DOI: 10.17816/KMJ2022-14

Features of the anti-erythrocyte antibodies screening results interpretation in patients with hematological diseases

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Abstract

Background. Screening for anti-erythrocyte alloantibodies is a mandatory pre-transfusion test. The correct interpretation of the screening results plays a key role in ensuring the immunological safety of hemotransfusion therapy.
Aim. To study the features of anti-erythrocyte antibodies detection in patients with hematological diseases.
Material and methods. The study was performed with blood samples of 1269 patients with hematological diseases (569 male and 700 female patients aged 18–85 with a median of 63 years). Screening and identification were carried out in indirect antiglobulin test using ID Coombs Anti-IgG gel cards with 4 and 15 samples of test erythrocytes as well as the salt agglutination method. The results were evaluated visually. The Pearson chi-squared test was used to check the statistical significance of differences in the alloimmune anti-erythrocyte antibodies frequency detection. The differences were considered statistically significant at $p \leq 0.05$.
Results. Interpretation of the anti-erythrocyte alloantibodies screening results was difficult in 6.55% of cases and was associated with the presence of autoantibodies in plasma (0.6%) and cross-reactive antibodies (5.9%). Anti-erythrocyte alloantibodies were detected in 2.05% of cases which required further identification of antibody specificity. Antibodies to Rh system antigens were detected in 68.2% of cases, to antigens of other erythrocyte systems — in 31.8% of cases. In Rh-negative patients only anti-D or anti-DC antibodies were detected. Rh-positive patients were more likely to have anti-K antibodies (30%). Anti-E antibodies were discovered in 20% of cases, anti-Cw and anti-Fya — in 10% each. Alloantibodies were detected most frequently in patients with β-thalassemia (20%), aplastic anemia (13%), hemophilia (9.2%) and thrombophilia (6.9%) and less frequently in patients with hemoblastosis and hematopoiesis depression (0.3–2.4%). Cross-reacting antibodies were detected more frequently in patients with multiple myeloma (74.7%; $p \leq 0.05$) than in patients with chronic lymphocytic leukemia (17.3%), myelodysplastic syndrome (5.3%), and acute leukemia (2.7%).
Conclusion. The reasons for the difficulties in interpreting the alloantibody screening results in hematological patients were the presence of autoantibodies (0.6%), alloantibodies (2.05%) and cross-reacting antibodies (5.9%).
Keywords: alloimmunization, red blood cell transfusion, Rh and Kell blood group systems, myelodysplastic syndrome, chronic myeloid leukemia, acute myeloid leukemia.

For citation: Krobinets II, Mineeva NV, Bodrova NN, Sisoeva EA, Gavrovskaya SV, Sidorkevich SV, Bessmeltsev SS. Features of the anti-erythrocyte antibodies screening results interpretation in patients with hematological diseases. *Kazan Medical Journal.* 2022;103(1):14–22. DOI: 10.17816/KMJ2022-14.

Background

In recent decades, the combat against hematological diseases has significantly improved, in association with the widespread use of new diagnostic and treatment methods for patients with diseases of the blood system. Moreover, transfusion therapy remains an important component of the complex treatment of patients with hematological disorders [1]. One of the complications of transfusion therapy is alloimmunization to blood group antigens of erythrocytes, human platelets (HPA), and human neutrophils and leukocytes (HNA and HLA, respectively) [2–4].

Screening of alloantibodies to erythrocyte antigens in the Russian Federation is obligatory. The correct interpretation of screening results is significant in ensuring the immunological safety of blood transfusion therapy. However, the peculiarities of the pathogenesis and treatment of hematological diseases lead to certain problems in the interpretation of the results of screening of anti-erythrocyte alloimmune antibodies.

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Received 22.11.2021; accepted 15.12.2021; published 15.02.2022.

Aim

This study aimed to examine the aspects of detection of anti-erythrocyte alloimmune antibodies in patients with hematological diseases.

