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OPTIMAL BLOOD GROUPING AND ANTIBODY SCREENING FOR SAFE TRANSFUSION

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A b s t r a c t: *Introduction.* Blood group antigens as integrated parts of the red cell membrane have many essential functions for the cell as well as for the organism, but they are recognized as unique antigens for the purpose of safe blood transfusion. Especially in the case of those with great clinical importance because of their involvement in haemolytic transfusion reactions and hemolytic disease of the newborn, it is very important that they be correctly, and some of them routinely, typed in blood donors as well as in patients.

Aim. Evaluation of Rh and Kell blood group antigen frequencies in blood donors as well as the incidence of alloimmunization in transfused patients in the Macedonian population. The need for routine typing of certain blood group antigens in addition to ABO and RhD was also evaluated.

Material and method. We evaluated data from 1600 ABO/Rh and Kell typed blood donors (from January 2003 to May 2008), as well as the data from pretransfusion testing (ABO/RhD blood typing, irregular red blood cell antibody screening and compatibility testing) and antibody identification in the period from January 2005 to November 2008. All tests were performed by the DiaMed micro tube gel system.

Results. The frequencies of ABO antigens were as follows: A (39.7%), O (38%), B (14.1%), AB (7.4%). The frequencies of Rh antigens were as follows: D pos. (84.2%), D neg. (15.8%), C (58.3%), c (82.4%), E (21.3%), e (97.1%). We found the following frequencies of Kell phenotypes: K+ k- (0.25%), K+ k+ (6.18%), and K- k+ (93.6%) with the total frequency of K antigen of 6.4%. Antibody screening and/or cross-match were positive in the sera from 150 transfused patients. In 75 (50%) sera the following 81 antibodies were identified: anti-K (26), -E (25), -e (1), -C (4), -c (6), -C^{w} (2), -k (1), -Fy^{a} (3), -Fy^{b} (1), -Jk^{a} (3), -Lu^{b} (1), -Le^{b} (2), -Le^{a} (1), -M (4), -P1 (1). The most frequent alloantibody was anti-K with 32%, and anti-E with 30.8% of all identified antibodies.

Conclusion. Alloimmunization to red cell antigens is still a current problem in our transfusion practice. It is obvious that the additional testing of blood donors for Rh and Kell antigens should be implemented as a routine to prevent as far as possible the incidence of alloimmunization. It would also be cost-effective, bearing in mind the additional laboratory testing necessary to provide compatible blood for alloimmunized patients. Extended blood typing should be implemented for some categories of poly-transfused patients as well. This strategy is another step forward to improve the safety of blood transfusion with optimal blood grouping.

Key words: Kell phenotypes frequency, alloimmunization, blood grouping, safe transfusion.

Introduction

The differences in human blood are due to the presence or absence of certain protein molecules called antigens and antibodies. The antigens are located on the surface of the red blood cells and the antibodies are in the blood plasma. Individuals have different types and combinations of these molecules. The blood group you belong to depends on what you have inherited from your parents.

The International Society of Blood Transfusion (ISBT) recognizes over 300 (302) red cell surface antigenic determinants; most of these (about 285) belong to one of 29 blood group systems. Each blood group system represents a single gene or cluster of 2 or 3 closely-linked homologous genes of related sequence and with little or no recognized recombination occurring between them, giving a total of 34 gene loci. There are 4 categories of blood group antigens: blood group systems (262 antigens in 29 blood group systems); collections (12 antigens); 700 series (19 antigens); 901 series (9 antigens) [1, 2].

Blood group antigens as integrated parts of the red cell membrane have many essential functions for the cell as well as for the organism (membrane transporters and protein canals, ligand receptors, adhesion molecules, enzymes, structural proteins) as well as different biochemical composition. For example: A, B and H (ABO system) are oligosaccharides; D, C, c, E, e, C^w (Rh system) are proteins, M, N, S, s (MNS system) are syaloglicoproteins; K, k, Js^a, Js^b, Kp^a, Kp^b (Kell system), Fy^a, Fy^b (Duffy system), Lu^a, Lu^b (Lutheran system) are glycoprotein; Jk^a and Jk^b antigens (Kidd system) are proteins and P1 antigen (P system) is glucolipid [3].

