Cold autoantibody interference in pretransfusion testing and its resolution: a case report

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ABSTRACT

We report a case of cold autoantibody detected in a 69-year-old gentleman diagnosed with chronic lymphocytic leukemia and cold autoimmune hemolytic anaemia. Pretransfusion testing showed panagglutination in ABO grouping, antibody screening and identification, with positive direct antiglobulin test (DAT) and positive control cells. A cold autoantibody was suspected, however, the test using a prewarm method and washed RBC with warm saline failed to resolve it. Further tests using DTT-treated cells resolved the grouping as AB RhD positive and DAT as positive with anti-C3d only (control negative). Cold autoadsorption was done and repeated antibody screening using cold-autoadsorped plasma was negative, excluding the presence of alloantibody. The cold-agglutinin titer was >1028 having a wide thermal range. Crossmatching using cold autoadsorped plasma with the AB-positive donor blood was compatible and the patient was transfused safely. For such cold autoantibody cases, an extensive immunohematological workup is required to exclude underlying alloantibody for transfusion safety and efficacy.

Keywords:

Cold autoantibody; pretransfusion testing; panagglutination, cold autoadsorption; dithiothreitol (DTT).

INTRODUCTION

Pretransfusion testing is a compulsory process to determine the appropriateness of blood products for transfusion to ensure patient safety and transfusion efficacy[1]. It includes multiple steps, starting from positive patient identification and correct sampling to immunohematological testing and finally, labelling the unit to be issued with product detail and patient identification information[2]. The pretransfusion immunohematological testing includes blood grouping of recipient and donor, antibody screening followed by antibody identification if screening positive. If clinically significant antibodies are identified, selection of corresponding antigen-negative blood is performed followed by crossmatching[3]. Any discrepancy in the pretransfusion procedure needs to be resolved before issuing the blood[4]. Presence of auto antibodies either cold, warm or mixed type, having high titer or wide thermal amplitude complicates the immunohematological

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test in blood bank and requires additional serologic work-up[5]. Although this challenging work of resolving pretransfusion discrepancies increases the turnaround time resulting in a delay in releasing compatible blood to the patient, it ensures the patient's safety[6, 7]. In this present case report, we describe a case of high-titer cold autoantibody complicating the pretransfusion testing in a chronic lymphocytic leukemia (CLL) patient. This case report may assist as a guide to resolve the cold autoantibody interference cases.

CASE REPORT

A 69-year-old gentleman was admitted to the hematological ward with upper respiratory tract infection having underlying CLL and cold autoimmune hemolytic anaemia. Upon arrival, full blood count showed hemoglobin: 5.8g/dl, RBC: 1.2x10^12/L, MCV: 109.9fl, MCH: 47.9pg, MCHC: 43.6g/dL, WBC: 112x10^9/L and platelet: 170x10^9/L. Full Blood Picture showed marked RBC agglutination, presence of 94% lymphocytes and no schistocytes or spherocytes seen. Due to low hemoglobin, three units of crossmatched packed cells were requested. Pretransfusion testing done by fully automated analyzers (Biorad IH-500) using column agglutination technology (CAT) showed uninterpretable ABO grouping with 4+ reactions in each forward grouping, reverse grouping, RhD typing and control. The positive control indicates spontaneous red blood cell (RBC) agglutination which invalidates the results. Direct antiglobulin test (DAT) with polyclonal AHG and antibody screening with three cells was positive (Figure-1).

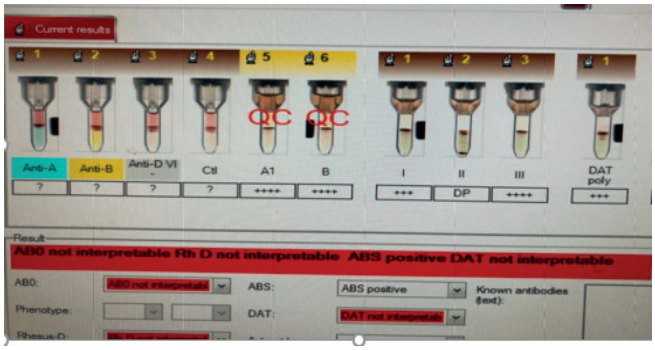

Figure 1: ABO grouping, antibody screening and DAT performed by Biorad IH-500.