Materials and methods of research

Screening results of anti-erythrocyte antibodies were analyzed and interpreted in blood samples of 1269 patients with hematological diseases treated at the Russian Research Institute of Hematology and Transfusiology of the Federal Medical and Biological Agency of Russia. The patients included 569 men and 700 women. The distribution of patients by disease is presented in Table 1.

Antibodies to erythrocyte antigens were determined through indirect antiglobulin test (Coombs's test) using manual methods and an automatic immunohematological analyzer IH-1000 (BIO-RAD, USA) according to the manufacturer's instructions. Alloantibodies were determined in two stages.

– Stage 1 included screening of anti-erythrocyte antibodies in the blood of patients and was performed using BIO-RAD ID Coombs Anti-IgG system cards with six microtubes that contained gel with polyspecific antiglobulin serum or monospecific serum containing anti-IgG antibodies, and three samples of standard erythrocytes ID-DiaCell I–II–III of the ccDEEK-(I), CCWDeeK-(II), and ccdeeK+(III) phenotype.

– Stage 2 included identification of antibodies and was performed by gel agglutination with a panel of test erythrocytes, consisting of 15 erythrocyte samples phenotyped for 36 antigens (Grifols, Spain).

Antibodies (immunoglobulins (Ig) of the IgM class) were detected through salt agglutination in the gel using BIO-RAD NaCl, enzyme test, and cold agglutinin system cards. The study results were recorded visually.

Statistical analysis was performed using Statistica 7.0 software (StatSoft, USA). To test the statistical hypothesis about the presence of significant differences in the frequency of alloimmune anti-erythrocyte antibodies, Pearson's χ^2 test was used. The differences were considered significant at a significance level of $p < 0.05$.

Results and discussion

Positive screening results for anti-erythrocyte alloantibodies were obtained in 109 patients, which accounted for 8.6%. After additional studies and repeated screening, a positive screening result was found to be associated with the presence of anti-erythrocyte alloantibodies in 26 patients, plasma autoantibodies in 8, and cross-reacting antibodies that are not clinically significant for transfusions in 75.

Table 1. Distribution of patients by nosology

Diagnosis	Number of patients
Multiple myeloma	291 (36*)
Chronic lymphocytic leukemia	216
Lymphoma	127
Polycythaemia vera	90
Chronic myeloid leukemia	180
Primary myelofibrosis	42
Myelodysplastic syndrome	77
Acute lymphoblastic leukemia	21
Acute myeloid leukemia	59
Hemophilia	87
Thrombophilia	29
β-Thalassanemia	5
Aplastic anemia	23
Other hematological diseases	13
Total	1269

Note: *patients were treated with daratumumab.

Table 2. Specificity of M immunoglobulins (IgM) antibodies

Number of patients who were alloimmunized	Number of patients with different specificities of IgM alloantibodies		
	Anti-c	Anti-E	Anti-E+K+Kpa
5	2	2	1

Specific anti-erythrocyte alloantibodies were detected in 26 patients, of which 14 patients (53.8% of the patients who were immunized) were Rh-positive and 12 (46.15%) were Rh-negative. Among the patients who were alloimmunized, 10 were men and 16 were women (0.8 and 1.3% of the total number of patients, respectively). Antibodies to antigens of the Rh system were detected in 68.2% of the cases, and those to antigens of other erythrocyte systems were revealed in 31.8%. The identified anti-erythrocyte antibodies were represented by IgM and IgG (0.4 and 1.65% of the total number of patients, respectively). The specificity of the identified IgM antibodies is presented in Table 2.

