Antibody screening and identification in donors and general patients at a tertiary care teaching hospital in Western India

Kamini Parshuram Gupta, Maitrey D. Gajjar, Tarak Ramesh Patel, Nidhi Manish Bhatnagar, Nihar Chaudhari, Mamta Chintan Shah

Abstract:

BACKGROUND AND OBJECTIVES: The aim of the blood transfusion service should be to provide effective blood and blood components, which are as safe as possible and adequate to meet patient's need. To achieve safe blood transfusion practice, many blood transfusion center in India follow routine type and screen protocol for all patient's and donor's blood samples to detect unexpected alloantibodies. The present study is aimed at assessing the frequency and type of unexpected red cell alloantibodies in general patient population and donors at a tertiary care teaching hospital in western India.

MATERIALS AND METHODS: In this prospective study, samples of patients as well as blood donors were processed for ABO and Rh "D" grouping as well as antibody screening with three cell screening panel on fully automated immunohematology analyzer. Positive sample in three cell screening panel was further evaluated for identification of specific alloantibody with eleven cell identification panel by column agglutination technique. Results were recorded, and data were analyzed to calculate the frequency of unexpected alloantibody.

RESULTS: A total of 74,214 patient samples and 80,173 donor samples were processed for type and screen. Out of which, 512 patients and 11 donors were identified with alloantibody. Most common alloantibody found in the present study is anti-D (0.075%), followed by anti-E (0.041%), anti-c (0.021%), anti-K (0.0205%) in Rh and Kell blood group system.

CONCLUSION: Antibody screening and identification of specific alloantibody help in identifying most appropriate blood unit that lacks the corresponding antigen and prevent alloimmunization.

Keywords: Alloimmunization, antibody identification, antibody screening

Introduction

Allogenic red cell transfusion is safer now than it has ever been. Increasingly, restrictive policies covering who may donate blood, increased and more sensitive testing of donated blood for evidence of transmissible infectious agents, and increased vigilance surrounding the process of transfusion have contributed to this improved safety. However each and every blood transfusion have potential risk of transmitting transfusion transmitted infections and adverse transfusion reactions resulting from alloimmunization against red cell antigen or other cellular blood components. Therefore, protection of recipient from adverse transfusion reactions due to alloimmunization against red cell antigen is one of the challenge against safe blood transfusion.

How to cite this article: Gupta KP, Gajjar MD, Patel TR, Bhatnagar NM, Chaudhari N, Shah MC. Antibody screening and identification in donors and general patients at a tertiary care teaching hospital in Western India. Asian J Transfus Sci 2019;13:34-8.

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com
Immunization to red cell antigens may result from pregnancy, transfusion, transplantation, or injections of immunogenic material. In some instances, no specific immunizing event can be identified. Antibodies detected in serologic tests may be passively acquired. These antibodies may be acquired from injected immunoglobulin (Ig), donor plasma, passenger lymphocytes in transplanted organs, or hematopoietic progenitor cells.[1,2]

The degree of clinical significance varies among antibody of same specificity; some cause destruction of incompatible red cells within hours or even minutes whereas others cause a decrease in the red cell survival by only a few days and still others cause no discernible shortened red cell survival.

Some antibodies are known to cause hemolytic disease of fetus and newborn (HDFN) whereas others may cause a positive direct antiglobulin test (DAT) in the fetus without clinical evidence of HDFN. Clinically significant antibodies are usually IgG antibodies that react at 37°C or in the antihuman globulin phase of the DAT.[1,2]

This study is aimed at assessing the frequency and type of unexpected red cell antibodies in the general patient population and donors at a tertiary care teaching hospital in Western India. This would help in selection of appropriate unit for transfusion, investigating for causes of hemolytic anemia, investigating for causes of HDFN, preventing any transfusion reaction, and preventing any serological changes during pregnancy by providing anti-D to patients.

### Materials and Methods

The study was carried out at the red cell serology laboratory of our Department of Immunohematology and Blood Transfusion, over a period of 2 years from January 2015 to December 2016. Blood samples collected in ethylenediaminetetraacetic acid (EDTA) and plain vacutainer from all patients and EDTA and plain vacutainer (Pilot tubes) from all donors were processed for ABO and Rh (D) blood grouping as well as antibody screening with the three cell panel on fully automated immunohematology analyzer “Qwalys-3” (Manufacturer: Diagast, France) working on the principle on erythrocyte magnetization technology. Samples giving positive antibody screen on three cell screening panel were subjected to antibody identification by extended eleven cell panel by column agglutination technology (Dia Med ID microtyping system-Bio-rad, USA). Any sample positive for antibody screening with the three cell panel on Qwalys-3 was subjected for repeat antibody screening by column agglutination technology (Gél card method) in commercially available three cell panel (Bio-Rad).

