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ANALYSIS OF REASONS FOR RED CELL CROSSMATCH INCOMPATIBILITIES IN A BLOOD BANK OF A TERTIARY CARE REFERRAL TEACHING INSTITUTE IN SOUTH INDIA.

Immunohematology

Praveen MD Senior Resident, Department of Transfusion Medicine, SVIMS, Tirupati

Sreedhar Babu KV* Professor & Head, Department of Transfusion Medicine,SVIMS,Tirupati *Corresponding Author

Anitha M Senior Resident, Department of Transfusion Medicine,SVIMS, Tirupati

Sriranjitha TVN Junior Resident, Department of Transfusion Medicine, SVIMS, Tirupati

ABSTRACT

Background: Pre-transfusion compatibility testing is performed in order to prevent the transfusion of incompatible donor RBCs. It can assure ABO compatibility between donor and patient blood as well as detect most clinically significant red cell alloantibodies.

Aim: To estimate the incidence and nature of red cell incompatibilities that were reported in the cross match lab.

Settings and Design: This single centre prospective study has been conducted at the Department of Transfusion Medicine, Sri Venkateswara Institute of Medical Sciences, Tirupati during the period from June 2016 to November 2018.The study population comprise of recipients of whole blood and packed red cells whose requests were sent to Immuno-Haematology laboratory for pre-transfusion testing.

Material and Methods: Two millilitre of plain blood sample from each recipient was collected separately for pre transfusion testing according to our departmental SOPs. Whenever an incompatibility was identified at any stage of pre transfusion testing, the reasons were analyzed. Results were expressed as percentage.. Statistical analysis was carried out using SPSS version 25, SPSS Inc, Chicago, USA.

Results: During the study period, 24,528 cross matchings have been done among which a total of 42 (0.17%) incompatibilities occurred. Incompatibilities occurred with sample of the patients from 4 years to 70 years with eight males and sixteen females. Majority of incompatibilities were due to unexpected antibodies and majority of them belong to O blood group.

Conclusion and Recommendations: For patients who are unlikely to require blood transfusion in a given medical or surgical setting, "type and screen" should be a common approach. This practice of performing type and screen only allows better inventory management of red blood cell supply.

KEYWORDS

Pretransfusion testing, incompatibilities, antibody, cross match.

INTRODUCTION:

The objective of pretransfusion testing is to ensure that donor red blood cells (RBCs) should survive when transfused. In a normal subject, the recovery of fresh, compatible red cells is 97% to 102% at 60 minutes and 95% to 100% at 24 hours (1). Pre-transfusion compatibility testing is performed in order to prevent the transfusion of incompatible donor RBCs that may lead to an immune-mediated haemolytic transfusion reaction (2). Pretransfusion testing can assure ABO compatibility between donor and patient blood as well as detect most clinically significant red cell alloantibodies that react with antigens on donor RBCs. But, it cannot always guarantee the normal survival of transfused cells as minute numbers of deleterious reactions due to serological incompatibility can still occur (3).

The goals of antibody screening are to detect as many clinically significant antibodies and few clinically insignificant antibodies as possible and to complete the procedure in a timely manner. The traditional method of doing compatibility testing is an Indirect Coombs' Test (ICT) performed in a test tube. Later, various enhancement reagents or potentiators were added before the 37 °C incubation phase in order to increase the sensitivity of the test system and also for a shortened incubation time. Several modifications of the Coombs' test like microplate, Solid Phase Red Cell Adherence Assay (SPRCA), Column Agglutination Technology (CAT) have come up lending to the introduction of semi and fully automated testing platforms. These systems are safe, reliable, and easy to read and are comparable and sometimes better to the conventional test (4).

Many of the factors that affect the invivo destruction are not taken into account during in vitro pretransfusion compatibility testing. At present, even by use of more elaborate tests, it is difficult to accurately predict the fate of a transfused unit of blood. By using some simple serological tests like ICT with and without potentiators, autocontrol, Direct Coombs' Test (DCT) and antibody screening, it is sometimes possible to predict the outcome of transfusing a unit of blood that is incompatible invitro (5). Invitro reactions not due to blood group antibodies are also sometimes encountered when typing RBCs or performing compatibility testing.

The transfusion services aim to provide a high quality facility with no to minimum risk to the recipients. However, a wide range of errors ranging from near miss events to errors those lead to patient injury and even mortality may occur at various steps of the transfusion process.

We performed a prospective study to estimate the incidence and nature of red cell incompatibilities that were reported in the Immuno haematology lab and investigated the contributing factors, and underlying system problems.

