ORIGINAL PAPER

Significance of Detecting Antierythrocyte Antibodies in Pretransfusion Testing

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ABSTRACT

Background: Transfusion treatment during life, as well as pregnancy in women, can stimulate the sensitization of the person who received blood, which after the transfusion of blood products can result in the occurrence of moderate to very severe posttransfusion reactions. **Objective:** The aim of this study was to examine the specificity and frequency of antierythrocyte antibodies during the pretransfusion treatment of patients depending on the gender, to determine the origin of antibodies in patients serum, as well as to examine their clinical significance. **Methods:** Retrospective analysis of documentation was performed in the Department for Pretransfusion Testing, therapy and distribution of blood products, Polyclinic for Transfusion, UKC Tuzla. Data was analyzed by reviewing the written and electronic documentation from the period of 5 years (2018-2022). **Results:** A retrospective analysis of 378 procedures for the identification of antierythrocyte antibodies was performed. It was evident that 140 of all detected antibodies belonged to the Rh-system (66.7%), of which 32.4% were anti RhD-antibodies, 20% anti Rh-E, anti Rh-c 7.1%, and in a low percent of anti-C, anti-e and anti-Cw antibodies. Combinations of anti-D and anti-C antibodies showed the highest frequency (34.2%), followed by a combination of anti-E and anti-c antibodies (21%). **Conclusion:** Pretransfusion testing represents a very important link in the safety of the use of blood. The identification of antierythrocyte antibodies and the use of phenotyped blood products significantly reduces the risk of posttransfusion reactions and facilitates the implementation of the safe blood policy.

Keywords: antierythrocyte antibodies, sensitization, transfusion.sensitization of the person who received blood, sen

1. BACKGROUND

Transfusion treatment during life, as well as pregnancy in women, can stimulate the sensitization of the person who received blood, which after the transfusion of blood products can result in the occurrence of moderate to very severe posttransfusion reactions. The antierythrocyte antibody screening test is a mandatory part of standard blood group testing and is based on IAT (indirect antiglobulin test) (2). Antierythrocyte antibodies are formed as an immune response against erythrocyte antigens that a person does not have on his erythrocytes.

Antierythrocyte antibodies are divided into natural and immune antibodies. Natural antibodies are found in the serum of people who have never received blood or been sensitized through pregnancy. Examples of these antibodies are antibodies from the ABO system, anti M, anti Lea, and many naturally occurring antibodies from other blood group systems (3). Immune antierythrocyte antibodies are produced by sensitization after blood transfusion or during pregnancy in women. They are detected by the indirect antiglobulin test (IAT) and are divided into three groups: clinically significant, potentially clinically significant and clinically insignificant antierythrocyte antibodies (1, 7, 8). Clinically significant antibodies are those that react at body temperature. The most clinically significant antierythrocyte antibodies include antibodies from blood group systems: Rh and Kell (4). They mainly belong to the IgG class, and are caused by immunization during pregnancy

and after transfusion with an incompatible dose of erythrocytes. IgG antibodies react optimally at 37 °C, and are also called warm antibodies. They may or may not activate complement, and due to their size, they may pass through the placenta of the child and cause hemolytic disease of the newborn (HBFN) (1). Potentially significant antierythrocyte antibodies are antibodies from the MNS, Jk, Kappa and Duffy blood group systems. These antibodies belong mainly to the class of IgM antibodies, and are produced by immunization of antigens from the environment. They do not pass through the placenta of the child, and become clinically significant only when they activate complement. They react optimally at a temperature of + 4 °C to + 20 °C and are therefore called cold antibodies. Clinically insignificant antibodies are antibodies from the Lewis, Lutheran and P systems, which are IgM class and almost never cause hemolytic reactions.

2. OBJECTIVE

The aim of this study was to examine the specificity and frequency of antierythrocyte antibodies during the pretransfusion treatment of patients, to determine their origin depending on the gender of the patients and to distinquish the type of antibodies (natural or immune), as well as their clinical significance.

3. MATERIAL AND METHODS

Retrospective analysis of documentation was performed to determine the number, origin and frequency of antierythrocyte antibodies in the Department for pretransfusion testing, therapy and distribution of blood products, Polyclinic for Transfusion, UKC Tuzla. The data was analyzed by reviewing the documentation (written protocols and computer system RGB-Renovatio) from a time period of 5 years (2018-2022). The analysis included all hospitalized patients for whom transfusion of blood and blood products was indicated in the given period, and for whom the presence of a specific antierythrocyte antibody in the serum was detected. Data on gender, existence of possible sensitization, type of sensitization (acute and chronic), and results of antierythrocyte antibody identification and their clinical significance was analyzed. No exclusionary criteria was taken into account, as the study included all patients regardless of age, gender, diagnosis, etc.