Repeated ABO/RhD grouping and DAT manually using CAT (ID-Card "LISS/Coombs") showed similar results as in IH -500. DAT with monoclonal anti-IgG and anti-C3d showed a 4+ reaction (control positive). Repeated antibody screening (3cell panels, Bio-Rad ID-DiaCell I-II-III Asia, CAT, manual) and identification (11 cell panel, Bio-Rad ID DiaPanel, ID DiaPanel-P, CAT, manual) showed panagglutination. Crossmatch was incompatible. Red cell acid elution (ELU-KIT™ Plus Kit, Immucor Gamma red cell elution system) showed negative results in the eluate.

In view of clinical history and test findings, we suspect the cold autoantibody as RBC heavily coated with IgM autoantibodies can spontaneously agglutinate during centrifugation, leading to false-positive reactions in the ABO typing and DAT tests. The IgM autoantibodies in the plasma also cause false positive reactions in antibody screening/identification and compatibility testing. We repeated all the tests using the prewarm method at 37◦C but failed to resolve the false positive reaction. Again, the forward grouping using washed red cells (four times washing with warm saline) also revealed similar findings.

The next step performed was dithiothreitol (DTT) treatment of the patient's RBCs and of known AB cells (used as a control). Forward grouping using DTT treated patient's cells showed 4+ reactions at anti-A, anti-B and anti-D with control as negative, confirming the patient's forward group as AB RhD positive. The AB control cells were used to check the validity of the test. AB cells should be strongly reactive with typing reagents after the DTT treatment. Inappropriate DTT treatment may destroy the ABO antigen that will show a negative result and invalidates the test result. Here, the AB cells showed strong positivity indicating a valid test (Figure-2a).

A cold autoadsorption was performed to remove the cold autoantibody from the plasma. During cold autoadsoprtion, it was found that the potent IgM cold autoantibody reacted with the patient's RBC and led to hemolysis, as evidenced by the reddish colour of the post-cold autoadsorped plasma. Therefore, our subsequent interpretation of positive reaction should be based on agglutination and not hemolysis. Reverse grouping using post-cold autoadsorped plasma showed a negative reaction (absence of agglutination) at the A1 cell, B cell and O cell (Figure-2b) which confirms the patient's blood group as AB.

Repeated DAT with DTT-treated patient's cells showed a 2+ reaction with anti-C3d, a negative reaction with anti-IgG and the control was negative (Figure-2c). This

