Distribution of Clinically Relevant Erythrocyte Antigens among Blood Donors of the Republic of Srpska

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ABSTRACT

Introduction: Identifying voluntary blood donors with rare phenotype characteristics is the basic precondition for creating a registry of blood donors with rare blood groups.

Aims of the study: Determining the presence of phenotypes in the clinically most relevant blood group systems in regular blood donors at the Institute for Transfusion Medicine of the Republic of Srpska, with the goal to create a national registry of blood donors with rare blood groups.

Patients and Methods: Determination of antigens in the Rh system was performed by the automatic microplate method, as well as by gel method. Determination of antigens in other blood group systems Kell, Kidd, Duffy, MNS, Lewis and Lutheran was performed by the gel method and test tube method. Altogether 384 blood donors were screened between 2012 and 2013.

Results: The analysis of Rh phenotypes showed that most of the examinees had the Rh phenotype CcDee, 29.7% and 26.0% the ccddee, and the least of them had the Rh phenotype ccddEe 0.8%, Ccddee 1.6% and ccDee 2.3%. The antigen Cw was proved to be in 13 donors (3.4%), while the antigen P1 was detected in 291 donors (75.8%). In analyzing the Lewis antigen system, most of the blood donors were found to have the phenotype Le (a-b+), 74.0%. By analyzing the Lutheran antigen system, the phenotype Lu (a-b+) was detected in most of the donors typed for Lutherean antigens, that is, in 93.0% of them. One individual was found to have the phenotype Lu (a+b-) (0.3%), and two donors the phenotype Lu (a-b-) (0.5%). The analysis of the Kell antigen system showed most of the donors were of the phenotype kk, 93.2%, one individual was found to have the phenotype KK (0.3%) and 25 donors the phenotype Kk (6.5%). In analyzing the Kidd antigen system, most of the donors were found to have the phenotype Jk(a+b+), 46.6% and 29.4% the Jk(a-b+), whereas 24.0% had the phenotype Jk(a+b-). According to the study of MN antigen in the MNS system, most of the donors were typed as MN, 50.0% and 33.9% as MM, while the phenotype NN was detected in 16.1% donors. Analyzing the S and s antigens in the MNS system, the phenotypes Ss were found in most of the donors, 49.0% and ss in 43.2%, whereas the phenotype SS was detected in 7.8% donors. The analysis of the Duffy antigen system showed most of the donors were of the phenotype Fy(a+b+), 46.6% and 34.6% of the Fy(a-b+), while the phenotype Fy(a+b-) was observed in 18.8% donors.

Conclusion: Data on the distribution of clinically relevant erythrocyte antigens among regular blood donors in the Republic of Srpska is in accordance with the data set out in the literature for the caucasian.

Key Words: blood groups, erythrocyte antigens, distribution of clinically relevant erythrocyte antigens

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Introduction

Blood groups are every variation or polymorphism in blood. According to the general definition, blood groups are inherited biological characteristics that, in healthy people, do not change throughout life. According to another definition, blood groups are antigens on the surface of red blood cells, which represent inherited characteristics of every individual and may be proved by specific antibodies, and they express refined structural difference in the erythrocyte cell membrane.¹

In practice, the term blood group refers to antigens. Immunohematology is a branch that studies erythrocyte antigen-antibody reactions.

Erythrocyte antigen can be divided according to: a) the clinical significance (they may cause hemolytic disease of the newborn or hemolytic transfusion reaction); b) the way of provoking immune response in the organism (exposure to similar antigens in the environment, when the so called natural antibodies are produced or immune response is stimulated by transfusion and pregnancy, respectively; when immune antibodies are produced); c) similarities in the biochemical structure.¹⁻⁵

All the proved blood group antigens have been divided into: systems (at this moment we know about 35 of them), collections (there are 7 of them), and series (there are 2).³

Blood group antigens are classified into systems or collections, as they are defined immunologically, genetically and biochemically. In practice, first an anti-erythrocyte antibody is detected in the serum of a multigravida or politransfused patient. When its unique specificity is verified, the antibody is used for conducting researches of biochemical features and structure of antigens against which it was produced. Inheritance of the antigen and its genetic frequency are studied due to potential connection with the other, already detected antigens. Every antigen that belongs to any blood group system is presented by a six-digit number. The first three numbers mark the system (001-035), collection (205-213) or series (low-frequency antigens 700, and high-frequency antigens 901), whereas the other three numbers refer to the specificity of a certain

antigen. For example, the Lutheran system is numbered 005, and the antigen Lu^a, as the first antigen of this system, has the number 001, i.e. 005001.