The identification of antierythrocyte antibodies was performed in microgel technology including 11 microtubes in three environments: NaCl, Liss-Coombs (indirect antiglobulin test-IAT) and enzymatic medium to which the enzyme bromelain was added. After incubation and centrifugation, the result was read by the presence or absence of agglutination in the microgel cards and by comparison with the standard panel of results.

In the conducted research, the data were presented in absolute and relative frequencies. The significance of differences between variables was tested using the $\chi 2$ test with a significance level of $p<0.05$.

4. RESULTS

A retrospective analysis of data in the period from the beginning of 2018 to the end of 2022 was performed. We analyzed total the results of 378 procedures for the identification of antierythrocyte antibodies, including 278 women and 100 men. As a result of 248 procedures, the presence of one type of immune antibody or a combination of two or more antibodies was determined. (65 %). The remaining number of procedures (35%) did not result in the detection of irregular antibodies, so it could not be determined whether it was the presence of immune antibodies in the serum, some non-specific antibodies or the positive result was the consequence of the interference of some therapy on the result of the findings.

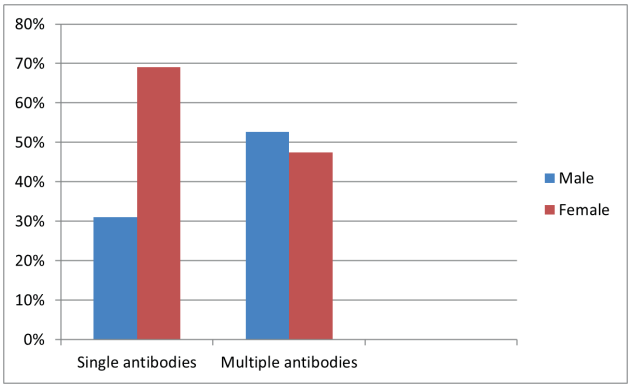

Figure 1. Gender distribution of antierythrocyte antibodies

Type of antibody	2018	2019	2020	2021	2022	TOTAL (n)	(%)
anti-D	19	12	8	16	13	68	32,40%
anti-E	12	6	13	8	3	42	20,00%
anti-C	1	0	3	2	2	8	3,81%
anti-c	3	0	4	6	2	15	7,10%
anti-e	2	0	1	2	0	5	2,40%
anti-Cw	1	1	0	0	0	2	0,95%
anti-K	11	5	3	4	4	27	12,90%
anti-k	0	1	0	0	1	2	0,95%
anti-M	2	3	3	4	0	12	10,00%
anti-N	1	0	0	0	0	1	0,47%
anti-S	1	2	0	0	0	3	1,43%
anti-Fy a	1	0	5	4	1	11	5,24%
anti-Fy b	1	0	0	1	0	2	0,95%
anti-Jka	0	0	1	1	0	2	0,95%
anti-Kpb	0	0	1	0	0	1	0,47%
anti-Lea	4	1	0	1	0	6	2,86%
auto anti-N	1	0	0	0	0	1	0,47%
auto anti-e	0	1	0	1	0	2	0,95%
TOTAL	60	32	42	50	26	210	100,00%

Table 1. Specificity of antierythrocyte antibodies proven by the identification procedure in microgel technology

According to the gender structure of the sample, out of the total number of patients in whom the presence of antierythrocyte antibodies was detected, women predominated. In 145 women, the presence of one antibody was determined (69%), and combined antibodies were detected in 18 women (47,4%) (Figure1).

The large representation of women in the sample is also explained by sensitizations during previous pregnancies, where the largest number of detected antibodies belonged to the Rh-system of blood groups, primarily anti-RhD antibodies, combined anti-RhD and anti-C antibodies, and anti-K-antibodies. Comparing the group of men and women with detected one

Clinical significance	N (%)	Female (%)	Male (%)	p-value
Clinically significant	172 (81,90%)	103 (59,89%)	69 (40,11%)	0,01
Potentially clinically significant	32 (15,24%)	20 (62,5%)	12 (37,5%)	0,16
Clinically insignificant	6 (2,86%)	4 (66,67%)	2 (33,33%)	0,41

