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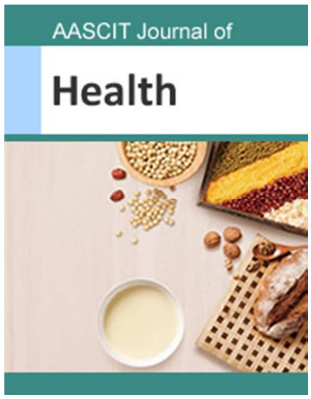


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# The Impact of Immunohaematology in Blood Transfusion Management

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## Abstract
Alloimmunisation occurs when an antigen is introduced into an immune-competent host. This commonly occurs following transfusion of blood or in pregnancy, when red cells that bear antigens absent in the individual's blood enters the circulation. Five patients' blood samples were brought for compatibility testing which includes blood grouping, antibody screening and identification in the event of positive screening and cross-matching with the available blood in the bank for transfusion. This narrative review emphasises the benefit and criticality of comprehensive serology in the provision of safe blood for transfusion. At the same time, this gives a clue on to how an efficient allocation and use of scarce blood resource for a patient cause.

## 1. Introduction

The provision of safe blood for transfusion does not only imply thorough testing for infectious agents, but also protection from haemolytic transfusion reactions emanating from alloimmunisation against red cell antigens. Ever increasing efforts at improving blood safety have led to the incorporation of routine sensitive screening protocols for detection of unexpected immune antibodies at the various transfusion centres across the globe. The goal is to determine the exact specificity of the antibody and to provide blood that lacks the corresponding antigen to the patient [3]. Alloimmunisation occurs when a foreign antigen introduced in an immune competent host which evokes an immune response. This commonly occurs following transfusion of blood or in pregnancy, when red cells that bear antigens absent from the individual's blood enter the circulation. The most important unexpected red blood cell alloantibodies in daily transfusion practice, in terms of frequency of occurrence, are the Rh (D, C, E, c and E), and Kell (K) antigens followed by other blood group antigens of the Duffy, Kidd, MNS, Le, Lu, P and other minor blood group systems [13]. These antibodies can cause acute and delayed haemolytic transfusion reactions as well as haemolytic disease of the foetus and newborn [9, 6]. Antibody detection and identification are fundamental to the practice of immuno-haematology. Antibody identification can be a guide to the clinical significance of the

antibody and provides information that aids in the selection of suitable blood for transfusion [8, 13]. In some circumstances, it can be a difficult and time-consuming process, and thus cause a delay in patient care. When a positive antibody screen is encountered, there are many pathways that can be followed and each laboratory has a policy outlining their procedures. It is important that a systematic approach is taken to assign specificity and to exclude the possibility of the presence of additional antibodies. It is also useful to know the ethnic background of the patient because some rare phenotypes are found almost exclusively in certain population e.g. In(b-) in Asians; S-s-U-, Js(b-) in blacks [10, 4]. White patients are more likely to make antibodies to high incidence antigens such as k, $Kp^b$, $Lu^b$ and Vel. Clinical and serological history of the patient is useful [10]. Strength and mode of reactivity is often a clue as to what type of antibody is present, and a very strongly reacting antibody is more likely to be clinically significant. Also, the reaction temperature, sensitivity and resistance to the enzyme are all to be considered [4]. Some antibodies react weaker with cells carrying a single dose of antigen, notably M, N, S, s, $Jk^a$ and $Jk^b$ [6, 14].

Therefore, pre-transfusion antibody screening of patients', with antibody identification in the event of a positive result prior to cross matching is an essential component of compatibility testing. The two principal techniques for unexpected antibody screening and identification are the indirect antiglobulin and enzyme methods. The main purpose of pre-transfusion screening, antibody identification in the event of positive screening result and final cross-matching is to prevent immune mediated haemolytic transfusion reactions [15]. Haemolytic transfusion reaction could be immediate or delayed; intravascular or extravascular [5].

Five patients' blood sample was brought for blood grouping, antibody screening and identification in the event of positive screening and cross-matching with the available blood in the bank for transfusion.

## 2. Methodology

### 2.1. Cell and Serum Blood Grouping

Cell and serum ABO, Rh and K blood grouping was performed on all the patients' sample using Diamed gel cards (UK) [8]. Standard reagent cells and the reagent antisera were controlled. Antibody screening by indirect antiglobulin test (column agglutination technology Diamed IAT gel Card, UK) was performed on all patients' plasma/serum. Two 0.8% screening cells R1R1 and R2R2 in Cell Stab reagent (NHSBT, Bristol, UK) was used for the screening. The reagent cell made up the following antigens; C, D, E, c, e, CW, M, N, S, s, P1, K, k, $Kp^a$, $Le^a$, $Le^b$, $Fy^a$, $Fy^b$, $Jk^a$ and $Jk^b$. Using 0.8% O $R_{1r}$ in Cell stab reagent, weak anti-D and AB serum were included as positive and negative control respectively. Incubation was done at $37^0$ for 15 minutes. Gel cards were spun, the result interpreted and recorded. Samples positive for screening are considered for antibody identification.