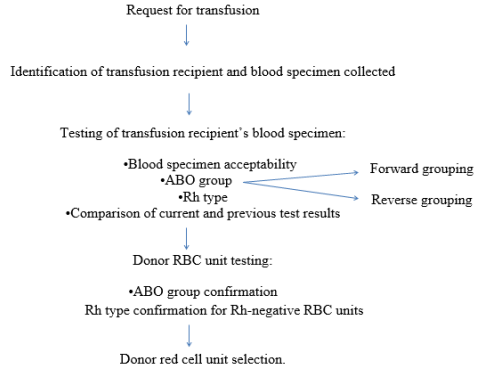
MATERIAL AND METHODS:

This single centre prospective cross-sectional analytical study has been conducted at the Department of Transfusion Medicine, Sri Venkateswara Institute of Medical Sciences, a tertiary care referral teaching hospital, Tirupati during the period from June 2016 to October 2018 to enumerate the red cell incompatibilities that were reported in the Immuno haematology laboratory.

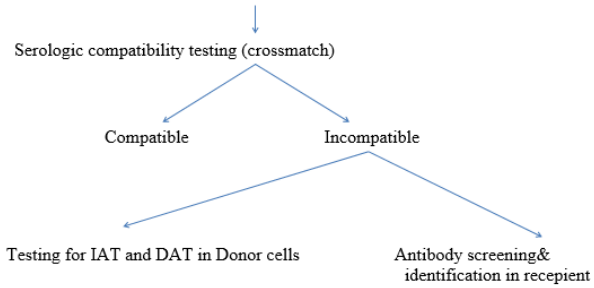
The study population comprise of recipients of whole blood and packed red cells whose requests were sent to Immuno-Haematology laboratory for pre-transfusion testing. Two millilitre of plain blood sample from each recipient was collected and utilized for ABO (forward and revers) grouping, Rh typing, Coombs'cross match according to department standard operating procedure by Column Agglutination Technology (CAT).

Whenever an incompatibility was identified at any stage of pre transfusion testing, the data was collected and entered on to a document. All incompatibilities were resolved before the issue of blood component from the Immuno-Haematology lab.

Steps followed in pre transfusion testing :



- **Selection of components of ABO group and Rh type that are compatible with the transfusion recipient**



For the purpose of the analysis, data collected on incompatibilities were divided into different categories: Coombs' positive, unexpected and cold antibodies, technical errors, subgroups of abo system.

RESULTS:

During the study period, 24,528 cross matching were performed among which a total of 42 incompatibilities observed. Majority of incompatibilities were due to unexpected antibodies and majority of them belong to O blood group (Table 1). Incompatibilities occurred with sample of the patients from 4 years to70 years with eight males and sixteen females. Presence of unexpected antibodies has been found to be the commonest reason for cross match incompatibilities (Table 2) and autoantibody being the commonest antibody identified followed by the antibodies against the gel matrix of the column being the second commonest (Table 3). Among the technical factors improper washing of the cells prior to testing being the commonest reason for red cell cross match incompatibility (Table 4).

Table :1 Cross match incompatibilities among different blood groups

Blood Group		Incompatibilities observed N (%)
A	A1	6 (19)
	A2	4(18)
B		14(35)
AB		3(04)
O		15(35)
Total		42(100)

Table:2 Reasons for cross match incompatibilities

Reasons	Incompatibilities observed N (%)
Donor Direct Coombs' Positive	4(9)
Un expected antibodies	29(70)
Technical errors	6(14)
Subgroups of A	3(7)
Total	**42(100)**

Table:3 Un expected antibodies identified

Antibody Identified	Number (%)
C	2(6)
K	1(4)
N	2(6)
M	2(6)
D	1(4)
E	1(4)
A2 with A1	2(6)
Cold antibody	3(9)
Auto antibody	13(40)
Antibodies To gel matrix	5(15)
Total	**32(100)**

Table :4 Technical errors

Reason	Number
Improper washing	5
Wrong sampling	1

DISCUSSION:

Many blood group antigens and their genes have been identified, and their physiological roles uncovered, and have been found to be important determinants in Transfusion Medicine. Approximately, 400 red blood cell antigens have been identified (7).

The introduction of the ICT in 1945 added a new dimension to the safety of blood transfusion. After that, there was an enormous increase in the identification of alloantibodies that caused transfusion reactions or hemolytic disease of the newborn (8). Pre transfusion blood grouping, red blood cell antibody screening, and compatibility testing are essential to prevent incompatible blood transfusion and alloimmunization (9).

Sensitive cross-matching protocols were developed to further increase transfusion safety, including minor cross matches, DCTs and auto controls. However, minor cross matching was given up since the introduction of antibody screening for donors. The recipient's serum or plasma must be tested for clinically significant unexpected antibodies.

The object of the antibody screening test is to detect as many clinically significant unexpected antibodies as possible. In general, “clinically significant unexpected antibody” refers to antibodies that are reactive at 37°C or in the antihuman globulin test and are known to have caused a transfusion reaction or unacceptably short survival of transfused RBCs (10).