Table 2. Clinical significance of detected antierythrocyte antibodies

Combined antierythrocyte antibodies	Male	Female	Total
Anti-D, anti-C	4	9	13
anti-E, anti-c	3	5	8
Anti-D, anti-E	1	1	2
anti-D, anti-C, anti-E	1	1	2
anti-E, anti-Cw	0	1	1
anti-D, anti-C, anti-K	0	1	1
anti-E, anti-M	1	0	1
anti-E, anti-Lea	0	1	1
anti-E, anti-c, anti-K	0	1	1
anti-E, anti-K, anti-Jkb	0	2	2
anti-E, anti-Fya	1	0	1
anti-E, anti-Jka	1	0	1
anti-c, anti-K	1	0	1
anti-Fya, anti-S	0	1	1
anti-Jkb, anti-Lea	0	1	1
anti-Jka, anti-K	1	0	1
TOTAL	14	24	38

Table 3. Combined antierythrocyte antibodies

single antibody, a statistically significant difference in favor of women was evident (p<0,0001). Multiple antibodies were detected almost equally in both gender groups.

From the analyzed data, it is evident that 140 of all identified antibodies belong to the antigen system of high frequency in the population, the Rh system (66.7%), of which 32.4% are anti RhD-antibodies, 20% anti RhE-antibodies, antiRh c- antibodies as the most immunogenic antierythrocyte antibodies 7.1%, and in a smaller number anti-C, anti-e and anti-Cw antibodies. Antibodies from the Kell blood group system were detected second in frequency and immunogenicity, namely 27 patients with anti K-antibodies (12.9%) and 2 patients with anti K antibodies (0.9%).

The next most frequent are antierythrocyte antibodies from the MNS system, anti M-antibody in the serum of 12 patients (5.7%). A high frequency was shown by the anti-Fya antibody, which was proven individually and in combination with other anti-erythrocyte antibodies, mostly in polytransfused patients. Other immune antibodies from the remaining blood group systems were detected in individual cases and belong to the group of potentially clinically significant and clinically insignificant antierythrocyte antibodies (anti Jka, Kpa, Fya, Fyb, MNS) (Table 1).

A statistically significant difference was demonstrated in the number of patients with detected clinically significant antibodies in favor of women (p<0.05), while the number of clinically potentially significant and insignificant antierythrocyte antibodies did not show a marked discrepancy depending on gender (Table 2).

Autoimmune antibodies were created as a result of the formation of antibodies against antigens on one's own erythrocytes. They very often occur as part of autoimmune hemolytic reactions, chronic lymphatic leukemia, but they can also occur isolated without a known cause. During the analyzed period, a total of 3 autoimmune antierythrocyte antibodies were detected, two with anti-e specificity and one with anti-N specificity. Both patients with autoimmune anti-e antibody had diagnostically verified autoimmune diseases, while the origin of auto-anti-N antibodies could not be established.

Out of the total number of proven antierythrocyte antibodies, 38 antibodies were a combination of two or more irregular antibodies (15.4%). Combinations of anti-D and anti-C antibodies showed the highest frequency (34.2%), followed by anti-E and anti-c antibodies (21%). Overall, antibodies against antigens from the Rh-system are present in the largest number of combinations, with anti E-antibodies dominating (89.5%). Antierythrocyte antibodies anti-Jka and anti-Jkb from the Kidd blood group system are also antibodies that can rarely be isolated alone, and more often come in combination with antibodies from other blood group systems (13.2%) (Table 3).

Out of a total of 248 analyzed blood samples, where antierythrocyte antibodies were proven, 74 patients were polytransfused patients, which means that they received more than one dose of erythrocyte concentrate during the last five years (30%). Out of a total of 38 recorded combined antierythrocyte antibodies, a total of 20 patients received a transfusion of two or more doses of erythrocyte concentrate, which led to sensitization (52.6%). Polytransfused patients were most likely to develop antierythrocyte antibodies (29,8%), followed by 27,4% of women with proved previous pregnancies. Other reasons for sensitization were mostly therapy that interfered and gave false positive results (14,5%). In 28,3% of cases there was no possibility to determine the reason of sensitization, since patients had no history data.

5. DISCUSSION

Pretransfusion testing is a very important segment of laboratory testing that enables the safe process of blood transfusion towards the final user (1). In addition to the existing manual methods of identification of irregular antibodies in the test tube, the test in microgel technology found a higher proportion of irregular antibodies than the test in the test tube (69,8% or 41,3%), and the identification procedure in microgel technology is today considered the "gold standard" in selection of adequate techniques for the detection of antierythrocyte antibodies in the serum of patients both in Bosnia and Herzegovina and in more developed countries of Europe and the world (4, 13).

