Antibody screening, identification and cross match

Antibody Screening, Identification and Cross Match: Case studies from Bristol Memorial Hospital

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Summary

Antibody screening, identification, and cross matching comprises an essential element of pre-transfusion testing procedure that is of paramount importance in blood bank establishments. Clinically significant antibodies can cause haemolytic transfusion reactions. Antibody screening is crucial for patients who require blood transfusions to detect the presence of any unexpected antibodies and ensure selection of the most compatible unit. We report on five patient case studies regarding the use of antibody screening and identification to select the most appropriate red cell units available. At this time, limited blood units were available. An analysis is provided with key emphasis on the importance of antibody cross matching and compatibility testing.

Keywords: Antibody Screening, Clinically Significant, Compatibility testing, ABO, RhD

Red Blood Cells (RBCs) carry a varying number of blood group antigens on their cell surface (Dean, 2005). To date, there are over 600 identified antigens within 30 distinguished blood group systems (Dean, 2005). To ensure the provision of safe blood for transfusion, antibody screening and identification is routinely performed in blood bank establishments in

accordance with pre-transfusion testing procedures (Makroo et al., 2014). This is primarily achieved through the microcolumn gel technique, which has become the most prevalent technique used in blood bank laboratories worldwide (Hwang Shin et al., 2009). The aim is to detect unexpected antibodies and quantify their specificity to provide blood that lacks the corresponding antigen, forming an element of fundamental importance in clinical transfusion (Makroo et al., 2014).

Alloimmunisation commonly occurs following blood transfusions and is defined as the immune response to antigens that are recognised as foreign (Yazdanbakhsh, 2012). The most important RBC alloantibodies in transfusion practice include the Rh (D, C, E, c, and e) and Kell antigens, in addition to the Duffy, Kidd, and MNS blood group antigens (Makroo et al., 2014; Dean, 2005). Antibodies that are considered clinically significant can cause haemolytic transfusion reactions, following the accelerated destruction and shortened survival of transfused RBCs (Garratty, 2012). Furthermore, clinically significant antibodies are associated with haemolytic disease of the fetus and newborn (Daniels et al., 2002). Therefore, it is critical to recognise and consider clinically significant antibodies present in a patient in order to select the most appropriate unit for transfusion (Makarovska-Bojadzieva, 2009). As a result, the blood service aims to provide a regular supply of all blood groups and blood types.

In this study, we present a case by case report of antibody screening, identification and cross matching for five patients, in addition to the management and use of blood units from a limited supply, highlighting the

importance of clinically significant antibodies and their detection in transfusion medicine.

Materials and Methods

1. Patients

The patients included in this study comprise five individuals with varying medical and transfusion history. The details of each patient are outlined in table 1.

2. IAT Gel Antibody Screening

DiaMed IAT gel cards were used to detect antibodies and performed on all five patients. Each well was labelled with the patient identification number (1-5) with 2 wells used for each patient. 50Î¼l of 0. 8% screening Cell Stab reagents and 25Î¼l of patients' plasma were added to the DiaMed IAT gel cards. Two controls, positive and negative, were prepared using 50Î¼l of 0. 8% O R1r in Cell Stab, with 25Î¼l of AB serum added to the negative control and 25Î¼l of weak anti-D added to the positive control. Cards were incubated at 37°C for 15 minutes and spun in the DiaMed ID-Centrifuge 12 S II for 10 minutes at 1030 rpm. Cards were analysed for agglutination and results were scored accordingly from 0 to 5, where a negative score of 0 indicates no agglutination and a positive score of 5 indicates agglutination.

3. Antibody Identification

Antibody identification was performed on patients 2, 3, and 4 with a positive antibody screen, using enzyme and IAT panels. A 1% red cell suspension was prepared from 10Î¼l packed red cells and 1mL DiaMed diluent. 50Î¼l was

added to each well followed by 25Î¼l of patients' plasma. Two controls were prepared. An IAT control was prepared from 50Î¼l of R1r control cells and 25Î¼l of weak anti-D. An enzyme testing control was prepared using R1R1 control cells and 25Î¼l of anti-K. Cards were incubated at 37°C for 15 minutes and spun in the DiaMed ID-Centrifuge 12 S II for 10 minutes at 1030 rpm. Cards were analysed using a light box and scored accordingly.