### 2.2. Antibody Identification

Antibody identification was performed by indirect antihuman globulin, LISS/Coombs (Diamed GmbH, Switzerland) and enzyme (Diamed GmbH, Switzerland) methods using 10 different set of 1% reagent identification panel cells in Cell stab reagent. Using 1% O $R_{1r}$ in Cell stab reagent, weak anti-D and AB serum were included as positive and negative control respectively. Incubation was done at $37^0$ for 15 minutes. Gel cards were spun. LISS/Coombs and enzyme methods results were interpreted and recorded. Probable antibody/antibodies were determined using the anti-gram/ID panel profile leaflet supplied with the panel stab cell.

### 2.3. Cross-Matching

Cross-matching was performed for patients who were positive in the events of the antibody screening. However, the choice of blood for the cross-match will depend on patient's ABO, Rh, K blood group, and antibody identified in the serum. The ethnicity might also play a role. The probable compatible unit was then chosen for the cross-match. Diamed IAT gel card cross-match was performed. 1% donor unit cells in Cell stab reagent was cross-matched with patient serum. Using 1% O $R_{1r}$ in Cell stab reagent, weak anti-D and AB serum were included as positive and negative control respectively. Incubation was done at $37^0$ for 15 minutes. Gel cards were centrifuged. And agglutination scored as 0 to 5. The same procedure was followed for all the cross-matching. Table 1 shows the available blood units in the bank. Table 2 and 3 shows the blood grouping, antibody screening and identification results and the compatible blood for the five patients.

## 3. Results

***Table 1.*** *The following are the blood units with their blood groups in the blood bank.*

| Unit ID | ABO phenotype | Rh | | K | Others antigens of known status |
|---|---|---|---|---|---|
| Q | O | Ccee | Rr | Negative | $Fy^{a+}$ |
| M | B | DCe/ce | $R_1r$ | Negative | $Fy^a$ |
| K | A | DCe/ce | $R_1r$ | Negative | |
| F | A | ce/ce | ce/ce | Negative | $M^{b-}$, $Jk^{b-}$ |
| S | O | Dce/DCe | $R_1R_1$ | Negative | $C^{W-}$ |
| P | O | DCe/Dce | $R_1R$ | Negative | $Kp^{a-}$ |
| R | O | ce/ce | Rr | Negative | $Fy^b$ |
| T | A | ce/ce | Rr | Negative | |

| Unit ID | ABO phenotype | Rh | | K | Others antigens of known status |
|---|---|---|---|---|---|
| I | O | ce/ce | Rr | Negative | |
| B | B | ce/ce | Rr | Negative | $Fy^-$ |

***Table 2.*** *ABO/Rh D blood grouping, antibody screening & identification and the compatible blood units for the five patients.*

| Patient | Forward Group | | | | Reverse Group | | ABO/RhD blood group | Antibody screening | Antibody identified | Compatible Blood units |
|---|---|---|---|---|---|---|---|---|---|---|
| | Anti-A | Anti-B | Anti-D | Ctl | A1 cells | B cells | | | | |
| 1 | 5 | 0 | Mf | 0 | 0 | 4 | A Rh D+ve* (D need to be confirmed) | Positive | Anti-k present | Unit S and Q |
| 2 | 5 | 0 | 5 | 0 | 0 | 5 | A RhD+ve | Negative | Nil | Unit K |
| 3 | 0 | 5 | 5 | 0 | 5 | 0 | B RhD+ve | Positive | Anti-$Fy^a$ present<br>Anti-$Lu^a$ (need to confirm) | Units M and R |
| 4 | 5 | 0 | 0 | 0 | 0 | 4 | A RhD-ve | Positive | Anti-D, C, $C^W$ present<br>Anti-G may be present | Units F, I and Q |
| 5 | 0 | 0 | 4 | 0 | 5 | 5 | O RhD+ve | Negative | Nil | Unit P |

***Table 3.*** *Rh CcEe & K blood grouping for the five patients with their probable Rh CE and K genotype.*

| Patient | Anti-C | Anti-c | Anti-E | Anti-e | Anti-K | Ctl | Probable Genotype |
|---|---|---|---|---|---|---|---|
| 1 | 5 | Mf | 0 | 5 | 0 | 0 | Cc*ee (c need to be confirmed) |
| 2 | 5 | 0 | 0 | 5 | 0 | 0 | CcEe |
| 3 | 5 | 5 | 0 | 5 | 0 | 0 | Ccee |
| 4 | 0 | 5 | 0 | 5 | 5 | 0 | ccee, K |
| 5 | 5 | 5 | 0 | 5 | 0 | 0 | Ccee |

Key;
Mf: mixed field reaction
0, 1, 2, 3, 4, 5: Various grades of increasing trend of agglutination.