Blood group antigens are recognized as unique antigens for the purpose of safe blood transfusion, foeto-maternal blood group incompatibility and the haemolytic disease of the newborn, and later to meet the needs of the transplantation practice, etc. ABO is the original blood group system first discovered by the Nobel Laureate Karl Landsteiner, who was involved in the discovery of

both the AB0 and Rh blood groups. The AB0 and Rh systems are the most important ones used for blood transfusions. Not all blood groups are compatible with each other. Mixing incompatible blood groups leads to blood clumping or agglutination, which is dangerous for individuals (haemolytic transfusion reactions and haemolytic disease of the newborn) [1, 4].

According to the Guidelines for BT Services in the UK, mandatory tests of blood donation (red cell immunohaematology) are: 1. The ABO blood group must be determined on each blood donation as well as antibody screening; in the case of a donor whose ABO blood group is unknown to the test centre, e.g. a first-time donor, the ABO blood group must be determined by testing the plasma/serum with group A1, and B red cells. The red cells of the donation must be tested twice with anti-A and anti-B as a minimum. The ABO group can only be accepted if the results are in agreement; 2. The D blood group must be determined on each donation of blood; in the testing of donors being grouped for the first time, two anti-D blood grouping reagents should be used capable of detecting between them D^{IV}, D^{V} and D^{VI} antigens. If two monoclonal anti-Ds are used, they should be from different clones. 3. Routine antibody screening: all donations must be tested for the presence of red cell antibodies. This is achieved by testing the donor's serum or plasma using a validated technique capable of detecting anti-D at 0.5 IU/mL or lower. As for additional testing, it should be mentioned that phenotyping beside ABO/RhD is not mandatory but an additional extended phenotyping which includes RhC, E, c, e and K antigens as well as other specificities if necessary [5].

The Council of Europe also recommends ABO/RhD typing and antibody screening on every first donation as mandatory tests. Antibody screening is an additional test only if the donor has a transfusion or pregnancy record in the period since the last donation. Extended phenotyping is also an additional test [6].

Considering the safety of blood transfusion and optimal blood testing we must bear in mind mandatory as well as additional blood typing tests which include antigens with great clinical importance involved in adverse immunologic reactions to transfusion. Because of this, the proper selection of mandatory and additional blood grouping tests and their correct performance and interpretation (validated techniques) in blood donors as well as in patients is very important. Besides the worldwide recommendations (CE or others), the decisionnas to what to test should be made, bearing in mind the local needs based on the local antigenic profile and pathology of the population, is also of great importance.

Material and method

We evaluated data from 1600 ABO/Rh (D, C, E, c, e) and Kell typed voluntary blood donors in the period from January 2003 to May 2008. ABO and

RhD testing was performed by tube test and by micro-plate technique (depending what was available at the time). RhC, c, E, e and K antigen typing was performed by the Micro Typing System column agglutination technique. We used an automated technique for donor tests.

We also evaluated the data from patient pre-transfusion testing (ABO/RhD blood-typing, irregular red blood cell antibody screening and compatibility testing), antibody identification, additional blood group typing and direct antiglobulin test (DAT) in the period from January 2005 to November 2008. Of 150 transfused patients with positive antibody screen and /or cross-match 108 (78%) were female and 42 (28%) were male. All tests for the patients were performed manually on corresponding ID-cards by the DiaMed ID-Micro Typing System.

Results

The frequencies of ABO antigens in blood donors were as follows: A (39.7%), O (38%), B (14.1%), AB (7.4%).The frequencies of Rh antigens were as follows: D pos. (84.2%), D neg. (15.8%), C (58.3%), c (82.4%), E (21.3%), e (97.1%) (Table 1). We found the following frequencies of Kell phenotypes: K+ k- (0.25%), K+ k+ (6.18%), K- k+ (93.6%). The total frequency of K antigen was 6.4% (Table 2). Antibody screening and/or cross-match were positive in the sera from 150 transfused patients. In 75 (50%) sera the following 81 antibodies were identified: anti-K (26), -E (25), -e (1), -C (4), -c (6), -C^w (2), -k (1), -Fy^a (3), -Fy^b (1), -Jk^a (3), -Lu^b (1), -Le^b (2), -Le^a (1), -M (4), -P1 (1). The most frequent alloantibodies were anti-K with 32%, and anti-E with 30.8% of all identified antibodies (Table 3). There were 6 sera with the presence of multiple antibodies: 3 (anti-K + E), 1 (anti-C + e), 1 (anti-E + C^w) and 1 serum containing anti-E, + C^w + c. All of the 75 transfused patients were antigen negative for the corresponding alloantibody. The DAT and the auto control were also negative excluding the presence of autoantibodies. 16 (21.3%) of the patients with identified irregular antibodies were male and 59 (78.6%) were female.