The most common antibodies from this category are antibodies against high-specificity antigens from the blood group system Rh (anti-D, anti-c, anti-C and anti-E) and Kell (5, 6). Their clinical importance is reflected in the fact that in the optimal environment of the bloodstream in-vivo they react very quickly with the appropriate antigens on the recipient's erythrocytes and may lead to an acute hemolytic reaction. Of all the antigens included in the Rh-system, antigen D is the

most frequent antigen and extremely immunogenic. 20% of RhD-negative people create anti-RhD-antibodies after one transfused dose, and 60% of people after transfusion of two or more doses, while 20% of people never even create antibodies (3).

Antigens c and E are more immunogenic than antigens C and e, and anti-c and anti-E antibodies are often detected after transfusion of blood components containing these antigens. Anti-E antibody is the most commonly detected antibody in patients who have received a blood transfusion, and anti-c antibody is the second most common antibody reported as a cause of hemolytic disease of the newborn in the UK (7). Antigen K is ranked right after RhD antigen in terms of immunogenicity. Studies have shown that 1 out of 10 people who do not have the K antigen develop anti-K antibodies after Kell positive blood, which means that the probability is 10%. By comparison, the probability of sensitization by antigen D is 80% (4).

According to Erikstein et al., anti-E antibody was also identified as the most common antibody in Norway (20 %), followed by anti-M (18 %), anti-K (10 %), and anti-D (9 %) (10). In a study conducted by Politou et al. in three Greek hospitals on a sample of 53,800 patients who were transfused from 2012 to 2016, the antibody with the highest frequency was anti-K (26,61%), followed by anti-E (16,02%), anti-D (15,02%), anti-Jka (5,87%) and anti-M antibody (5,72%) (5).

The results of our research do not deviate much from the European and world average. The most frequent antierythrocyte antibody was anti-D antibody 68%, anti-E42%, anti-c 7,1%, anti-C 3,81%, anti-K 12,9% and anti-M antibody 10 %.

Outside the European continent the results are similar with small variations due to the frequency of erythrocyte antigens on erythrocytes. With regard to gender, according to Reyhaneh et al., in Iran, the most frequently detected antibody among the male population is anti-K (33%), while in women it is anti-E (27%) (12).

When single antierythrocyte antibody is identified, it is very important not to miss the identification of other clinically significant antibodies, given that the immunized patient is at increased risk of creating additional antierythrocyte antibodies of different specificities after repeated transfusions (9). The most common combinations of antibodies are from the Rh-system, where in most cases the anti-E antibody is present in combination with some other antibody. This is supported by a study published by Makarovska Bojadžijeva in 2017, where the presence of anti-E antibodies in combination with other antibodies was proven in more than 50% of detected combined antierythrocyte antibodies (15). Perreira Bueno et al. in 2021, studied the sample of 29,128 tested patients and found the presence of multiple antibodies in 25,32 cases, where the dominant was also anti-E antibody (14).

The results of our research showed the presence of anti-E antibodies in combination with some other antibody in 89,5% of cases. The second most frequent antibody in combination with other antibodies was anti-K antibody with the frequence of 15,8%. There are also autoantibodies of immune origin, which are formed by an immune reaction to erythrocyte antigens of one's own erythrocytes (anti-D, E, c, and anti M-autoantibodies) and are mainly detected in the serum of patients suffering from autoimmune hemolytic anemias and autoimmune connective tissue diseases. In our study, the most frequently present autoantibody was the anti-e autoantibody detected in two patients with previously diagnostically verified autoimmune diseases (CLL).

For this reason, pretransfusion testing represents a very important link in the safety of the use of blood and blood products, and the identification of antierythrocyte antibodies, as well as the use of phenotyped blood products significantly reduced the risk of posttransfusion reactions, and facilitated the implementation of the safe blood policy (4).

6. CONCLUSION

Pretransfusion testing is the key link in the chain of ensuring safe blood. Our study showed that every transfusion carries a certain risk of sensitization of the patient. Even a small dose of blood can sensitize the patient to antigens of high frequency in the population, and can cause problems when finding adequate doses of blood in the future. In this regard, phenotyping of erythrocyte concentrates for antigens of high frequency in the population (Rh and Kell system) and issuance to patients with matching antigens is recommended. In all other cases of sensitization to antigens of lower frequency, an adequate procedure of identification of antierythrocyte antibodies and administration of phenotyped blood to certain antigens is necessary.

- **Author's contribution:** SJL and all coauthors gave a substantial contribution to the conception and design of the work. SJL, SDC, AZ and ASM gave a substantial contribution to acquisition of data. SJL, SDC, AZ and ASM gave a substantial contribution to the analysis, or interpretation of data for the work. SJL, SDC, AZ and ASM had a part in article preparing for drafting or revising it critically for important intellectual content. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
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