The clinically relevant erythrocyte antigens are, by definition, those that may cause erythrocyte hemolysis *in vivo*, when symptoms and signs of direct, i.e. delayed hemolytic transfusion reaction or clinical picture of the hemolytic disease of the newborn occur. The antigen systems Rh, Kell, Kidd, Duffy, MNS, etc. are considered to be clinically relevant erythrocyte antigens.⁴ Distribution of erythrocyte antigens was tested in various races and populations. The results of those tests indicate that there are differences in the distribution of some erythrocyte antigens among different races, as well as within certain nations of the same race.

Aim of the study

To determine the distribution of clinically relevant erythrocyte antigens C, c, E, e, C^w, K, k, Kp^a, Kp^b, M, N, S, s, Fy^a, Fy^b, Jk^a, Jk^b, Lu^a, Lu^b, as well as the antigens P, Le^a and Le^b in the population of regular blood donors on the territory of Banja Luka and the Republic of Srpska.

To compare the found frequencies of the distribution of erythrocyte antigens with the frequencies in other tested white populations.

Patients and Methods

In this prospective study a total of 384 examinees were studied during 2012 and 2013, the population of regular blood donors at the Institute for Transfusion Medicine Banja Luka, males and females, the age of 18 through 55, with the blood groups A and O, RhD-positive and RhD-negative. Blood donors of the blood group A b were tested, as that was the most common blood group in this climate, as well as the blood group O, as those were regarded as „universal” erythrocyte donors (Table 1.).

The examinees belonged to the category of regular blood donors, who gave their blood at least twice before the screening, and their results upon the testing of presence of the markers for transfusion transmissible diseases had been

Table 1. Distribution of blood donors by blood transfusion centers at the Institute for Transfusion Medicine Banja Luka in 2013

2013	BL	DO	PR	GR	BN	ZV	TR	KS	FO	NE	TOTAL
Number of taken doses of blood	10114	4830	2327	2583	4078	2074	1690	1524	1332	50	30602
Number of voluntary blood donors	5710	2541	1820	1824	3460	1014	1712	642	373	50	19146
	57%	53%	78%	71%	73%	40%	99%	42%	28%	100%	64,04%
Number of women	1595	236	407	264	543	160	163	120	86	6	3580
	16%	5%	17%	10%	11%	6%	9%	8%	7%	9%	9, 88%

negative. In 2013, there were 5710 blood donors in Banja Luka, covering 7% of the total number of blood donors on this territory.

The frequency of the clinically most relevant erythrocyte antigens was studied in this sample, including implicitly Rh phenotypes, C, c, E, e, also including the C^w antigen, K, k, Kp^a and Kp^b antigens in the Kell system, M, N, S, and s antigens in the MNS system, Fy^a and Fy^b antigens in the Duffy system, Jk^a and Jk^b antigens within the Kidd system, Lu^a and Lu^b of the Lutheran system, as well as Le^a and Le^b antigens of the Lewis system and P₁ antigen. Blood samples were taken in the appropriate vacutainer with anticoagulant EDTA, as prescribed on the Techno device instructions.

To determine the blood groups and red blood cell/erythrocyte antigen typing, the following methods were applied: a) test tube method or agglutination in an aqueous environment,⁶⁻⁸ b) gel method,⁹ c) microplate method.^{10, 11}

In this study, the process implied automatic testing of blood groups ABO and RhD antigens, as well as Rh phenotypes from donors' samples in the Techno device (DiaMed)¹², by microplate method, and gel method, test serums DiaMed. In this way we had electronic entry in the Lira reader and any error in copying was avoided. As for the determining of other blood group systems, the first time it was performed by gel method (DiaMed), and the second time by test tube method, by using the serum Biognost Zagreb, Novaclone Canada, Sanguin Holand, Immucor USA, Diagast France.

The primarily obtained data was analyzed by descriptive statistic methods. As descriptive statistic method the following was used: central tendency measures (arithmetic environment), variability measures (standard deviation) and structure indicators expressed in percentages.

Results

In the tested sample the highest percentage of blood donors were those of group A, RhD+ 35.9% (Figure 1.).

Figure 1. Distribution of blood groups in relation to the ABO system and RhD antigen

Analysis of **Rh phenotypes** in the tested sample of blood donors showed that most of the examinees were found to have the Rh phenotype CcDee, 29.7% and 26.0% the ccddee, and that the least of them had the Rh phenotype ccddEe 0.8%, Ccddee 1.6% and ccDee 2.3%. (Table 2.).