4. Compatibility testing

DiaMed IAT gel cards were used to perform compatibility tests for each patient against donor units. Each well was labelled accordingly with patient number and donor unit. 50Î¼l of 1% donor unit cells in Cell Stab reagents and 25Î¼l of patients' plasma were added to the corresponding wells. Two controls, positive and negative, were prepared using 50Î¼l of 1% O R1r in Cell Stab, with 25Î¼l of AB serum added to the negative control and 25Î¼l of weak anti-D added to the positive control. Cards were incubated at 37°C for 15 minutes and spun in the DiaMed ID-Centrifuge 12 S II for 10 minutes at 1030 rpm. Cards were analysed and scored for agglutination, 0-5.

Results

Patient	Gender & Age	Transfusion History	Additional Medical Details
1	Female, 70 years old	No history of blood transfusions	• Scheduled for repair of fractured hip joint

		following a fall	
2	Female, 34 years old	Undergone several surgeries to treat disease. Received blood during last surgery 5 years ago.	• Crohn’s disease • Undergoing evaluation for unexplained anaemia
3	Male, 58 years old	Received 4 units of RBCs during surgery 8 years ago.	• History of cardiovascular disease • Undergone heart bypass surgery
4	Male, 14 years	Receives frequent blood	• Sickle cell anaemia

		transfusions for the management of his condition. Last transfusion dated 6 months ago.	• History of anti-K
5	Female, 19 years old	No history of blood transfusions	• Involved in a road traffic accident

Table 1 – The medical history of each patient, including transfusion history.

Patient	ABO/ RhD Type	Screening Cell 1	Screening Cell 2	Interpretation
1	A+ *	0	0	No antibody detected
2	A+	0	5	Antibody

				detected
3	B+	3	0	Antibody detected
4	O+	0	4	Antibody detected
5	O–	0	0	No antibody detected

Table 2 – The ABO and RHD typing of each patient and results obtained from the antibody screening panel. Interpretation of results is also provided.

* A mix field reaction was detected for patient 1 in the ABO/RHD screening.

Patient	Antibody Present	Probable Genotype	Further Patient Information
1	–	Dce/dce – R^1r (31%)	May require more units of red cells in the future but not today
2	Anti-c, Anti-E	DCe/Dce – R^1R^1	Requires 2 units today

		(18%)	
3	Anti-Fy [a] , Anti-K	Dce/dce – R0r (<1%)	Requires 2 units of red cells as soon as possible
4	Anti-K	Dce/dce – R [1] r (31%)	Requires 3 units of red cells
5	–	Dce/dce – r’r (14%)	No longer needs any blood

Table 3 – Results of the antibody identification screening panel and transfusion requirements for each patient.

Patient	Unit	ABO/RhD	Antigens
1	G	A / RhD Positive D+C+E-c+e+	K Fy [a] , S, M – Negative
	M	A / RhD Negative D-C-E-c+e+	Fy [a] , JK [a] – Negative

		A / RhD Positive D+C+E-c-e+	K, Fy ^{a} , S, M – Negative
2	S		
	F	O / RhD Positive D+C+E-c-e+	K, Fy ^{a} , S, M, HbS – Negative
	Q	B / RhD Positive D+C-E-c+e+	K, Fy ^{a} , S, M, HbS – Negative
3	R		
		B / RhD Negative D-C+E-c+e+	K, Fy ^{a} , S, s, M – Negative
	J	O / RhD Positive D+C+E+c+e+	K, Fy ^{a} , S – Negative
4	K	O / RhD Positive D+C+E-c+e+	K, Jk ^{a} , S, M – Negative
	I	O / RhD Positive D+C-E-c+e+	K, Fy ^{b} , S, Le ^{a} – Negative
5	T	O / Rhd Negative D-C-	Fy ^{a} , HbS –

E-c+e+	Negative

Table 4 – Compatibility testing of each patient against selected donor units.