Antibodies with specificity to anti-c and anti-E were detected with equal frequency at 7.7% each, whereas anti-E+K+Kpa was identified in 3.8% of the patients who were alloimmunized. These antibodies were not detected in each hospitalization, which indicates the non-immune nature of the antibodies. The reasons for the appearance of such antibodies can be contact with group-specific substances of plant, animal, and bacterial origins, in which the structure of the cell wall is similar to these erythrocyte antigens, and mutations in genes

Table 3. Specificity of anti-erythrocyte antibodies detected in IgG

Number of patients who were alloimmunized	Number of patients with different specificities of IgG alloantibodies					
	Anti-D	Anti-DC	Anti-E	Anti-Cw	Anti-K	Anti-Fya
22	7 (31.9%)	5 (22.7%)	2 (9.1%)	1 (4.5%)	6 (27.3%)	1 (4.5%)

Note: The percentage represents the proportion of cases of the total number of patients who were alloimmunized.

Table 4. Detection rate of anti-erythrocyte antibodies depending on gender and Rhesus factor (Rh).

Specificity of antibodies	Number of patients with detected antibodies				
	Total	Rh^-		Rh^+	
		Women	Men	Women	Men
Anti-D	7	3 (2**; 1*)	4*	—	—
Anti-DC	5	4 (3**;1*)	1*	—	—
Anti-E	2	—	—	2**	—
Anti-C^w	1	—	—	1*	—
Anti-K	6	—	—	3**	3*
Anti-Fya	1	—	—	1**	—
Total	22	7 (31.9%)	5 (22.7%)	7 (31.9%)	3 (13.6%)

Note: *Patients with a history of transfusions. **Patients with a history of pregnancies and transfusions.

that control the synthesis of immunoglobulins [5]. In one patient, IgG antibodies were revealed, in addition to IgM antibodies. The specificity of the identified IgG antibodies is presented in Table 3.

Antibodies to the Rh system antigens and antigens of other erythrocyte systems were detected in 68.2% and 31.8% of the cases, respectively. Anti-D (31.9%) and anti-K (27.3%) antibodies were predominant in IgG antibodies. Antibodies were detected in both women and men. The detection rate of antibodies depending on gender and Rh factor is presented in Table 4.

Alloimmunized women had a history of both transfusions and pregnancies, whereas men only had transfusions (probably K^+ donor erythrocyte components before 1998, when transfusions were performed without taking into account the K antigen). The identified specific anti-erythrocyte IgG alloantibodies were developed only after the stimulation of the immune response by pregnancy and/or transfusion of blood components incompatible with erythrocyte antigens. IgG antibodies that emerge without stimulation of the immune response were not detected in this study.

In Rh-negative group, only anti-D or anti-DC antibodies were detected, which confirms the high immunogenicity of these antigens. Our research results are consistent with the findings of Russian researchers [2, 6], but differ from data of international researchers [7]. Such differences are caused by mandatory Rh prophylaxis with anti-D immunoglobulin in women who were Rh-negative during pregnancy in developed countries [8]. In our study, the presence of anti-D antibodies in women attributed to the development of alloimmunization during pregnancy. In men, the presence of anti-D antibodies was probably associated with either the transfusion of erythrocyte blood components from a donor with the D antigen or transfusion of fresh-frozen plasma without taking into account Rh factor. An error in the D antigen typing could be the result of the non-observance of the method for determining the Rh factor of the recipient's or donor's blood and low quality of diagnostic reagents [9].

Anti-K antibodies were detected more often in patients who were Rh-positive, including both men and women. They accounted for 30% of the number of patients who were Rh-positive and alloimmunized. Anti-E antibodies accounted for 20%, and anti-Cw and anti-Fya accounted for 10% each. Antibodies to Duffy system antigens are rare, and in our study, they were identified in 4.5% of the patients who were immunized. Transfusions incompatible for this antigen always lead to the development of post-transfusion complications. The severity of these complications depends on the levels of antibodies in the recipient, qualitative and quantitative compositions of the antigen (homo- or heterozygote) in donor erythrocytes, and number of transfusions [10]. We did not detect antibodies to the antigens of the erythrocyte systems MNS, Kidd, Lewis, and Lutheran.

Our data on the specificity of antibodies (except for anti-D antibodies) are consistent with the data of international authors. Thus, Stiegler et al. and

Leisch et al. showed that anti-K and anti-E alloantibodies are the most common specificities [11, 12].