Table 2. Frequency of Rh phenotypes in the sample of blood donors in the Republic of Srpska

Rh fenotip	n (%)
CcDEe	45(11.7)
ccDEe	36 (9.4)
CcDee	114 (29.7)
ccDEE	8 (2.1)
ccddee	100 (26.0)
CCDee	63 (16.4)
ccDee	9 (2.3)
ccddEe	3 (0.8)
Ccddee	6 (1.6)
Total	384 (100.0)

In the tested sample of blood donors, the antigen C^w was verified in 13 of them (3.4%).

In the tested sample of blood donors the antigen P₁ was detected in 291 of them (75.8%).

After analyzing the **Lewis** antigen system in the tested sample of blood donors, most of the blood donors were found to have the phenotype Le(a-b+), 74.0%.

By analyzing the **Lutheran** antigen system in the tested sample of donors, the phenotype Lu(a-b+) was detected in most of the donors typed for Lutheran antigens, in 93.0%. One individual was found to have the phenotype Lu (a-b-) (0.3%), and two donors the phenotype Lu(a-b-) (0.5%).

The analysis of the **Kell** antigen system in the tested sample of blood donors showed that most of the donors had the phenotype kk, 93.2% of them, one individual was found to have the phenotype KK (0.3%) and 25 donors the phenotype Kk (6.5%).

In analyzing the **Kp** antigen in the Kell system in the tested sample of blood donors, the phenotype Kp(a-b+) was detected in most of the examinees, in 97.9% of them, whereas the phenotype Kp(a+b+) was observed in 8 donors (2.1%).

The analysis of the **Kidd** antigen system in the tested sample of blood donors showed that most of the had the phenotype Jk(a+b+), 46.6% and 29.4% the phenotype Jk(a-b+), whereas the phenotype Jk(a+b-) was observed in 24.0% donors (Table 3.).

Table 3. Distribution of the Kidd antigen system in the sample of blood donors in the Republic of Srpska

Antigen system Kidd	n (%)
Jk(a+b+)	179 (46.6)
Jk(a+b-)	92 (24.0)
Jk(a-b+)	113 (29.4)
Jk(a-b-)	0 (0.0)
Total	384 (100.0)

By studying the **MN** antigen in the **MNS** system, in the tested sample of blood donors, most of the donors were typed as MN, 50.0% and 33.9% as MM, while the phenotype NN was detected in 16.1% donors. (Table 4.).

Table 4. Distribution of the MNS antigen system in the sample of blood donors in the Republic of Srpska

Antigen MN system MNS	n (%)
MN	192 (50.0)
MM	130 (33.9)
NN	62 (16.1)
Null fenotip	0 (0.0)
Total	384 (100.0)

Analyzing the **S** and **s** antigens in the **MNS** system, in the tested sample of blood donors, the phenotypes Ss were found in most of the donors, 49.0% and ss in 43.2%, whereas the phenotype SS was detected in 7.8% donors.

As for the **Duffy** antigen system, most of the donors were found to have the phenotype Fy(a+b+), 46.6% and 34.6% the Fy(a-b+), while the phenotype Fy(a+b-) was observed in 18.8% donors.

Discussion

By analyzing the data of the observed sample, it was determined that 50% examinees belonged to the blood group A, and nearly 50% to the blood group O. The situation was similar in regard to the ABO and RhD: about equal percentage of donors belonged to the blood group A, RhD+(poz) and O, RhD+(poz)- about 36%. As donors who gave blood at least twice were selected for the study, and they were supposed to belong to the blood groups A and O, no wonder there was such a distribution. The most common blood group in populations of the former Yugoslavia was A¹ and people with blood group O were said to be universal erythrocyte donors.¹ The frequency of blood groups ABO in white donors was as follows: O-44%, A- 43%, B-9%, AB-4%. As for black donors, the frequency was the following:

O-49%, A-27%, B-20%, AB-4%, whereas the blood group O frequency in the yellow race was 43%, A-27%, B-25%, and AB-5%.^{13,14}

When it comes to the distribution of antigens of the Rh phenotype, in the examined donor population, the most common phenotype was CcDee, with the frequency of about 30%. The second most frequent was the phenotype CCDee with about 16%, followed by CcDEe with about 12%, ccDEe with about 9%, ccDee and ccDEE with about 2% frequency. In RhD-negative people, the phenotype ccddee frequency was found to be about 26% in the tested sample, which was considerably more than the D antigen frequency in the white population. This may be due to the fact that this examination included the selected regular blood donors of blood groups A and O, RhD-pos. and RhD-neg, according to the need for examination of the distribution of erythrocyte antigens, and not according to the observance of D antigens in the white population, ranging from 12% to 18%.¹⁵⁻¹⁷ Frequency of RhD-negative persons in the population of soldiers of the former Yugoslavia was 15.5%, whereas RhD-positive 84.5%.¹

