PREVALENCE OF UNEXPECTED RED BLOOD CELL ANTIBODIES IN BLOOD DONORS

Makarovska Bojadzieva T, Ismani E
Institute for transfusion medicine, Macedonia

Abstract:

Red blood cell (RBC) antibody screening is an obligatory part of our national blood testing strategy. It has been performed on regular basis, on every donation from each donor. In the last decade we have introduced more sensitive methods for detecting RBC antibodies.

Aim of the study: To estimate the prevalence and the nature of the irregular RBC antibodies detected in the period from 2009 to 2014 and to analyze demographic characteristics of blood donors with specific antibodies.

Material and methods: A total of 158170 blood units were screened for irregular RBC antibodies using pooled screening cells (2 donors) in combination with the indirect antiglobulin test (IAT), performed by gel technique and the automated system Techno Twin Sampler. Samples with confirmed positive antibody screening were subjected to antibody identification with commercial red cell panels (DiaMed and Ortho). We used blood donor data from the donor information system.

Results: The total number of samples with positive antibody screening was 122 (0.078%). The ratio of female to male donors with positive antibody screening was 64 (52%): 58 (48%) respectively. Specific antibodies were identified in 67 (55%) out of 122 samples from which 53 (79.1%) were clinically significant (CS). The overall prevalence rate of the specific antibodies was 0.04%. The specificity of antibodies was as fallows: anti-D, 15 (22%); -E, 11 (16%); -C, 4 (6%); -c, 3 (4.5%); -C^w, 2 (3%); -K, 13 (19.5%); -Kp^a, 2 (3%); -Fy^a, 1 (1.5%); -Lu^a, 2 (3%); -M, 11 (16.5%); -P, 2 (3%) and anti-Le^b, 1 (1.5%). The average age in donors with CS antibodies was 48.2 years. The average number of donation prior to the antibody detection was 1.7 with mean interval between donations of 1.8 years.

Conclusion: The prevalence of RBC irregular antibodies in our blood donors is very low mainly due to the good donor selection programme, as well as to the currently used screening method which contributes to the decrease of false positive and nonspecific reactions. The low prevalence of antibodies raises the question of cost-effectiveness of red cell antibody screening on regular basis. However, permanent donor education and further analysis of the possible impact on the safety of our blood supply is essential to establish cost-effective and safe RBC antibody screening model by targeting of donors which are at particular risk of RBC alloimmunization.

Key words: blood safety, red blood cell, antibody screening, antibody specificity

Introduction

Red blood cell antigens are polymorphic immunogenic determinants on the erythrocyte membrane. They have substantial biological and clinical importance because they can provoke immune response in the recipient by production of red blood cell antibodies (1, 2).

The purpose of the antibody screening is to detect red blood cell (RBC) antibodies other than the "natural" anti-A or anti-B antibodies which are present in every individual according to the inherited ABO blood group. These antibodies are called "unexpected" or "irregular" because only 0.3 to 2 % of the general population has positive antibody screening due to immunization against RBC after blood transfusion, pregnancy or transplantation (3, 4).

Speaking of blood donors the prevalence of irregular RBC antibodies is much lower (usually less than 0.1%) than the general population because of the process of selection of the donors who are eligible to donate. In certain groups of patients, especially those who are chronically transfused (hereditary or acquired hematopoietic disorders, chronic renal failure, etc.) the prevalence is much higher, from 5% up to 60% (5, 6, 7).

RBC antibody screening is an obligatory part of our national blood testing strategy. It has been performed on every donation from each donor in order to exclude blood units with positive antibody screen from clinical use, thus preventing hemolytic transfusion reaction in the recipient. Different manual screening methods such as tube technique with enzyme treated screening cells were employed through the time. In the last decade we have introduced more sensitive and fully automated methods for detection of irregular RBC antibodies in blood donors, especially those which are clinically significant (CS), capable of causing hemolytic transfusion reactions (HTR) and hemolytic disease of the fetus and newborn (HDFN).