All patients received hemocomponent therapy with consideration to the alloantibodies identified. Post-transfusion reactions and complications were not registered.

The probability of developing antibodies depends on a number of factors, which are mainly genetic and epigenetic factors, number of transfusions, and disease characteristics. The detection rate of alloantibodies depending on the disease is presented in Table 5.

The data presented show that the highest frequency of detection of antibodies was recorded in patients with β-thalassemia, aplastic anemia, hemophilia, and thrombophilia (6.9%–20%). In patients with hemoblastosis, antibodies were identified with a frequency of 0.3%–2.4%. Thus, antibodies were detected significantly more often in patients with β-thalassemia than in patients with acute myeloid leukemia (p = 0.024), chronic lymphocytic leukemia (CLL; p = 0.0001), lymphoma (p = 0.023), myelodysplastic syndrome (MDS; p = 0.009), and multiple myeloma (MM; p = 0.0001). Antibodies were detected more often in patients with aplastic anemia than in patients with acute myeloid leukemia (p = 0.033), CLL (p = 0.0001), lymphoma (p = 0.017), MDS (p = 0.012), and MM (p = 0.0001). Antibodies were detected more often in patients with thrombophilia than in patients with CLL (p = 0.018) and MM (p = 0.0001). Antibodies were identified more often in patients with thrombophilia than in patients with CLL (p = 0.0001), lymphoma (p = 0.027), MDS (p = 0.027), and MM (p = 0.0001).

In four patients, the production of alloantibodies was probably associated with a pre-existing pregnancy incompatible with erythrocyte antigens and lack of full-fledged prophylaxis with anti-D immunoglobulin. Patients who were alloimmunized and had aplastic anemia and hemophilia were over 40 years old, while no alloantibodies were detected in younger patients. In our opinion, the main reason for such a significant difference in alloimmunization is (among other things) a change in the approach of treating hemophilia and aplastic anemia. Thus, replacement therapy with factor VIII drugs in hemophilia and immunosuppressive therapy in aplastic anemia lead to a decrease in transfusion dependence in these patients.

The low incidence of alloimmunization in patients with hemoblastosis, who received multiple transfusions, can be explained by the nature of the disease itself and its therapy. Patients with hemoblastosis and hematopoietic depression receive long-term immunosuppressive and high-dose chemotherapy, which blocks the normal immune response [11].

Table 5. Detection rate of antibodies depending on the disease

Disease	Number of patients with alloantibodies	
	n	% of the number of patients with the disease
Acute myeloid leukemia (n = 59)	1*	1.7
Chronic lymphocytic leukemia (n = 216)	2**	0.9
Lymphoma (n = 127)	3(2*; 1**)	2.4
Myelodysplastic syndrome (n = 77)	1*; ***	1.3
Multiple myeloma (n = 291)	1**	0.3
Aplastic anemia (n = 23)	3(2**; 1***)	13
β-Thalassemia (n = 5)	1***	20
Thrombophilia (n = 29)	2**	6.9
Hemophilia (n = 87)	8***	9.2

Note: *Patients with a compromised obstetric history (children with hemolytic neonatal disease). **Ppatients with a history of pregnancies and transfusions. ***Patients with a history of transfusions.

Our data differ from those of Russian researchers. Butina et al. revealed the highest level of alloimmunization in patients with MDS, chronic myeloid leukemia, and acute leukemia, and the lowest level was found in patients with aplastic anemia [6]. Probably, the high incidence of alloimmunization in these patients is associated with individual characteristics. Immunization could occur at a young age as a result of transfusion of components without taking into account Rh and Kell affiliation or pregnancy. It is also possible that the blood products contained these antigens at a concentration sufficient for alloimmunization.

Another problem in interpreting alloantibody screening results is the presence of nonspecific cross-reacting antibodies. Such antibodies were found in the blood serum of patients with CLL, MM, MDS, and acute leukemia (Table 6).