In the sample of blood donors, it was determined that the C^w antigen was in 13 of them (approx. 3%). The C^w and C^x antigens were alleles of the high-frequency MAR antigen, and not, as it was originally believed, the alleles of C and c antigens. C^w was an antigen with relatively low frequency in all populations. In the population of England the C^w antigen had 2.6% frequency, and similar data was found also for other white nations.¹⁵⁻¹⁷

In the sample of blood donors, 291 (75.8%) of them were found to have the P₁ antigen. The P₁ antigen frequency of blood donors in the group of males was 76.4%, whereas in the group of females it was 70.3%. The P₁ (P₁-positive) and P₂ (P₁-negative) phenotypes were present with the frequency of over 99% in the blood donor population. To show both phenotypes it was necessary to have the synthesis of P_k and P antigens, which differ from one another only in the expression of P₁ antigen. Furthermore, three rare autosomal recessive phenotypes (p, P₁^k, P₂^k) were identified, as well as poor variants of the P₁ antigen.^{6,18} The P₁ phenotype frequency was in accordance with the presence of this phenotype in other white nations.

In analyzing the Lewis antigen system, most of the examined blood donors in the Republic of Srpska were found to have the phenotype Le(a-b+) 74.0%, followed by phenotype Le(a+b-), with about 16% of presence, and phenotype Le(a-b-) with the distribution of about 10%. The Le (a-b-) phenotype frequency was higher in people originated in Africa, as compared to other populations.⁶⁻⁸ Distribution of the Lewis antigens among the blood donors in the Republic of Srpska was in accordance with the data released for the white race.

In analyzing the Lutheran antigen system, most of the examinees of the tested sample of blood donors in the Republic of Srpska were observed to have the phenotype Lu(a-b+) 93.0%, one person was found to have the phenotype Lu(a+b-) (0.3%) and two donors the phenotype Lu(a-b-) (0.5%). The Lu^a (LU1) antigen frequency was about 8% in Europeans and Africans, but it was rare in all other parts of the world. Its antithetical antigen Lu^b (LU2) was present in all populations.^{6, 18} The presence of Lu^a and Lu^b antigens was in accordance with the presence in other white populations.

The analysis of K and k antigens in the Kell system in the tested sample of blood donors in the Republic of Srpska showed that most of the examinees had the phenotype kk 93.2%, one person was found to have the phenotype KK (0.3%) and 25 donors the phenotype Kk (6.5%). Distribution of K and k antigens in the examined population was in accordance with the published data.^{6, 18}

Analyzing the Kp antigen in the Kell system in the tested sample of blood donors, the phenotype Kp(a-b+) was detected in most of the examinees, in 97.9% of them, whereas the phenotype Kp(a+b+) was observed in 8 donors (2.1%). According to the available data, such distribution was fully in correlation with the results of examinations performed on white donors in other countries.^{6, 18}

In analyzing the Kidd antigen system in the tested sample of blood donors, most of the donors were found to have the phenotype Jk(a+b+), 46.6% and 29.4% the phenotype Jk(a-b+), whereas the phenotype Jk(a+b-) was observed in 24.0% donors. The results were similar to the ones published in available literature.^{6, 18}

By studying the MN antigen in the MNS system, most of the donors were typed as MN, 50.0% and 33.9% as MM, while the phenotype NN was detected in 16.1% donors.

Analyzing the S and s antigens in the MNS system, in the tested sample of blood donors, the phenotypes Ss were found in most of the donors, 49.0% and ss in 43.2%, whereas the phenotype SS was detected in 7.8% donors. This was based on results and comparisons made with the usage of similar examinations of the white population in other countries.^{6, 18}

As for the Duffy antigen system, most of the donors were found to have the phenotype Fy(a+b+), 46.6% and 34.6% the Fy(a-b+), while the phenotype Fy(a+b-) was observed in 18.8% donors. Based on the presented data, it may be concluded that the results of this study are in correlation with the findings received for the phenotypes of the Duffy system in the previous examinations.^{6, 18}

Examining the distribution of erythrocyte antigens among blood donors is certainly a significant measure taken in

setting up the Registry of Blood Donors with Rare Blood Groups. Naturally, it first starts with the clinically relevant and most present erythrocyte antigens in a given population. Nonetheless, the examination needs to be expanded on antigen frequency that in the examined populations are said to be rare.