The aim of this study is to estimate the prevalence and the nature of the irregular RBC antibodies detected in blood donors in the period from 2009 to 2014 and to analyze demographic characteristics of blood donors with specific antibodies.

Material and methods

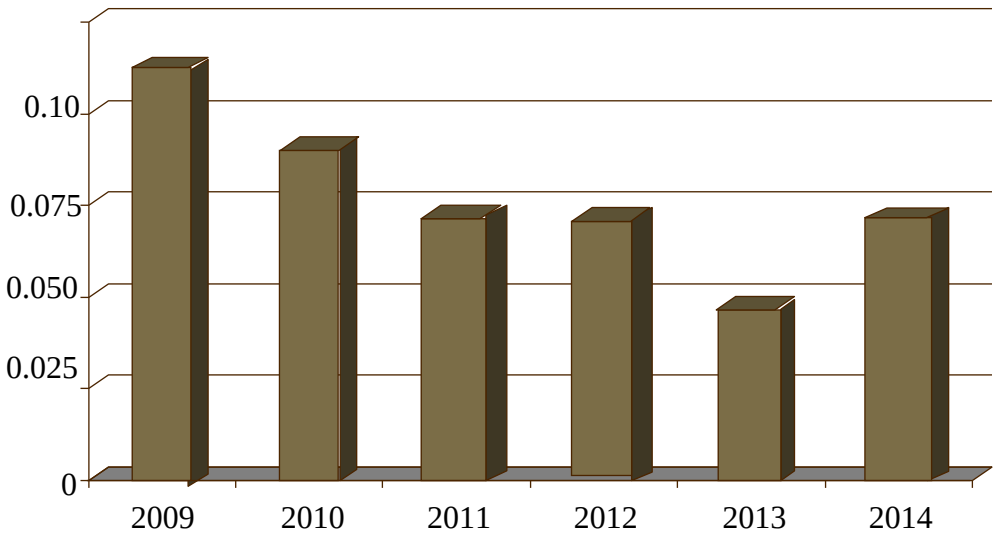
A total of 158.170 blood donors were screened for irregular red blood cell antibodies. Blood samples were taken in K2EDTA tubes. Donor plasma was tested against group O pooled reagent red blood cells with known antigens (2 screen cells). The principle of the method is

based on the the indirect antiglobulin test (IAT) performed in gel (column agglutination technique-CAT) using the automated system Techno Twin Sampler (DiaMed). Samples with positive antibody screening were subjected to antibody identification with commercial reagent red cell panels (DiaMed and Ortho) using antiglobulin (IAT) and/or enzyme test. Antibody specificity was interpreted according to the antigen table from the panel.
Corresponding blood group antigens were typed using specific blood group typing sera.
We used data from the donor information system to analyze the demographic characteristic of the blood donors with irregular RBC antibodies.

