100%). However, as expected, the patient group reported numerous conflicting results for RhCE and Kell.

Our results are consistent with that of other studies, where the discrepancies between the results of genotyping and phenotyping ranged from 10% to 90% in patients who received transfusions of blood components [14–16]. Thus, in 1999, T.G. Legler et al. evaluated the use of molecular genetic methods for typing blood groups of the Rh system in patients who depended on transfusions. In 2 out of 27 cases with patients with the D-negative phenotype were D-positive after genotyping; in 4 patients with the RhCc phenotype, the genotype was identified as *CC*; and in 1 patient with the Rhee phenotype, the *Ee* genotype was revealed [17].

Later, in 2013, another group of researchers also conducted a comparative analysis of genotyping and phenotyping erythrocyte antigens of the Rh and K systems in patients requiring continuous lifelong transfusions [7]. In 51% of the patients examined, discrepancies were identified thereby leading to the synthesis of alloimmune antibodies. Thus, the *CC* genotype was determined in five patients with the RhCc phenotype, and the *EE* genotype was established in two patients with the Rhee phenotype. Moreover, two patients with no K antigen in their phenotype obtained a genotypic result with *Kk*, which expanded the search for compatible donor blood components. The authors emphasized the significance and relevance of molecular genetic typing as a standard for patients dependent on transfusions [7].

However, molecular genetic typing is quite complicated and expensive for routine practice; therefore, it is not realistic to assume that donor centers can provide all recipients with donor blood components compatible with transfusion-important antigens.

Nevertheless, extended typing by PCR-based methods of donor blood components for alloimmunized recipients and patients with diseases such as sickle-cell anemia and thalassemia have been widely implemented in routine practice [3, 18, 19]. Despite the importance of molecular genetic typing, serological methods still have their place and are still relevant [1, 2]. The choice of methodology will depend on the treatment profile and objectives of a particular health service. The use of molecular genetic typing of blood groups in addition to serological ones will become another stage in improving the system for ensuring the immunological safety of blood transfusion therapy.

CONCLUSION

Firstly, the use of molecular genetic typing of erythrocytes in blood donors and recipients with transfusion chimerism is of great relevance and importance.

Secondly, we registered 45.3% of cases with discrepancies in the results of serological and molecular genetic typing of RhCE and Kell blood groups in blood samples of those who received 3 and/or more erythrocyte transfusions within 3 months.

Finally, molecular typing enables us to obtain a significant result in cases where the interpretations of the results of serological typing were problematic.

Author contributions. N.V.M. was the project supervisor and formulated the work concept and wrote the text; S.V.G., E.A.S., and N.N.B. conducted the research; I.I.K. and S.S.B. collected, analyzed, and interpreted the results, as well as wrote the text; A.V.Ch. and S.S.B. provided consultation on clinical issues and gave the final approval to the manuscript.

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