**Patients' findings in details**

Patient 1;

Female,

29 years old woman

Had a knee cap repair few days ago. Transfused with 2 units of blood during surgery.

Two more units of blood required for her by the surgeon.

ABO blood group; A,

K negative

Rh typing; there is a complete reaction with anti C, anti-e (Rh Ce) and mixed field reaction with anti-D and anti-c which cast ambiguity about her Rh D and c status. Probably this was caused by the previous blood transfusion. The transfused blood might be positive for D and/or c and hers being negative for the D and/or c. Otherwise, the transfused blood might be negative for D and/or c and hers being positive for D and/or c. So, in addition to her A, Rh C and e antigens, she may have RhD+ c+, D+ c-, D- c+ or D-c-.

Antibody screening is positive, and the antibody identification panel suggested that it is anti-K. All Kell system antibodies should be considered clinically significant, and where possible, the antigen-negative red cell should be selected for transfusion. Anti-K can cause severe and fatal haemolytic transfusion reaction. Anti-K can cause haemolytic disease of the newborn and foetuses, and unlike RhD, no prophylaxis is available for the prevention of K alloimmunisation during pregnancy/delivery for the women who are K-ve with K+ve foetus. The antibody screening neither shows anti-D nor anti-c which also suggest that she might be RhD+c+ that was recently transfused with RhD-c- cells. Or else she is D-c- yet to produce anti-D and anti-c. Her Rh group could be resolved by Rh genotyping. In most case, such patient is to receive the RhDc negative blood because of fear of alloimmunisation. However, cross-matching is important. Based on the findings units S, K, T and Q were chosen for cross-match. None of the unit chosen is K positive because the patient has a significant antibody, anti-K.

Blood units chosen for cross-match;

a. Unit S: Blood group O $R_1R_1$, K negative, CW negative
b. Unit K: Blood group A $R_1r$, K negative,
c. Unit T: Blood group A rr, K negative
d. Unit Q: Blood group O rr, K negative, $Fy^a$ positive

Units S and Q were found to be compatible with the patient. While K and T units were not compatible, that may be due to the presence of antibody in patient's serum corresponding to a low frequency antigen on donor's cell. The patient might be ABO blood group $A_2$ having anti-$A_1$ (2% of $A_2$ have anti-$A_1$) which makes units K and T incompatible provided K and T are $A_1$. Antibody screening couldn't detect anti-$A_1$ using O panel cell. Probably the patient is blood group $A_2$. Anti-$A_1$ is usually cold agglutinin that rarely causes haemolytic transfusion reactions. However, Anti-$A_1$ is considered clinically significant when it reacts at 37°C [1].

Patient 2;

Female,

A 59-year-old woman

To undergo an elective myomectomy in two days' time. She had never been transfused with blood.

Her blood group is A Rh D+ve, Rh C+, c+, E+, e+ and K negative. Her probable Rh genotype is $R_1R_2$. As a precaution, two units of blood is requested to be kept in advanced.

Antibody screening was negative indicating absence of the

atypical antibody in her serum. Blood units can be arranged by electronic cross-match, and no wet cross-match is necessary [7]. On the day of the transfusion, a new sample might be necessary to re-group and re-screen before the electronic cross-match and issuance. This is just for confirmatory purpose and in the case of antibody emanating within the short period. Blood group A Rh D+ve, K negative will be given to this patient.

Patient 3;

Female,

A 24-year-old lady. She is an athlete.

Had a bleeding from the head injury in a domestic accident.

She had a very low Haemoglobin level secondary to the head injury.

Her family doctor requested for three units of blood.

She had transfusion at the age of two secondary to road traffic accident.

Her blood group is B Rh D+, Rh C+, c+, e+ and K negative. Her probable genotype is R1r.