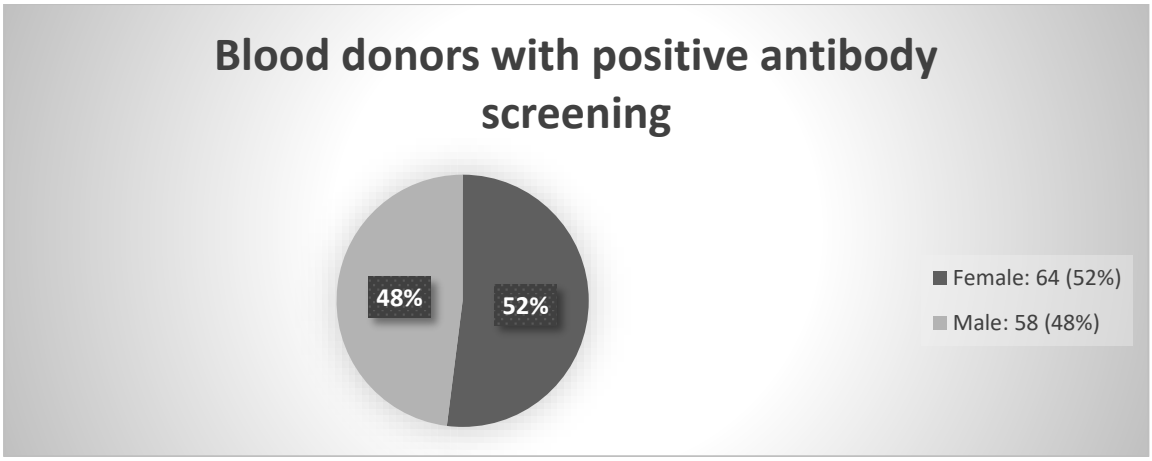
Results

The total number of donors with positive antibody screening was 122 (0.078%) or less than 1 in 1000 donors. The prevalence of irregular antibodies was decreasing until 2013, starting with 0.12% in 2009, 0.08% in 2010, 0.07% in 2011, 0.07% in 2012, 0.04% in 2013 and 0.07% in 2014 as shown on Graphic 1.

Graphic 1. The prevalence of irregular RBC antibodies



Graphic 2. Distribution of the donors with positive antibody screening according to the sex



The average number of donation prior to the antibody detection was 1.7 with mean interval between donations of 1.8 years.

Specific antibodies were identified in 67 (55%) out of 122 samples (Table 1). In the rest of the 55 (45%) samples with positive RBC screening the antibodies were non specific. Clinically significant for blood transfusion were 53 (79.1%) out of the 67 identified antibodies. The average age in donors with clinically significant antibodies was 48.2 years. The overall prevalence rate of the specific antibodies was 0.042%.

Table 1. Specificity of the antibodies

Antibody specificity	No (%)	Clinical significance
D	15 (22)	Yes
E	11 (16.0)	Yes
C	4 (6.0)	Yes
c	3 (4.5)	Yes
C^w	2 (3.0)	Yes
K	13 (19.5)	Yes
Kp^a	2 (3.0)	Yes
Fy^a	1 (1.5)	Yes
Lu^a	2 (3.0)	Yes
M	11 (16.5)	Yes*
P	2 (3.0)	No
Le^b	1 (1.5)	No
Total	67 (100)	

* Most of them naturally occurring; clinically significant if reactive on 37ºC

The donor RBC were typed antigen negative for the corresponding antibody.

Discussion

The sensitivity and specificity of the antibody screening depends on the screening cells as well as on the method. For blood donor screening, reagent RBC may be pooled, obtained from maximum two group O donors with Rh phenotype CCDee and ccDEE. The fallowing antigens must be present on at least one of the cell sample: D, C, E, c, e, M N, S, s, P, Le^a, Le^b, K, Fy^a, Fy^b, and Jk^b. The indirect antiglobulin test (IAT) performed using column agglutination technology (CAT) nowadays is considered to be most sensitive for the detection of clinically relevant RBC antibodies (8, 9). The usage of enzyme treated RBC for antibody screening in blood donors may lead to detection of non specific, clinically insignificant RBC antibodies to a greater extent which means additional testing and laboratory overload (10, 11). Until 2007, the antibody screening in our blood donors was performed using enzyme test which is one of the reasons for the high prevalence of irregular RBC antibodies of about 1% at that time. Nowadays, the prevalence of RBC antibodies in our blood donors is much lover, being 0.078% which is due to the currently used technique (CAT with IAT) which contributed to the decrease of false positive and nonspecific reactions. In spite of that there are still some limitations to antibody detection method such as:

- Will not detect antibodies to antigens that are not present on the screen cells.
- May not detect antibodies exhibiting dosage.
- May not detect antibodies that are low in titer.
- Complement-dependent antibody/plasma specimen.