Cross-reacting antibodies were significantly more common in patients with MM than in patients with CLL (p = 0.0001), MDS (p = 0.004), and acute leukemia (p = 0.003). These results can be explained by the disease pathogenesis (synthesis of pathological paraproteins) and an increase in the adhesive properties of cells, disease stage, and type of therapy [13, 14]. With the onset of remission in such patients, the production of cross-reacting antibodies decreases [6].

To clarify the class of antibodies detected, the patient's serum was incubated with a 5% unitiol solution for 24 h. Serum treatment with a 5%

Table 6. Detection of cross-reacting antibodies in various diseases

Diseases	Number of patients with cross-reacting antibodies		
	n	% of patients with disease	% of patients with cross-reacting antibodies
Multiple myeloma	56	19.2*	74.7*
Chronic lymphocytic leukemia	13	6	17.3
Myelodysplastic syndrome	4	5.2	5.3
Acute leukemia	2	2.5	2.7
Total	75		

Note: *$p < 0.5$.

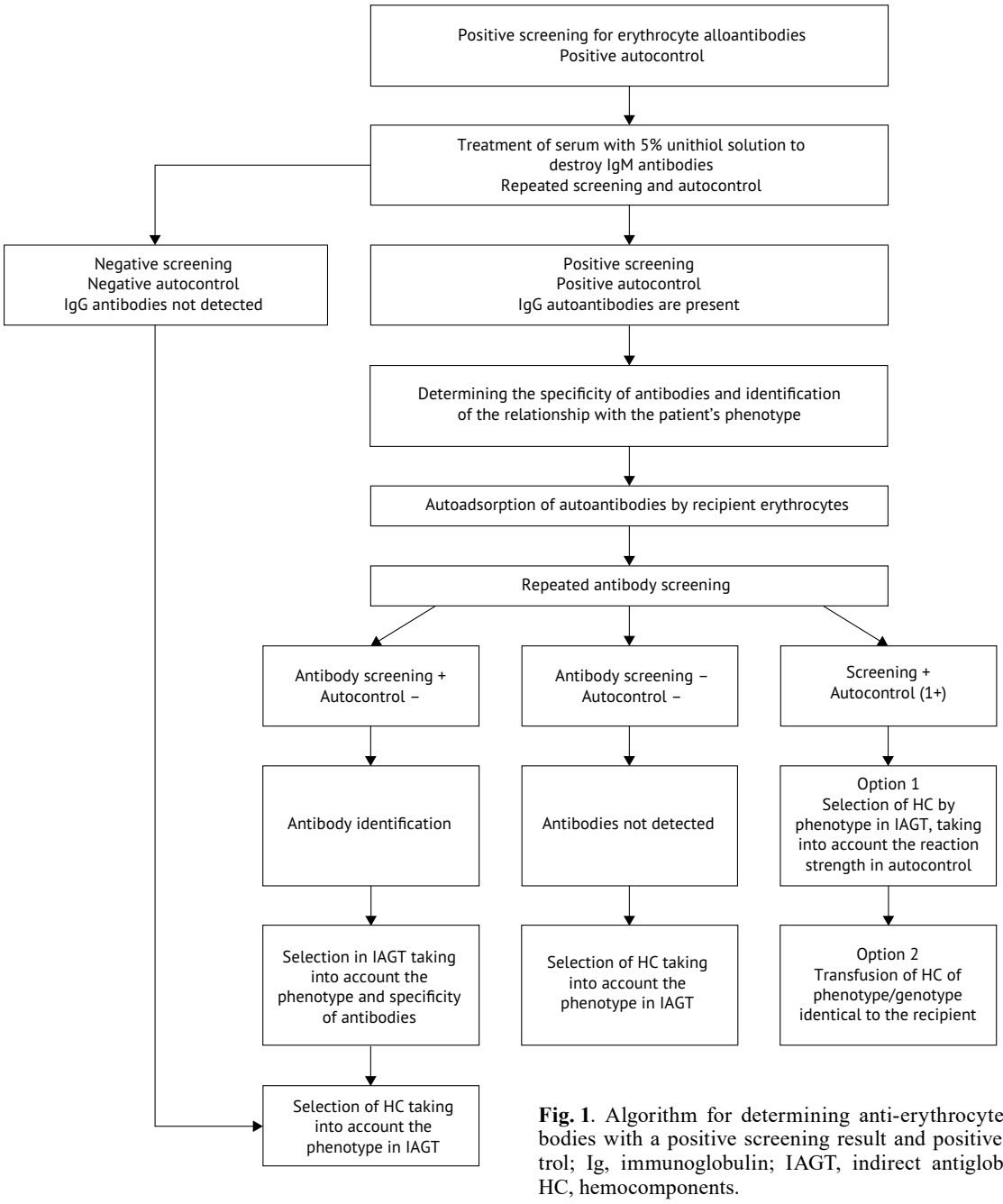

Fig. 1. Algorithm for determining anti-erythrocyte alloantibodies with a positive screening result and positive autocontrol; Ig, immunoglobulin; IAGT, indirect antiglobulin test; HC, hemocomponents.

unithiol solution disrupts both the agglutinating and complement-binding activities of IgM molecules because of the destruction of disulfide bonds, which enables to identify parallel IgG antibodies.

Cross-reacting antibodies detected in patients with CLL, MDS, and acute leukemia were represented by IgM immunoglobulins in 100% of cases. In patients with MM, IgM antibodies were detected in 17.86% of cases and IgG antibodies in 82.14% of cases. Of the 46 MM patients with cross-reacting antibodies, 36 were treated with anti-CD38 drug antibodies (daratumumab). The IgM antibodies identified had no clinical significance. However, nonspecific cross-reacting antibodies complicate the selection of compatible hemocomponents. Thus, the required doses of hemocomponents can be selected for only 75% of patients with hematological diseases requiring transfusions [8].