Data were examined to calculate the frequency of unexpected alloantibodies.

### Results

During the study period from January 2015 to December 2016, a total of 154,387 samples were type and screened for the presence of unexpected antibodies which was included, 74,214 patient samples and 80,173 donor samples. A total of 87,707 male patients and 86,507 female patients were screened; out of 74,214 patient samples, 512 patients (0.20%) were positive for antibody screening and the antibody was also identified. Out of 80,173 donors samples 11 donors (0.004%) were positive for antibody screening and the antibody was also identified. Alloimmunization rate in male patient was 0.19% and in female patient was 0.39% [Tables 1-3].

Two hundred and fifty patient samples with suspected autoantibody or autoantibody with underlying alloantibody were excluded from the present study as we were unable to do further immunohematological workup to differentiate between autoantibody and autoantibody with underlying alloantibody.

Most common alloantibody found in the present study is anti-D (0.075%), followed by anti-E (0.041%), anti-c (0.021%), anti-K (0.0205%) in Rh and Kell blood group system. In MNS group, the most common antibody found is anti-M followed by anti-S followed by anti-N> and anti-s. The other significant alloantibodies

| Parameters                                      | n     | Patient samples | Donor samples |
|-------------------------------------------------|-------|-----------------|---------------|
| Total number of blood samples underwent type and screen | 154,387 |
| Patient samples                                 | 74,214 |
| Patient samples having antibody screening positive and identified | 512 |
| Donor samples                                   | 80,173 |
| Donor samples having antibody screening positive and identified | 11 |

### Table 2: Gender-wise distribution of unexpected antibodies

| Parameter                      | Gender | Number |
|--------------------------------|--------|--------|
| Antibody identified in patients| Male    | 170    |
|                                | Female  | 342    |
|                                | Total   | 512    |
| Antibody identified in donors  | Male    | 11     |
|                                | Female  | 00     |
|                                | Total   | 11     |
such as Anti-Jk<sup>a</sup>, Anti-Jk<sup>b</sup>, Anti-Fy<sup>a</sup>, Anti-Fy<sup>b</sup>, Anti-Lu<sup>a</sup>, and Anti-Lu<sup>b</sup> were found in lower frequency [Table 4 and Figure 1].

In 29 patient samples, the alloantibodies could not be accurately characterized. These samples contain either multiple alloantibodies or antibody against high-frequency antigen.

As our aim of the study is to identify unexpected antibody in donors and patients, we have included anti-H antibody in the study. However, it was found as naturally occurring antibody in Bombay blood group patients.

**Discussion**

In the present study, a total of 174,214 patient samples and 80,173 donor samples were processed for type and screen as per our routine protocol. Antibody screening was positive in 512 patient’s (0.20%) and 11 donor’s samples (0.004%). The present study has excluded 250 patient’s samples demonstrating autoantibody or autoantibody with underlying alloantibody. The overall alloimmunization rate was 3%. The most frequent unexpected alloantibodies identified were against the Rh antigen system (69.21%), followed by Kell antigen system (9.94%). Anti-D was the most frequent alloantibody (36.32%) identified in the present study due to routine type and screen protocol of all antenatal patients as per our institution policy and may be due to lack of universal Rh immunoprophylaxis of all Rh “D-” negative mothers.[3]

Fewer studies have been done worldwide on alloimmunization in general patient population. Makroo et al. studied a total of 49,077 patient samples for the presence of unexpected antibodies. Antibody