Table 1 – Табела 1

Frequencies of D, C, c, E, e antigens in blood donors
Фреквенции на D, C, c, E, e антигените кај крводарителите

Antigen	Frequency (%)
D	84.2%
C	58.3%
c	82.4%
E	21.3%
e	97.1%

Table 2 – Табела 2

Frequency of Kell system phenotypes in blood donors
Фреквенции на фенотиповите на Kell системот кај крводарителите

Phenotype	Frequency (%)
(K+ k-)	0.25
(K+ k+)	6.18
(K- k+)	93.6

Table 3 – Табела 3

Frequencies of specific alloantibodies in patients
Фреквенции на специфични алоантитела кај пациенти

Specificity	Antibodies detected No (%)	Found in combination
anti-K	26 (32)	3 (+ anti-E) 2 (+ anti-D)
anti-E	25 (30.8)	3 (+ anti-K) 1 (+ anti-C^w + c)
Anti-c	6 (7.4)	1 (+ anti-E + C^w)
Anti-C^w	2 (2.4)	1 (+ anti-E) 1 (+ anti-c + E)
Anti-C	4 (5)	1 (+ anti-e)
Anti-e	1 (1.2)	1 (+ anti-C)
Anti-Jk^a	3 (3.7)	
Anti-Fy^a	3 (3.7)	
Anti-Fy^b	1 (1.2)	
Anti-M	4 (5)	
Anti-P1	1 (1.2)	
Anti-Le^a	1 (1.2)	
Anti-Le^b	2 (2.4)	
Anti-Lu^b	1 (1.2)	
Anti-k	1 (1.2)	
Total	81	

Discussion

The frequencies of ABO, RhD, C, E, c, e antigens in our group of typed blood donors are not significantly different in comparison with other white race populations, except for the RhC and RhE antigens that are not lower (not significantly) in the Macedonian population than in English blood donors. Ho-

wever, the frequency of Kell (KEL1) antigen is significantly lower (6.4%) in comparison with about 9% in Northern Europeans [1, 7].

In our Institution, the mandatory tests for blood donations concerning red cell immunohaematology at present are: ABO (forward and reverse typing), RhD typing (two monoclonal sera), antibody screening with antihuman globulin (AHG), as well as test for D weak which includes AHG for first-time donors. Antibody screening is performed on every donation. An automated test system for mandatory tests is in use. Routine typing for A_1 and A_2 phenotypes of A and AB blood group has been abandoned since August 2008. In accordance with the National Guidelines. Routine typing for other Rh (C, c, E, e), Kell or other antigens is not performed. They are performed as additional tests when necessary. The patients are also routinely typed for ABO and RhD antigen only. Antibody screening is not performed on a regular basis. Pretransfusion testing consists of ABO/RhD typing and compatibility testing ("Type and cross-match [x] number of units").

The average annual number of compatibility tests is 19,576 and the average number of patients receiving blood transfusion (erythrocyte concentrates) is about 10,000 per year. the estimated X-M versus transfused units ratio is about 2, so the rate of alloimunization in the above-mentioned period was almost 1%. We consider that this number is much higher because antibody screening is not performed routinely in transfused patients as a “type and screen” procedure, so irregular antibodies are only detected if the compatibility test is positive.

Among the most common risks of red-blood cell (RBC) transfusion is the development of RBC alloantibodies. The incidence of RBC alloimmunization is not insignificant, ranging from 4% to as high as 60% in some patient populations [8, 9]. Clinically, RBC alloimmunization can result in delays in patient care, haemolytic transfusion reactions, haemolytic disease of the foetus and newborn and possibly increased morbidity following organ transplantation [10]. In addition, RBC alloimmunization has a significant negative impact on laboratory and institutional resources associated with a need for increased laboratory testing, difficulties in identification and procurement of compatible blood units for transfusion, obstetrical management, and with the evaluation and management of transfusion reactions. Proposals to minimize RBC alloimmunization through extended antigen matching for all patients are logistically impractical and would add significant costs to the health care system [11].