Antibody screening is positive. So, antibody identification is necessary. Antibody identification by IAT/LISS suggested that there is anti-$Fy^a$ which turns negative with enzyme treated cell panel. However, anti-$Lu^a$ status cannot be ascertained because there is an only one panel cell that is positive for $Lu^a$ antigen. Another panel of cells that is $Lu^a$ positive and $Fy^a$ negative is required to exclude or include the anti-$Lu^a$. Alternatively, the patient red cell can be phenotype for $Lu^a$ antigen by molecular genotyping. Anti-$Fy^a$ is a clinically significant antibody as it can cause mildly delayed or immediate haemolytic transfusion reaction. Anti-$Lu^a$ is not clinically significant. However, there was a report that Anti-$Lu^a$ had caused mild delayed haemolytic transfusion reactions [11]. Base on her blood group, antibody screening and identification status, the following units were chosen for cross-match.

a. Unit M: Blood group B, R1r, $Fy^a$ negative and K negative
b. Unit R: Blood group O, rr, $Fy^a$ negative and K negative
c. Unit B: Blood group B, rr, Fy negative and K negative

Two of the units, M and R were found to be compatible. Unit B is not compatible; this might be due to the presence of an antibody in patient serum that corresponds to a donor's antigen. The incompatibility could be due to $Lu^a$ antibody, though not clinically significant.

Patient 4

Female,

A 35-year-old singer

Her blood group is A Rh D negative, K positive. Probable genotype; Rh rr (ce/ce).

She had a postpartum haemorrhage which necessitated the request of three units of blood by the gynaecologist in charge. This is her first child. She had transfusion twice in her village due to other illness.

Antibody screening test was positive with increasing strength in enzyme treated cells. The following antibodies were identified in her serum; anti-D and anti-C. The status of anti-CW cannot be ascertained because there is only one cell panel having the antigen CW. CW antigen is a low-frequency antigen, so anti- CW is not clinically significant. The patient may possess anti-G which is associated with anti-D and anti-C. Anti-G is a Rh system antibody that is directed against an antigen found on most erythrocytes which are C+ and/ or D+. The potential of this antibody to cause Haemolytic Disease of Newborn is still not clear, as all cases that have been described in pregnant women have been in association with anti-D or anti-C [2]. Anti-D and -C are clinically significant antibodies. They are usually IgG type capable of causing haemolytic disease of the newborn and foetuses. Her baby's blood group is A Rh D-ve, C-ve. Baby is normal with no sign of jaundice or anaemia. The anti-D and anti-C present in the woman might be due to an immunisation from the previous transfusion in her village.

Base on her blood group status, antibody screening and identification, the following blood groups were chosen for cross-matching;

a. Unit F; Blood group A, rr, K negative, M-, Jkb-
b. Unit T; Blood group A, rr, K negative
c. Unit I; Blood group O, rr, K negative
d. Unit Q; Blood group O, rr, K negative, Fya+

Units F, I and Q were compatible. Unit T is not compatible. Patient contains antibody against an antigen present on donor cells unit T. This could be due to anti- CW. Neither the presence of anti-CW in patient serum nor the presence of antigen CW on donor cells is known. Occasionally, molecular typing might be needed to resolve some antigens.

Patient 5

Male,

A 35-year-old man

He is a cricket player undergoing an elective appendectomy in a week's time. He has never been transfused with blood or blood products. Need one unit of red cell.

He is blood group O Rh D+ve, C+, e+, K negative.

Probable Rh phenotype is $R_1R_1$.

Antibody screening is negative. There is no atypical antibody. Blood can be issue base on computer cross-match. Wet cross-matching is not necessary [7]. On the day of the transfusion, a new sample might be necessary to re-group and re-screen before the electronic cross-match and issue. This is just for confirmatory purpose and in a case of antibody emanate within the short period. Blood group O Rh D+ve, K negative will be given for this patient.

## 4. Conclusion

Overall, based on what is available at that moment of the request, the immediate demand for patient 1 was met. For patient 3, only 2 units of compatible blood were available as against the requested 3 units. The clinician was promptly notified about the case to take extra precaution such as cell salvage, alternate source of blood from sister blood bank or the use of crystalloid if the need arises.

The clinician was also advised to monitor the haemoglobin

(Hb) level of the patients after each unit's transfusion in case it had reached the target haemoglobin level required. For instance, if two units suffice to reach the target Hb level in patient 4, the third unit might not be necessary hence be allocated to another patient such as patient 1. The request for patient 2 and 5 was also met.

Another probable solution is that unit (Q) for Patient 1 would be made to be transfused last. If the second unit (Q) may be declined due to sufficient Hb level in patient 1, unit Q would be transferred to patient 4. Antibody screening and identification are crucial and fundamental in the practice of immuno-haematology to prevent blood transfusion reactions and to ensure safe blood for patients. These concise observations give a clue on how to efficiently allocate necessary blood for the betterment of patient especially, where blood is a scarce resource for patients' care.

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