All red cell antibodies other than naturally occurring anti-A and anti-B antibodies are defined as “unexpected antibodies.” There are 2 types of unexpected antibodies: alloantibodies and auto antibodies. Production of alloantibodies may result from pregnancy, transfusion, transplantation, or injections of immunogenic material (11). This occurs by allogeneic transfusion of RBCs, pregnancy, or transplantation. Therefore, the incidence of unexpected antibodies in the general patient population is 1.64% and 0.78% in published studies (12,13). The incidence of unexpected antibodies in the present study is 0.17%. The more frequently a patient is exposed to foreign RBC antigens, the more likely that patient will produce unexpected alloantibodies. This is evidenced by a study of multiply transfused sickle cell patients in which 29% of pediatric and 47% of adult patients developed clinically significant alloantibodies (14).

In the last few years, pre transfusion testing practices have shifted from tube to CAT. This technique is more sensitive than the conventional tube method (15). Currently, routine pre transfusion tests focus primarily on potential clinical significant antibodies that only react in the antiglobulin phase after incubation at 37°C. CAT improves productivity, increases standardization and addresses regulatory issues. Compatibility testing or antiglobulin tests are performed in a prefilled card containing dextran acrylamide gel particles or glass beads combined with antiglobulin reagent along with potentiators like Poly Ethylene Glycol (PEG) and preservatives. In the present study we found 5 cases where in the patients had an antibody against the ingredients of the matrix of column agglutination which is similar to a study done at SVIMS, Tirupati (16) where in a patient developed antibodies against matrix of column agglutination .

The cells of approximately 80% of all group A (or AB) individuals are A_1 (or A_1B), and the remaining 20% are A_2 (or A_2B) or weaker subgroups. The differences between A1 and A_2 are both quantitative and qualitative. Since 1 to 8% of A_2 individuals produce anti- A_1 in their serum, and 22 to 35% of A_2B individuals produce anti- A_1. This antibody can cause discrepancies between forward and reverse ABO grouping and incompatibilities in cross matches with A_1 or A_1B cells. Because anti- A_1 is a naturally occurring IgM cold-reacting antibody, it is unlikely to cause a transfusion reaction because it usually reacts only at temperatures well below 37°C. It is considered clinically significant only if it is reactive at 37°C (17).In the present study, two incompatibilities occurred due to A_2 group with A_1antibody.

The estimated number of A antigen sites on the few agglutinable RBCs is approximately 3,500 per RBC, where as no detectable A antigens are demonstrated on RBCs that do not agglutinate. No A glycosyltransferase is detectable in the serum or in the RBC membranes of Aend individuals. Aend is inherited as an allele at the ABO locus (18). Secretor studies detect the presence of only H substance in the saliva of Aend secretors. Anti-A1 is found in some Aend sera (19).

The DAT is not a required test in routine pre transfusion protocols. Eder performed DATs with anti-IgG on 15,662 pre transfusion patient samples out of which 15% were positive(20). Judd and coworkers revealed similar findings on 65,049 blood samples in a 29-month period, where only 5.5% of samples resulted in a positive DAT (21). In

the present study, 0.02% were positive for DAT.

Positive DAT without immune mediated red cell destruction is reported in 1 in 1000 to 1 in 14,000 in donors and 1-15 percent hospitalized patients. Most blood donors with positive DAT appears to be healthy (22). In the present study, the incidence of incompatibilities due to DAT positivity is 4/42 (9%).

The incidence of incompatibilities due to technical errors during the study period is 0.02% which is comparable to reported incidence of 0.22%in a study by Sudipthasekhar et al., (23).

CONCLUSION AND RECOMMENDATIONS:

For patients who are unlikely to require blood transfusion in a given medical or surgical setting, "type and screen" should be a common approach (determine the recipient's ABO and Rh type and perform an antibody screen). If this screen is negative, no further testing would recognize, but a crossmatch could be performed and blood components can be provided quickly in the event that they were needed. This practice of performing type and screen only allows better inventory management of red blood cell supply, as there are more units left available in inventory, and few units being held for a specific patient, unavailable to others. For patients where a positive antibody screen is detected on type and screen request, further testing as outlined above together with a crossmatch would be undertaken to ensure timely availability of compatible blood products.

A crossmatch should be requested for those patients for whom a blood transfusion is intended or definitely anticipated. This order should include the number of red blood cell units required. In the laboratory a crossmatch order results in blood grouping and antibody screening as well as compatibility testing with preparation and labelling of the red blood cell units for transfusion to the particular recipient.

To avoid technical errors the following are recommended:

a. Induction of automation in blood bank cross-match laboratory for compatibility testing.
b. Encouragement for reporting of medical events and transfusion reactions by promoting "no blame" working environment.
c. Mechanism for mandatory verification of information on blood compatibility documents with the requisition form before the issue of blood component,
d. Strict adherence to working as per SOPs.
e. Mandatory reporting of any and all events immediately with complete documentation
f. Regular competency assessment program for all personnel involved in the entire process of transfusion.
g. Implementation of quality assessment and quality improvement program in transfusion practice to enhance transfusion safety.

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