Despite the advances achieved by modern transfusion practice, as well as improvement in the diagnostic tests, haemolytic transfusion reactions (HTR) remain the most common cause of immediate life-threatening events associated with transfusion. Delayed haemolytic transfusion reactions (DHTR) were diagnosed with a frequency of 1 per 4,000 units of whole blood or erythrocytes

transfused, which represents an increased frequency [12]. The later studies showed that the incidence of DHTR is 1 per 12,000 [13]. According to the annual report (2004) for EHA (European Haemovigilance Network) from France, the overall risk to transfusion was 1: 163,910 labile blood products. There were 1216 case reports of alloimmunization following red blood cell transfusion with the risk of 1: 1,890 labile blood products. The haemovigilance network from Spain reported 52 (3.5%) cases of DHTR from a total of 1,500 adverse reactions to transfusion for 2006 [14].

The SHOT (Serious Hazards of Transfusion) report for the period from 1996 to 2007 showed the following data: out of 4,335 reported cases, 534 (12.3%) were due to ATR (acute transfusion reactions) and 342 (7.93%) were due to DHTR. According to the SHOT report for 2007, the incidence of HTR in the UK was 1 in 19,600 units of red blood cell (RBC), 4.1% of all adverse reactions. Recent data in the US *(E. Kardon, e-medicine haematology, Transfusion reactions, 2008)* showed the incidence of HTR as 1 in 40,000 transfused units of RBC [15].

The alloimmunization to red cell antigens and HTR is still an actual problem in our transfusion practice.

Total prevention of all haemolytic transfusion reactions is unrealistic, because current immunohaematological methods of testing are not sensitive enough to detect all antigens and antibodies or to predict an anamnestic response. Prevention is further hampered by unfortunate and frequently avoidable human errors. Errors at any stage of performing the tests for ABO/RhD grouping on donor and patient, antibody screening and compatibility testing can lead to incompatible or inappropriate blood transfusion. The implementation of a quality management system should help to reduce the number of technical and procedural errors made in the laboratory.

Bearing in mind that the K (KEL1) is a low frequency antigen, and the E (RH3) has the lowest frequency of the clinically most important Rh antigens, we found the highest rate of corresponding alloantibodies in our group of transfused patients. According to the data, the routine Rh and Kell typing for blood donors (which is not performed routinely) and for polytransfused patients (only performed when compatible blood is to be found) is necessary to prevent alloimmunization and improve the safety of blood transfusion.

Conclusion

Alloimmunization to red cell antigens is still an actual problem in our transfusion practice. It is obvious that the additional testing of blood donors for Rh and Kell antigens as a routine should be implemented to prevent as far as

possible the incidence of alloimmunization. It would also be cost-effective, bearing in mind the additional laboratory testing necessary to provide compatible blood for alloimmunized patients. Extended blood typing should be implemented for some categories of polytransfused patients as well. This strategy is another step forward to improve the safety of blood transfusion with optimal blood grouping.

REFERENCES

1. Daniels G., Bromilow I. (2007): *Essential Guide to Blood Groups;* Blackwell publishing, 1st Ed.; 1–5.

2. Daniels G. (2007): Functions of red cell surface proteins. *Vox Sang;* 93: 331–340.

3. Cartron JP., Colin P. (2001): Structural and functional diversiy of blood group antigens. *Transfus Clin Biol;* 8: 163–99.

4. Contreras M. (1998): Cellular antigens as immunogens in blood transfusion and pregnancy: Immunogenetic aspects of blood transfusion and bone marrow transplantation. *Proceedings of the European School of transfusion medicine;* 10–15.

5. *Guidelines for BT Services in the UK*. Chapter 13, Donation testing (red cell immunohaematology). 7th Ed. (2007).

6. *Guide to the Preparation, Use and Quality assurance of Blood Components*, Chapter, A Quality system in Blood Transfusion Services. Council of Europe, 12th Ed. 2006.

7. Daniels G. (2002): *Human Blood Groups*, 2nd edn. Oxford: Blackwell Science.

8. Schonewille H., Brand A. (2005): Alloimmunization to red blood cell antigens after universal leucodepletion. A regional multicentre retrospective study. *Br J Haematol;* 129 (1) 151–6.

9. Schonewille H., van de Watering LMG, Brand A. (2006): Additional red blood cell alloantibodies after blood transfusion in a nonhematologic alloimmunized patient cohort: is it time to take precautionary measures? *Transfusion;* 46: 630–635.

10. Boyd SD., Stenard F., Lee DK. *et al*. (2007): Alloimmunization to red blood cell antigens affects clinical outcomes in liver transplant patients. *Liver Transplantation;* 13: 1654–1632.

11. Lau FY., Wong R., Chan NPH., Chui CH. *et al.* (2001): Provision of phenotype-matched blood units: no need for pre-transfusion screening. *Haematologica;* 86: 742–748.

