

Case Report:

Red cell incompatibility due to antibody against ingredient in column matrix: a rare entity

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ABSTRACT

An essential goal in transfusion medicine is that transfused blood be compatible with the patient. Several problems arise while performing pre-transfusion compatibility testing. Rarely, *in vitro* reactions not due to blood group antibodies are sometimes encountered which can pose difficulty in routine immunohematology work up. Though these antibodies are clinically insignificant, proper work-up is indicated, before labelling such antibodies as clinically insignificant. In the report we describe a rare case wherein the patient had an antibody against the ingredients of the matrix of column agglutination.

Key words: Red cell antibody, Gel column, Antibody identification

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INTRODUCTION

The objective of pretransfusion testing is to ensure that donor red blood cells (RBCs) will 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, column agglutination technology (CAT) have come up leading 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 *in vivo* 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

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transfused unit of blood. By using some simple serological tests like ICT with and without potentiators, autocontrol, direct Coombs' test and antibody screening, it is sometimes possible to predict the outcome of transfusing a unit of blood that is incompatible *in vitro*.⁵

Invitro reactions not due to blood group antibodies are sometimes encountered when typing RBCs or performing compatibility testing. Many of these problems are because the patient has an antibody that reacts with a chemical present in the commercial RBC suspension media, commercial antisera, or commercial antibody potentiators⁶. In this report we describe the rare occurrence of antibodies against the gel column matrix.

CASE REPORT

A 61-year-old male was diagnosed to have well differentiated squamous cell carcinoma of left leg. His blood group was A₁ positive. There was no history of previous blood transfusions. Peripheral smear showed normocytic normochromic anaemia with neutrophilic leucocytosis. The total serum protein level was within normal limits (6.6 g/dL). Three units of packed red cells were requested for surgery. All units came as incompatible (4+ reaction) in CAT (Biovue Ortho Clinical Diagnostics) (Figure 1). There was no evidence of auto-agglutination in the blood sample. Autocontrol (AC) and ICT were positive (4+ reaction) whereas direct Coombs' test (DCT) was negative (Figure 1). Red cell antibody screening and identification was panreactive. We repeated AC, ICT and DCT in tube technique and in different manufacturer's CAT (Biorad GmbH, Switzerland). AC, ICT and DCT were negative in these two platforms (Figure 2). Red cell antibody screening done in Biorad card was negative. Blood units incompatible in Biovue were compatible in tube and Biorad (Figure 2). The test was also done by adding Biovue low ionic strength solution (BLISS) in tube which



Figure 1: Biovue gel card showing cross-match incompatibility with three donor units

ICT= indirect Coombs' test; AC = Auto control, DCT = direct Coombs' test.

is an additive solution used in Biovue technology. There was no agglutination seen which rules out the presence of antibodies to BLISS. Thus the antibody was against the ingredient added in column matrix of Biovue cards.

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

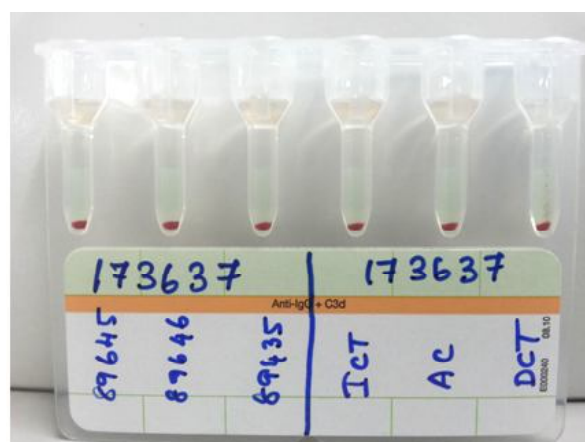


Figure 2: Biorad gel card showing cross match compatibility with three donor units

ICT= indirect Coombs' test; AC = auto control; DCT = direct Coombs' test

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.⁷ Pretransfusion 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 crossmatches, DCTs and ACs. However, minor crossmatching was given up since the introduction of antibody screening for donors. Additives such as bovine albumin, low ionic strength media,¹⁰ polybrene,¹¹ and polyethylene-glycol¹² (PEG) and enzyme¹³ treated red blood cells were used to enhance agglutination and to further shorten incubation times. In the last few years, pretransfusion testing practices have shifted from tube to CAT. This technique is more sensitive than the conventional tube method.¹⁴ Currently, routine pretransfusion tests focus primarily on potential clinically significant antibodies that only react in the ICT phase after incubation at 37°C. Hemagglutination is still the classical method for antigen testing and antibody screening.¹⁵

CAT is based on the principle of controlled centrifugation of red cells through a dextran-acrylamide gel or glass beads that contains predispensed reagents. It 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 PEG and preservatives.

Our patient sample showed incompatibility with all units and a positive autocontrol and positive ICT only when Ortho Biovue gel cards were used. Tube method and Biorad cards

showed negative results. However, the DCT was negative in all the three platforms. This made us suspect the presence of antibody in the patient serum against the reagents used in the Biovue system. Antibody against the BLISS solution was ruled out as the reaction came as positive irrespective of the presence of BLISS solution. As these biovue cards are predispensed with potentiators like PEG and preservatives like sodium azide in the matrix (glass beads), the antibody could be against these ingredients or against the matrix per se. The exact specificity could not be made out because of the unavailability of other ingredients in the matrix. Since tube method is considered as gold standard and since all the reactions were negative in Biorad too, we went ahead with the transfusion with close monitoring. It was an uneventful transfusion with no adverse reactions. There was no evidence of hemolysis post transfusion.

Antibodies that react with an ingredient in the solution used to preserve reagent red cells (eg, chloramphenicol, neomycin, tetracycline, hydrocortisone, ethylene diamine tetra acetic acid EDTA, sodium caprylate, or various sugars) may agglutinate red cells suspended in that solution. The AC will be often nonreactive.¹⁶ However, in our case AC was reactive thereby ruling out the presence of antibody against any preservative used in reagent red cells.

Antibodies that react with ingredients in other reagents, such as Parabens in commercially prepared LISS additives, can cause agglutination in tests using reagent red cells, donor red cells, autologous red cells. Antibody to ingredients in enhancement media may be suspected if the autologous control is positive but the DCT is negative. In some cases, antibodies to reagent ingredients show blood group specificity (eg, paraben-dependent anti-Jka, paraben-dependent antibody to Rh protein,

and caprylate- dependent auto anti-e).^{17,18} PEG which is incorporated in column matrix is immunogenic and antibodies against PEG have been reported.¹⁹ Antibodies against preservatives like sodium azide were also been reported.⁶ Since our patient had a positive AC with negative DCT, the antibody is against the ingredients in the column matrix; the specificity of which is unknown because of the unavailability of type of ingredients present in column matrix. However, the antibody found in our patient did not show any blood group specificity.

These reactions rarely cause erroneous interpretations of ABO typing that could endanger the patient when these antibodies are against the dyes used in grouping antisera. Fortunately, our patient didn't show any grouping discrepancy. Antibodies to a variety of drugs and additives can cause positive results in antibody detection and identification tests. Most of these anomalous reactions are in-vitro phenomena and have no clinical significance in transfusion therapy, other than causing laboratory problems that delay transfusions. Thus, the antibody detected in our patient was clinically insignificant. In such situations, we can go ahead with the transfusion with close monitoring of the patient without wasting much time for the work-up of the antibody.

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