In fact, our study revealed 142 (0.09%) samples with initially reactive RBC screening from which 20 (0.012%) were false positive and 122 (0.078%) confirmed positive. There is an algorithm according to which donors with positive screening are retested in order to exclude false positive results. For that purpose, the initially reactive sample is retested with two different reagent RBC using the same technique. If the RBC screening is confirmed positive, the blood is discharged and the donor is temporary deferred from donation. The donor is informed in written and asked for additional blood sample. The donor status depends on the nature of the identified antibody. In case of a clinically significant antibody the donor is permanently deferred from blood donation. Naturally occurring antibodies such as anti-M, anti-Lea, are also frequently detected in younger donors under the age of 30. If these antibodies are reactive at 37^{o}C, they are considered as potentially capable of hemolysis which may be a reason

for permanent donor deferral as well as is the permanent presence of non specific unexpected RBC antibodies.

Similar low prevalence of irregular RBC antibodies is observed in most of the European countries in which blood donation is voluntary and non remunerated and there is a good donor selection practice as it is in our country. Because of that they have introduced different selective RBC screening strategies which include only first time donors, female donors and donors with a history of previous blood transfusion. Having in mind that the majority of detected antibodies (79%) were clinically significant and developed as a result of previous blood transfusion and pregnancy (anti-D is still being the single most frequent antibody) we should be very careful in consideration the changes in the current screening strategy.

Conclusion

The prevalence of irregular RBC antibodies in our blood donors is very low mainly due to the good donor selection programme, as well as to the currently used screening method which contributes to the decrease of false positive and nonspecific reactions. The low prevalence of irregular antibodies raises the question of cost-effectiveness of red cell antibody screening on regular basis. However, permanent donor education and further analysis of the possible impact on the safety of our blood supply is essential in order to establish cost-effective and safe RBC antibody screening model by targeting of donors which are at particular risk of RBC alloimmunization.

References

1. Daniels G. Functions of red cell surface proteins. Vox Sang 2007; 93:331-340.
2. Cartron JP. Structural and functional diversiy of blood group antigens. Transfus Clin Biol 2001; 8: 163-99.
3. Daniels G, Bromilow I. Essential Gide to Blood Groups. Blackwell publishing, 1st Ed. 2007; 1-5.
4. Poole J, Daniels G. Blood group antibodies and their significance in transfusion medicine. Transfus Med Rev. 2007;21:58–71.
5. Makarovska-Bojadzieva T, Blagoevska M, Kolevski P, Kostovska S. Optimal blood gouping and antibody screenining for safe transfusion. Contributions, Sec.Biol.Med. Sci. MASA. 2009; 30 (1): 119-128.

6. Sanz C, Nomdedeu M, Belkaid M, Martinez I, Nomdedeu B, Pereira A. Red blood cell alloimmunization in transfused patients with myelodysplastic syndrome or chronic myelomonocytic leukemia. Transfusion. 2013; 53(4):710-5.
7. Azarkeivan A, Ansari S, Ahmadi MH, Hajibeigy B, Maghsudlu M, Nasizadeh S, Shaigan M, Toolabi A, Salahmand M. Blood transfusion and alloimmunization in patients with thalassemia: multicenter study. Pediatr Hematol Oncol. 2011; 28(6):479-85.
8. de Castilho LM, Pellegrino J, Jr, Bechelli AP, Le Pennec PY, Mendes NF. Evaluation of recent techniques for detection of red blood cell antibodies in sera of reference samples, patients, pregnant women, and blood donors. J Clin Lab Anal. 1996;10:250–256.
9. Shin JH, Lee JY, Kim JH, Kim HR, Lee JN,Screening and Identification of Unexpected Red Cell Antibodies by Simultaneous LISS/Coombs and NaCl/Enzyme Gel Methods. J Korean Med Sci. 2009 Aug;24(4):632-5.
10. Kretschmer V. Immunohaematological basis for blood transfusion (blood grouping, antibody screening, compatibility testing). Proceedings of the ESTM residential course, Skopje, Macedonia, 10^{th} – 14^{th} September, 2008; 1: 205:212.
11. Walker PS. Identification of red cell antibodies to red cell antigens. In AABB Technical manual. Roback JD., Combs MR., Grossman BJ., Hillyer CD. 16th ed. American Association of Blood Banks, Bethesda, Maryland 2008.