| Blood group system | Antibody | n (%) | Frequency (%) |
|--------------------|----------|-------|---------------|
| Rh                 | Anti-D   | 190 (36.32) | 0.075         |
|                    | Anti-C   | 4 (0.76)  | 0.0015        |
|                    | Anti-c   | 54 (10.32) | 0.021         |
|                    | Anti-E   | 106 (20.26) | 0.041        |
|                    | Anti-e   | 6 (1.14)  | 0.002         |
|                    | Anti-C<sup>W</sup> | 2 (0.38) | 0.0007       |
| Kell               | Anti-K   | 52 (9.94) | 0.020         |
|                    | Anti-k   | 0       | 0             |
| Duffy              | Anti-Fy<sup>a</sup> | 1 (0.19) | 0.0004       |
|                    | Anti-Fy<sup>b</sup> | 3 (0.57) | 0.0011       |
| Kidd               | Anti-Jk<sup>a</sup> | 4 (0.76) | 0.0015       |
|                    | Anti-Jk<sup>b</sup> | 1 (0.19) | 0.0004       |
| Lewis              | Anti-Le<sup>a</sup> | 2 (0.38) | 0.0007       |
|                    | Anti-Le<sup>b</sup> | 2 (0.38) | 0.0007       |
| MNS                | Anti-M   | 25 (4.78) | 0.0098        |
|                    | Anti-N   | 8 (1.52)  | 0.0031        |
|                    | Anti-S   | 18 (3.44) | 0.0070        |
|                    | Anti-s   | 7 (1.33)  | 0.0027        |
| Bombay blood group | Anti-H   | 9 (1.72)  | 0.0035        |
| Multiple antibodies/antibody against high frequency antigen | 29 (5.54) | 0.0114 |
screening was positive in 403 patient samples (0.82%). Out of 403 patient samples, 212 patient samples had only alloantibodies suggesting overall alloimmunization rate of 0.49%. Antibody against the Rh system was the most frequent (64.1%), the most common alloantibody identified being anti-E (37.2%) followed by anti-D (19.2%) [Table 5]. A study done by Chaudhary and Agarwal in total 2026 patient samples for the presence of unexpected antibodies. screening was positive in 26 patient samples. Anti-E was the most frequent alloantibody identified. Transfusion-dependent thalassemia patients and patient having anemia requiring transfusion were also shown to have higher alloimmunization rate in the study. Dhawan et al. reported 5.64% alloimmunization rate in 319 transfusion-dependent thalassemia patients.

The development of alloantibodies can significantly complicate transfusion therapy and results in difficulties in selection of compatible blood unit. After the introduction of DAT by Coombs in 1945, which added a new dimension to the safety of blood transfusion, there was a rapid increase in the identification of alloantibodies that caused transfusion reactions or hemolytic disease of the newborn. Alloimmunization occurs when an incompatible antigen introduced in an immunocompetent host evokes an immune response. The way the immune system reacts depends on several factors such as type of antigen, dose of antigen exposure, and frequency of antigen in given population. The complications of alloimmunization that may occur are many; some antibodies may become nondetectable over time endangering future transfusions and placing the patient at risk for anamnestic antibody production, which may lead to delayed hemolytic transfusion reactions. They may even present with a delayed transfusion reaction that may go unrecognized and/or be masked by features of their underlying disease. The frequency of alloantibodies is expected to be relatively low in blood donors compared with patients in hospital requiring blood transfusion; blood donors have a lower average age and are far less likely to have received blood transfusions in the past. The frequency with which particular antibodies are found depends on the sensitivity of testing (usually relatively low in screening healthy donors) and on the ethnic group being tested. The risk of alloimmunization is higher in patients who have received multiple blood transfusions such as patients of thalassemia and other hemoglobinopathies, hematological disorders, renal failure on dialysis and females with bad obstetric history [Table 6].

Limited data are available on frequency of unexpected alloantibodies in general patients and blood donors. The rate of alloimmunization in our general patient population was 0.19% and in blood donor was 0.013%. Different studies in general patients have estimated the presence of unexpected alloantibodies against red cell antigens between 0.46% and 2.4%, while studies in multitransfused patients has reported higher frequency for unexpected alloantibodies.

There are only a few studies for incidence of irregular red cell alloantibody in healthy donors in India. One study done on alloimmunization in donor population, In Makroo et al. total of 3073 donors were typed during the study period. The most common Rh antigen observed in the study population was e (98%) followed by D (93.6%), C (87%), c (58%) and E (20%). A study by Pahuja et al. shows incidence of 0.05% in their donor population. In another study, Garg et al. found 0.09% prevalence of red cell alloantibody. Although there is a low prevalence in normal healthy donor population, the significance of irregular erythrocyte antibodies can be appreciated only when large amount of plasma or whole blood is transfused as in massive transfusions and in pediatric patients. In another study on antenatal women performed by Varghese J et al. alloimmunization was found in 1.48%. Anti-D was the most frequent antibody found.

From the results of Table 5, we can conclude that the frequency of alloimmunization was higher in antenatal patients, multitransfused patients, for example, thalassemia, and in patients suffering from severe anemia [Table 5].
Conclusion

Red blood cell (RBC) alloimmunization is an immune response against foreign RBC antigens; this generally occurs after sensitization due to blood transfusion and pregnancies. Universal prophylaxis of all Rh “D-” negative mothers during first pregnancy can prevent alloimmunization of Rh “D” mother and subsequent HDFN.

