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Original Research Paper

Immunohematology

WARM AUTOANTIBODY WITH UNDERLYING ANTI-E ALLOANTIBODY IN A CHILD: A DIAGNOSTIC CHALLENGE AND ITS IMPLICATIONS

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ABSTRACT Every immuno-hematologist is faced with two challenges: 1) Reduce the risk of alloimmunisation in patients and 2) Identification of alloantibody in immunized individuals; so that corresponding antigen-negative blood unit is transfused in future to avoid hemolytic transfusion reaction. However, sometimes the alloantibody may be masked by coexistent autoantibody leading to difficulty in alloantibody identification and cross-match. We hereby present a case of warm autoantibody with alloantibody presenting as cross-match incompatibility with special emphasis on diagnostic work-up.

KEYWORDS : Alloantibody, autoantibody, adsorption, transfusion

INTRODUCTION

Alloimmunisation is a common problem encountered in transfusion medicine. Incidence of alloimmunisation varies from 0.3% to 60% depending on the study population with incidence being higher in multi-transfused patients like thalassemics. [1-3] However, in general patient population, the incidence is 0.46% to 2.4% only. [4,5] Alloimmunisation is slightly more common in females particularly associated with pregnancy, anemia and previous transfusion. [3-6] Once alloimmunized, alloantibody must be identified. However, coexistence of alloantibody with autoantibody leads to difficulty in cross-matching and alloantibody identification.

Case History

A 9 years old girl presented to our hospital with severe pallor and passage of red colored urine since 2 days. Patient had received single unit of blood transfusion 15 days back. Therefore, delayed hemolytic transfusion reaction (HTR) was suspected. Laboratory investigations revealed severe anemia (Hb - 5 g/dL) with reticulocytosis (corrected reticulocyte count - 8%) and mild unconjugated hyperbilirubinemia (Total bilirubin – 2.2 mg/dL, indirect bilirubin – 1.6 mg/dL) suggestive of hemolysis. Urgent requisition for packed red blood cells (PRBCs) was received in our Regional Blood Transfusion Centre (RBTC).

MATERIALS & METHODS

The patient's blood group was AB Rh positive by tube method. However, multiple group-specific PRBCs put-up for cross-match were incompatible. Fresh sample was collected in EDTA vial under strict warm conditions. The RBCs and plasma were separated immediately by centrifugation at 2000rpm for 5minutes. The cells were washed with warm normal saline. Thereafter, extended forward and reverse blood grouping was done at 4°C, 22°C and 37°C by tube method. The blood group was confirmed as AB positive by both forward and reverse blood grouping. Polyspecific Direct Antiglobulin Test (DAT) was strongly positive (4+). Further, DAT profiling (DC-Screening I gel card, DiaMed) revealed strong reaction (4+) with IgG. [**Figure 1(a) & 1(b)**]

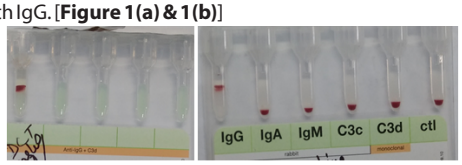
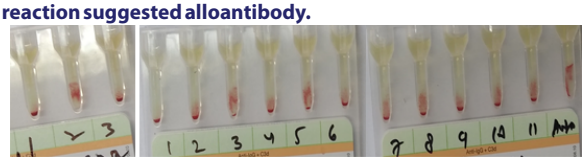


Figure 1: (a) Polyspecific Direct Antiglobulin Test and (b) DAT profile showing strong positivity (4+) for IgG.

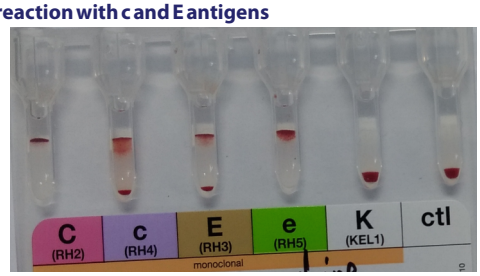
The patient's plasma was screened for presence of antibodies by IAT using three-cell antigen panel (ID-DiaCell I-II-III Asia, Diamed) using LISS/Coombs ID-cards, at 37°C in AHG (anti-human globulin) phase. The cards were incubated for 15 minutes and centrifuged in ID-centrifuge for 10 minutes. The three-cell panel showed reaction (2+) with 2nd cells. An extended 11-cell antibody identification panel (ID-DiaPanel, DiaMed) showed gradation reaction by IAT with negative reaction with 1st cells, using ID-cards at 37°C suggesting an alloantibody. Possibility of an underlying anti-c &/or anti-E alloantibody was considered. [**Figure 2**] Autocontrol was positive (1+) in Coomb's phase suggesting the presence of warm IgG autoantibody.