Discussion

Our first case study is a 70-year-old female who has been admitted for an operation to repair a fracture to her left hip joint, following a fall. The patient has no history of previous blood transfusions and appears in good health. Her son reports that she has been healthy throughout her life and only admitted to hospital for child birth. Pre-transfusion testing procedures were carried out to order blood for her upcoming surgery. The results for this patients ABO and RhD typing revealed a mixed field reaction for anti-D. Extended Rh typing also revealed a mixed field reaction for anti-c. Antibody identification was performed to determine if this patient has any clinically significant antibodies, in which none were detected.

It is therefore possible that this patients ABO type may be A_3, a subgroup of the A blood type. Weak subgroups of A_3 are known to cause mixed field reactions (Dean, 2005), therefore we have requested this patients' serum to be typed against A_1, A_2 and A_3 cells. However, extensive ABO and RH typing is required to precisely determine this patients' blood phenotype. This patient requires red cell units in the future for a planned operation. The units that have been designated for this patient include unit G and unit B, which are both A RhD positive red cell units. However, a full assessment of this patients' blood type must be analysed before the administration of these components.

Patient 2 forms our second case study, a 34-year-old female who suffers from Crohn’s disease. This patient has been admitted regarding unexplained anaemia. Patient 2 has previously undergone several surgeries to manage her condition. Her last surgery was 7 years ago, in which she received a blood transfusion. This patient has a haemoglobin level of 7. 9 g/dL and 2 units of RBCs have been ordered for transfusion today. The antibody identification revealed clinically significant antibodies, including anti-c and anti-E. Most Rh blood group antibodies are warm reacting IgG antibodies that cause haemolytic and delayed transfusion reactions and haemolytic disease of the fetus and newborn; therefore, they are considered clinically significant. Anti-C and anti-E are most commonly found together in patients, as most patients who have developed anti-E often go on to develop anti-c. The c antigen is highly immunogenic in comparison to the E antigen. As a result, anti-c may cause severe haemolytic disease of the fetus and newborn in this patient, whereas anti-e may cause a mild reaction. However, as the patients RhD type is positive, it is unlikely that she will require anti-D prophylaxis. This patient requires two RBCs units today. The units that have been designated for this patient include unit S and unit F. Unit S is A RhD positive and unit F is O RhD positive, in which both units are negative for anti-c and anti-E.

Our third patient is a 58-year-old male who has been admitted into hospital after complaining of chest pains and shortness of breath. This patient has a history of cardiovascular disease and underwent heart bypass surgery 8 years ago, in which he received 4 RBC transfusions. Upon arrival, a diagnosis of heart failure was determined and need for immediate surgery. Antibody

testing for this patient revealed the patient is both positive for anti-Fy ^{a} and anti-K. Furthermore, the probable genotype of this patient suggests African descent, therefore the patient will also receive anti-c and anti-e positive red cells. This patient requires two units of blood as soon as possible, in which unit Q and unit R have been allocated.

The fourth patient in our case report is a 14-year-old male that suffers from sickle cell anaemia and has a history of anti-K. This patient receives frequent blood transfusions for the management of his condition, with his last transfusion dated 6 months prior to admission. The patient was brought in by his family regarding fatigue and shortness of breath. The patient has been kept in hospital for observation pending suspicion of sickle cell crisis. Three RBC units have been allocated for this patient including units J, K, and I. Each unit is O RhD positive and negative for anti-K.

Finally, the fifth patient featured in this report is a 19-year-old female that was involved in a road traffic accident. This patient has no history of previous blood transfusions and has never been admitted to hospital prior to this occasion, with her parents citing excellent health. The patient was admitted with trauma to the head, in which a single blood unit was allocated – unit T. However, the patient no longer requires the unit at this time. The unit will be kept for the patient whilst she remains in hospital following any complications. Unit T was selected for this patient and is O RhD negative. This patient does not have any clinically significant antibodies.

Throughout the treatment and assessment of these 5 patients, only 12 of blood were available. A total of 10 units were used to treat all 5 patients.

Severe weather across the United Kingdom has impacted the distribution of blood from the NHS Blood and Transplant manufacturing sites located in Bristol, London, and Manchester. Access by road, rail, and air have all been affected by severe storms and rendered transport at a halt. The nearest blood bank could not be accessed and therefore a limited number of RBC units were available.

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