Similarly, Rh, Kell phenotyping of thalassemia patients and multiple transfused patients can help in identifying most suitable blood unit for transfusion and prevent alloimmunization.

Limitation

Every sample was first run in Qwalys-3 with screenlys plate and three cell panel, which detect only IgG antibodies. So many IgM alloantibodies would have missed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Fung MK, Brenda J, Hillyer D, Westhoff M. AABB. Technical manual. 18th ed., Ch. 16. USA: American association of blood bank; 2014. p. 391-3.
2. Denise M. Harmening, Modern Blood Banking and Transfusion Practices. 6th ed., Ch. 9. United states of America: F.A. Davis company Publication 2012. p. 217-9, 232-3.
3. Pahuja S, Gupta SK, Pujani M, Jain M. The prevalence of irregular erythrocyte antibodies among antenatal women in Delhi. Blood Transfus 2011;9:388-93.
4. Makroo RN, Bhatia A, Hegde V, Chowdhry M, Thakur UK, Rosamma NL, et al. Antibody screening & identification in the general patient population at a tertiary care hospital in New Delhi, India. Indian J Med Res 2014;140:401-5.
5. Chaudhary R, Agarwal N. Safety of type and screen method compared to conventional antiglobulin crossmatch procedures for compatibility testing in Indian setting. Asian J Transfus Sci 2011;5:157-9.
6. Harvey G. Klein MD and David J. Anstee PhD FRCPath FMedSci. Mollison’s Blood Transfusion in Clinical Medicine. 11th ed. United states of America and U.K., Blackwell publishing-2005. p. 74.
7. Agrawal A, Mathur A, Dontula S, Jagannathan L. Red Blood Cell Alloimmunization in Multi - transfused Patients: A Bicentric Study in India. Glob J Transfus Med [serial online] 2016;1:12-5. Available from: http://www.gjtmonline.com/text.asp?2016/1/1/12/178005. [Last cited on 2018 Apr 20].
8. Pahuja S, Pujani M, GuptaSK, Chandra J, Jain M. Alloimmunization and red cell autoimmunization in multitransfused thalassemics of Indian origin. Hematology 2010;15:174-7.
9. Makroo RN, Bhatia A, Gupta R, Phillip J. Prevalence of Rh, Duffy, Kell, Kidd and MNs blood group antigens in the Indian blood donor population. Indian J Med Res 2013;137:521-6.
10. Garg N, Sharma T, Singh B. Prevalence of irregular red blood cell antibodies among healthy blood donors in Delhi population. Transfus Apher Sci 2014;50:415-7. [Pubmed]
11. Varghese J, Chacko MP, Rajaiah M, Daniel D. Red cell alloimmunization among antenatal women attending a tertiary care hospital in South India. Indian J Med Res 2013;138:68-71.

Table 5: Comparison of different studies

| Study                     | Years | Total number of patient samples tested | Positive | Most common alloantibody detected |
|---------------------------|-------|---------------------------------------|----------|----------------------------------|
| Chaudhary and Agarwal     | 2011  | 2026                                  | 26       | Anti-E                           |
| R.N. Makroo               | 2012  | 49,077                                | 304      | Anti-E                           |
| Present study             | 2016  | 174,214                               | 512      | Anti-D                           |

Table 6: Various clinical conditions encountered during study

| Diagnosis                               | Number of cases |
|-----------------------------------------|-----------------|
| Antenatal cases                         | 151             |
| Hemolytic disease of fetus and newborn  | 11              |
| Severe anemia                           | 105             |
| Thalassemia                             | 115             |
| Sickle cell anemia                      | 43              |
| Acquired/congenital heart disease       | 19              |
| Gallbladder calculus                    | 2               |
| Septicemia                              | 4               |
| Thrombocytopenia                        | 4               |
| Burns                                   | 2               |
| Tuberculosis                            | 3               |
| Fracture                                | 9               |
| Trauma                                  | 14              |
| Preoperative anemia correction          | 17              |
| Hemophilia                              | 3               |
| Hemet emesis                            | 2               |
| Fibroid                                 | 1               |
| Liver cirrhosis                         | 3               |
| PLHA                                    | 2               |
| Renal stone                             | 2               |

PLHA=People living with HIV/AIDS