Figure 2: Indirect Antiglobulin Test (IAT) with patient's serum at 37°C (a) 3-cell panel and (b) 11-cell panel showing gradation reaction suggested alloantibody.



Rh/Kell extended antigen profile of the patient using DiaClon gel cards showed mixed-field reaction with c and E antigens. [**Figure 3**]

Figure 3: Rh/Kell antigen profile of the patient showing mixed-field reaction with c and E antigens



To determine the specificity of alloantibody (whether anti-c &/or anti-E), three O Rh+ donor cell lines R_1R_1 (CCee), R_2R_2 (ccEE) and rr (ccee) were prepared for allogeneic adsorptions. [**Figures 4**] Low ionic strength solution (LISS) was used to enhance the reactions with adsorbed plasma. Following the allogeneic adsorption onto cell lines (R_1R_1 and rr) negative for E-antigen, IAT with adsorbed plasma revealed an anti-E reaction pattern. [**Figures 5**] Whereas IAT was negative with adsorbed plasma left after allogeneic adsorption

onto R_2R_2 (ccEE) cells. Thus confirming anti-E alloantibody. [**Figures 6**]

Figure 4: Flow chart of allogeneic adsorption for differentiation & identification of anti-c & anti-E

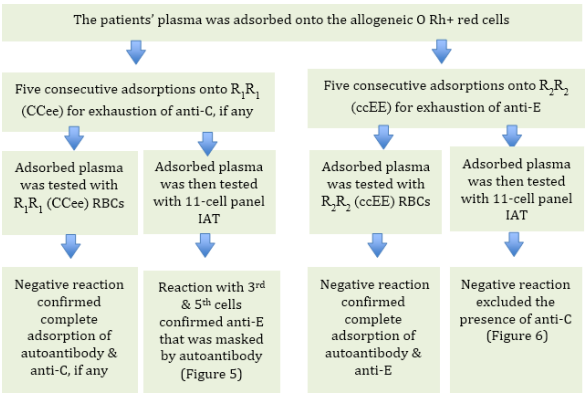


Figure 5: IAT with plasma adsorbed onto R_1R_1 allogeneic (CCee) cells revealed 2+ reaction with 3rd cells (ccDEE) & 1+ reaction with 5th cells (ccddEe) of 11-cell panel confirming anti-E alloantibody.

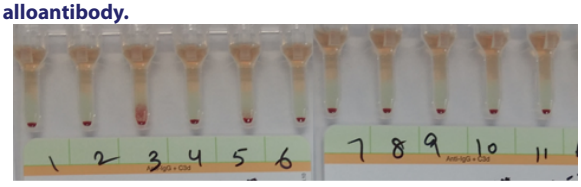
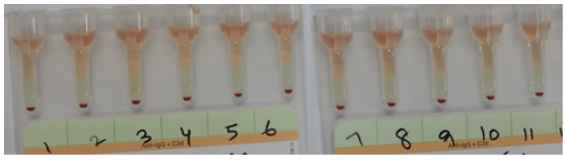


Figure 6: IAT with plasma adsorbed onto R_2R_2 allogeneic (ccEE) cells was negative.



Indirect Antiglobulin Test (IAT) with eluate of first adsorbed R_2R_2 allogeneic (ccEE) cells was performed. Elution was done using DiaCidel acid elution kit, Diamed. A uniform strong (4+) pan-agglutination reaction with both three-cell and 11-cell panels at 37°C confirmed a broad-spectrum, warm autoantibody coated on patient's RBCs that was masking the anti-E. [**Figure 7**]

Figure 7: IAT with eluate at 37°C (a) 3-cell panel and (b) 11-cell panel showing uniform pan-agglutination confirming warm autoantibody

Retrospectively checking the transfusion details of the patient, it was found that the patient was exposed to E antigen during previous transfusion with compatible AB positive ©-, E+, K-) PRBCs. The IAT of the donor plasma of that blood unit was negative. Also, there was no history of intravenous immunoglobulin therapy. Thus, passive acquisition of alloantibody was ruled out.