12. Moore SB., Taswell HF., Pineada AA. (1980): Delayed hemolytic transfusion reactions. Evidence of the need for an improved pretransfusion compatibility test. *Am J Clin Pathol;* Jul. 74 (1): 94–7.

13. Pineada AA., Vamvaks LD., Gorden LD. *et al*. (1999): Trends in the incidence of delayed hemolytic and delayed serologic transfusion reactions. *Transfusion;* 39: 1097–1103.

14. Cardenas JM. (2008): The Haemovigilance system in Spain. *Proceedings* of the ESTM residential course, Skopje, Macedonia, 10th–14th September, 1: 189–191.

15. Walterova L. (2008): Safety issues in blood administration: management of transfusion reactions. *Proceedings* of the ESTM residential course, Skopje, Macedonia, 10th–14th September, 1: 213–221.

Резиме

БЕЗБЕДНА ТРАНСФУЗИЈА НА КРВ СО ОПТИМАЛНА КРВНОГРУПНА ТИПИЗАЦИЈА И ДЕТЕКЦИЈА НА АНТИЕРИТРОЦИТНИ АНТИТЕЛА

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Вовед. Крвногрупните антигени претставуваат составен дел на еритроцитната мембрана и како такви имаат бројни значајни функции како за самата клетка така и за организмот во целост. Како посебни биохемски и антигенски структури се откриени и препознати за потребите на безбедната трансфузија на крв. Ова особено се однесува на антигените кои имаат големо клиничко значење поради нивната улога во несаканите трансфузиски реакции, хемолитичката болест на новороденото и потребите на ткивната и органската трансплантација.

Цел. Евалуација на ABO, Rh и Kell крвногрупните антигенски фреквенции кај дарителите на крв, како и евалуација на појавата на алоимунизација кон истите кај трансфундираните пациенти. Потребата од рутинска типизација на одредени крвногрупни антигени покрај ABO и RhD исто така беше предмет на испитувањето.

Материјал и методи. Беа евалуирани податоците од 1600 крводарители типизирани на ABO/Rh и Kell антигените (од јануари 2003 до мај 2008 год.), податоците од преттрансфузиското тестирање (ABO/RhD типизација, детекција на ирегуларни антиеритроцитни антитела и тестови на компатибилност-интерреакција) и податоците за идентифицираните алоантитела со конкретна специфичност (од јануари 2005 до ноември 2008 год.). Сите тестови беа изведени со микроаглутинирачката техника на микрогел картички (DiaMed).

Резултати. Фреквенциите на АВО антигените изнесуваат: А (39,7%), О (38%), В (14,1%), АВ (7.4%). Фреквенциите на Rh антигените се следниве: D позитивни (84.2%), D негативни (15,8%), C (58,3%), c (82,4%), E (21,3%), e (97,1%). Беа добиени следниве фреквенции на Kell фенотиповите: K+ k- (0,25%), K+ k+ (6,18%), и K- k+ (93,6%) со самостојна фреквенција на K (KEL1) антигенот од 6.4%. Детекцијата на антитела и/или тестот на компатибилност беа позитивни во серумите на 150 трансфундирани пациенти. Кај 75 (50%) од испитуваните серуми беа идентифицирани 81 антитело: anti-K (26), -E (25), -e (1), -C (4), -c (6), -C^{w} (2), -k (1), -Fy^{a} (3), -Fy^{b} (1), -Jk^{a} (3), -Lu^{b} (1), -Le^{b} (2), -Le^{a} (1), -M (4), -P1 (1). Од сите идентифицирани антитела, најголема честота покажа алоантителото anti-K со 32%, и anti-E со 30,8%.

Заклучок. Алоимунизацијата кон еритроцитните антигени е сè уште актуелен проблем во нашата трансфузиска практика. Потребно е воведување на дополнителна рутинска типизација кај крводарителите, и тоа на Rh и Kell антигените. Ваквиот обем на имунохематолошко тестирање на крвта истовремено е и рационално и исплатливо имајќи ги предвид дополнителните лабораториски тестови и продолженото чекање на сензибилизираните пациенти за обезбедување на компатибилна крв. Проширено крвногрупно типизирање треба да се воведе и за некои категории политрансфундирани пациенти. Ваквата стратегија е уште еден чекор кон зголемување на безбедноста на крвната трансфузија со оптимално крвногрупно типизирање.

Клучни зборови: Фреквенција на Kell фенотипови, алоимунизација, крвногрупна типизација, безбедна трансфузија.

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