Examinations performed in this study constitute a starting point for creating the Registry of blood donors with rare blood groups in the Republic of Srpska, as it refers to a show-sample of regular blood donors, and they will to a large degree instantly facilitate the provision of compatible blood to sensibilised patients.

Conclusion

Distribution of clinically relevant erythrocyte antigens of the Rh phenotype (C, c, D, E, e) and C^w antigen within the Rh system, K, k, Kp^a, Kp^b antigens within the Kell system, M, N, S, s antigens within the MNS system, Fy^a, Fy^b antigens within the Duffy system, Jk^a and Jk^b antigens within the Kidd system, Lu^a and Lu^b in the Lutheran system, and P₁, Le^a and Le^b antigens in the Lewis system in the examined population of regular blood donors on the territory of Banja Luka was determined and was in accordance with the examined and published frequencies for the white population of different countries.

Examinations performed in this study represent a starting point for creating the Registry of blood donors with rare blood groups in the Republic of Srpska, as it refers to a show-sample of regular blood donors, and they will, to a large degree, instantly facilitate the provision of compatible blood to sensibilised patients.

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Distribucija klinički značajnih eritrocitnih antigena u populaciji davalaca krvi Republike Srpske

SAŽETAK

Uvod: Identifikovanje dobrovoljnih davalaca krvi sa rijetkim fenotipskim karakteristikama je osnovni preduslov za formiranje registra davalaca krvi sa rijetkim krvnim grupama.

Cilj rada: Utvrđivanje procentualne zastupljenosti fenotipova klinički najznačajnijih krvnogrupnih sistema kod redovnih davalaca krvi u Zavodu za transfuzijsku medicinu Republike Srpske, sa ciljem da se formira nacionalni registar davalaca rijetkih krvnih grupa.

Ispitanici i metode: Određivanje antigena Rh sistema je obavljeno automatskom metodom u mikropločama, kao i metodom u gelu. Određivanje antigena drugih krvnogrupnih sistema Kell, Kidd, Duffy, MNS, Lewis i Lutheran je rađeno metodom u gelu i metodom u epruvetama. Tokom 2012. i 2013. godine ispitana su 384 davaoca krvi.

Rezultati: Analizom Rh fenotipa najviše ispitanika je Rh fenotipa CcDee 29.7% i ccdee 26.0%, a najmanje Rh fenotipa ccddEe 0.8%, Ccddee 1.6% i ccDee 2.3%. Antigen Cw je dokazan kod 13 davalaca (3.4%), a antigen P1 kod 291 davaoca (75.8%). Analizom antigena sistema Lewis, najviše davalaca je sa fenotipom Le (a-b+) 74.0%. Analizom antigena sistema Lutheran, najviše davalaca je sa fenotipom Lu (a-b+) 93.0%. Kod jedne osobe je pronađen fenotip Lu (a+b-) (0.3%), a kod dva davaoca fenotip Lu(a-b-) (0.5%). Analizom antigena sistema Kell, najviše davalaca je sa fenotipom kk 93.2%, kod jedne osobe je pronađen fenotip KK (0.3%) i kod 25 davalaca fenotip Kk (6.5%). Analizom antigena sistema Kidd, najviše davalaca je sa fenotipom Jk(a+b+) 46.6% i sa Jk(a-b+) 29.4%, a fenotip Jk(a+b-) ima 24.0%. Ispitivanjem antigena MN sistema MNS, najviše je davalaca sa fenotipom MN 50.0% i sa MM 33.9%, a fenotip NN je zastupljen kod 16.1% davalaca. Analizom antigena S i s sistema MNS, najviše davalaca je sa fenotipovima Ss 49.0% i ss 43.2%, a kod 7.8% davalaca je pronađen fenotip SS. Analizom antigena sistema Duffy, najviše davalaca je sa fenotipom Fy(a+b+) 46.6% i sa Fy (a-b+) 34.6%, a fenotip Fy(a+b-) je zastupljen kod 18.8% davalaca.

Zaključak: Podaci o distribuciji klinički značajnih eritrocitnih antigena u populaciji redovnih davalaca krvi Republike Srpske su u skladu sa podacima iz literature za bijelu rasu.

Ključne riječi: krvne grupe, eritrocitni antigeni, distribucija klinički značajnih eritrocitnih antigena