Least incompatible, AB Rh(D) positive &"c, E, K" triple negative, fresh PRBCs were slowly transfused to the patient under strict clinical supervision & steroid cover. Transfusion was uneventful. Patient was started on corticosteroid therapy and discharged in clinically stable condition. Repeat RBC phenotype was advised at least three months after the transfusion. However, patient was lost to follow-up.

DISCUSSION

Alloantibodies are commonly directed against Rh, Kell, Lewis and MN antigens. [3-5,7] Anti-E alloantibody accounts for almost one-third cases of alloimmunization; suggesting E-antigen is highly immunogenic. Moreover, E-antigen shows variable expression in general population due to genetic disparity. Hence, patient's RBC Rh/Kell phenotyping should be done prior to transfusion in all patients as far as possible to avoid exposure. [3-4] To make matters worse, alloimmunization is often complicated by autoantibody formation, a phenomenon first described by Dameshak W et al in 1943. [8] Since then, various authors have reported coexistent autoantibody and alloantibody in approximately upto 30% of IAT positive cases. [3]

Castellino et al reported presence of warm (IgG) autoantibodies in 7.6% (n=14/184) cases of multi-transfused pediatric sickle cell anemics, all of which were associated with alloantibodies. Clinically significant haemolysis was reported in four patients only. [9] Similarly Ahrens N et al reported an incidence of 28% (n=200/717) for coexistent auto- & allo-antibodies in general patient population, of which 73 had history of transfusion. This further highlights that transfusion is associated with autoimmunization also, similar to our case. Nine patients were primarily alloimmunized and subsequently developed autoantibody whereas 10 patients developed vice versa. However, only 6 patients developed autoimmune hemolytic anemia (AIHA). [10] Though the risk of AIHA is low, the pan-agglutinating autoantibody is a double-edged sword. It complicates the identification of underlying alloantibody; leading to delay in arranging compatible blood. On the other hand, autoantibodies decrease RBC survival; precipitating the need for transfusion. Also, subsequent blood transfusion further exacerbates autoimmunization. Therefore, further management may include recombinant human erythropoietin and corticosteroid therapy to minimize need for transfusion. [11]

Exact kinetics of autoantibody formation in alloimmunized patients remains unclear. Post-transfusion immune activation may be one of the factors. [12] Alloantibody binding to transfused RBCs may cause a conformational change in the antigen. This neo-antigen may stimulate the production of antibodies that cross-react with self-antigens. Similarly repeated deformation of the RBC membrane in sickle cell anemia may lead to RBC neo-antigen exposure. [9] Epitope-spreading may also be involved. [12] Some patients might have naturally occurring antibodies such as anti-M, anti-E or anti-K. [13,14] But these antibodies are usually IgM type, hence clinically insignificant.

Alloantibody identification is the foremost concern for immunohematologist. Adsorption techniques are widely used to remove autoantibodies from serum and subsequently specify the underlying alloantibody. Autoadsorption is a safe & cost-effective method that does not alter the alloantibody titre. However, in severely anemic patients, enough autologous RBCs are not available for complete adsorption. Often many patients are recently transfused like our patient and even a small number of transfused RBCs might consume the alloantibody leading to false-negative results. Alloadsorption is helpful in such cases. Alloadsorption also helps in ruling out 'autoantibody with mimicking specificity' on IAT that are also usually directed against Rh system and are possibly commoner (21% to 26.8%) than true alloantibodies in patients with warm autoantibody. Autoantibodies with mimicking specificity don't maintain specificity after alloadsorption. Also, in contrast to alloimmunized patients, transfusion of antigen-negative RBCs may not be necessary in these patients. Therfore least incompatible blood may be issued especially in emergencies. [15,16] But, use of allogeneic adsorption is limited if alloantibody is against high prevalence antigen. Some authors have employed an alternative quicker dilution method with/without RBC phenotyping, however it should be performed only when the alloantibody titer is higher than the autoantibody titer. [16-18]

CONCLUSION

The coexistence of autoantibody and alloantibody is not uncommon and poses a serious challenge to immunohematologist.

Though adsorption studies are labor-intensive & time-consuming, they are highly useful in such cases for alloantibody identification and ensuring safest possible blood transfusion. Once developed, these alloantibodies may also have long-term consequences especially in young females like our patient who may develop HTR or HDN in future. Therefore, alloantibody specificity should be determined in all cases.

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