Based on the work performed, the developed research algorithm enables in difficult cases to differentiate cross-reacting antibodies that have no clinical significance and anti-erythrocyte autologous antibodies and/or alloantibodies that have clinical significance and to identify the specificity of alloantibodies in patients with autoantibodies (Fig. 1).

The use of the proposed algorithm enabled selection of donor erythrocytes compatible for recipients in 100% of cases.

Conclusions

1. Interpretation of screening results was difficult in 6.55% of cases due to the presence of autoantibodies (0.6%) and cross-reacting antibodies (5.9%). Anti-erythrocyte alloantibodies were found in 2.05% of cases.

2. Anti-erythrocyte alloantibodies were detected more often in patients with β-thalassemia, aplastic anemia, hemophilia, and thrombophilia (6.9%–20%). In patients with hemoblastosis and hematopoietic depression, alloantibodies were rarely identified (0.3%–2.4%).

3. In the range of the antibodies detected, anti-D and anti-K antibodies were predominant.

4. Nonspecific cross-reacting antibodies were significantly more common in patients with MM (74.7%) than in patients with CLL (17.3%), MDS (5.3%), and acute leukemia (2.7%).

5. Cross-reacting antibodies detected in patients with CLL, MDS, and acute leukemia in 100% of cases were represented by IgM. In patients with MM, IgM antibodies were detected in 17.86% of cases and IgG antibodies in 82.14% of cases.

Author contributions. N.W.M. was the project supervisor, created the concept of work, and wrote the text. I.I.K. and E.A.S. conducted the research, collected, analyzed, and interpreted the results and wrote the text. N.N.B. and S.V.G. conducted the research. S.V.S. performed consultation on clinical issues and gave final approval of the manuscript. S.S.B. collected, analyzed, and interpreted the results, wrote the text, performed consultation on clinical issues, and gave final approval of the manuscript.

Funding. The study had no external funding.

Conflict of interest. The authors declare no conflict of interest.

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