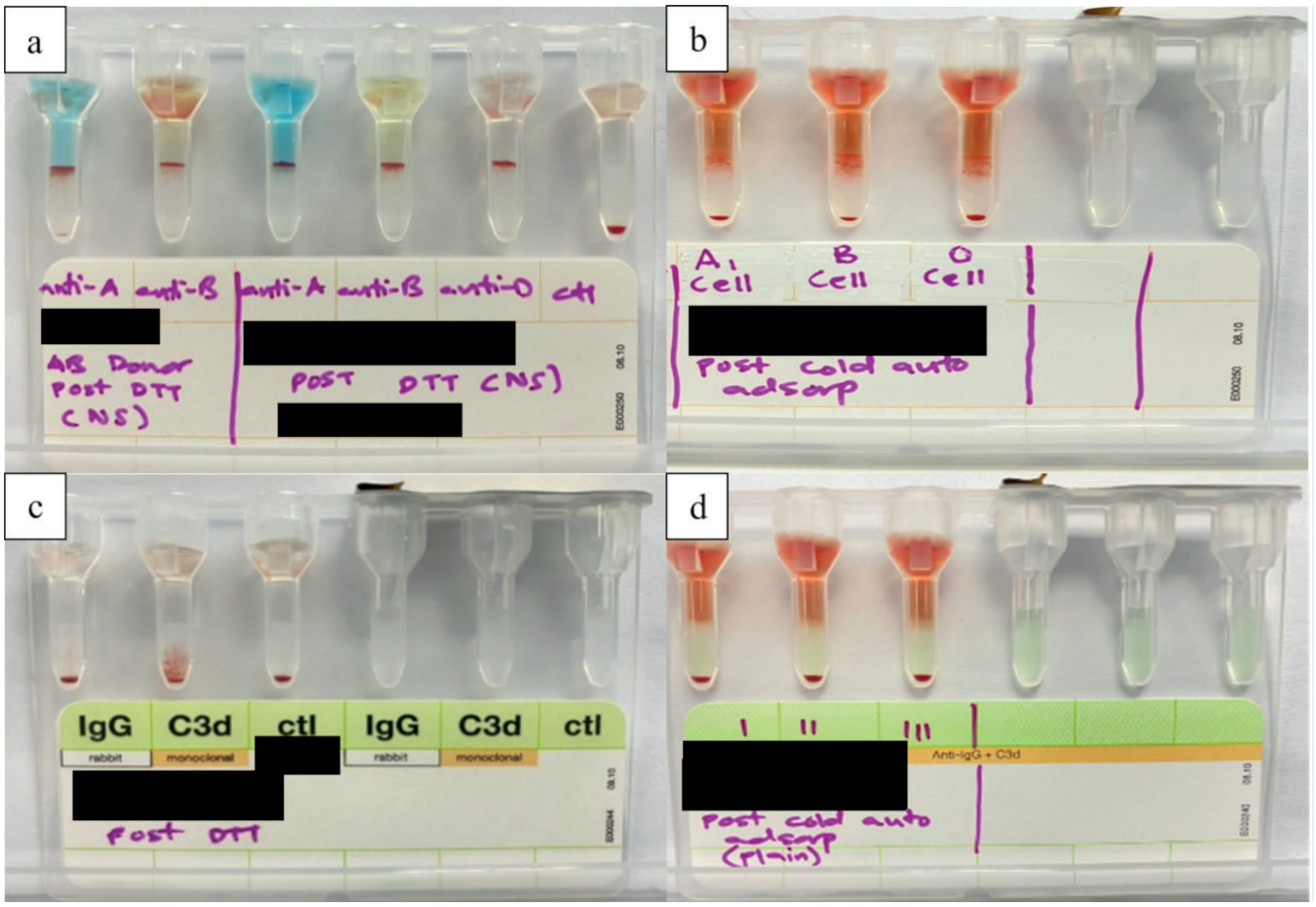

Figure 2: (a) Forward grouping using post-DTT treated cell with AB control cell. (b) Reverse grouping using post-cold autoadsorped plasma (c) DAT using post-DTT treated cells. (d) Antibody screening using post-cold autoadsorped plasma.

confirms the positive DAT was due to the presence of complement on RBC surface. The negative DAT with anti-IgG were compatible with the negative reaction in the eluate following the acid elution test. Repeated antibody screening using post-cold autoadsorped plasma showed a negative reaction (absence of agglutination) and excluded the presence of alloantibody (Figure-2d).

Red cell phenotyping done using post-DTT treated patient's cells, showed the patient as R1R1 (DCe/DCe), MNss, Jk(a-b+), and Mi(a-). Kell system antigens are being destroyed by DTT, we could not determine the K/k status in the patient (Figure-3a). Crossmatching using cold autoadsorped plasma with the AB RhD positive, R1R1, kk, MNss, Jk(a-b+), Mi(a-) phenotype matched packed cells was compatible (absence of agglutination) (Figure-3b). The patient's cold agglutinin titer was found to be>1028 with anti-I specificity having a wide thermal range up to 37◦C.

The packed cells were transfused slowly while the patient was kept warm and closely monitored. The transfusion was completed without any adverse reaction. The patient was discharged home after 15 days of admission with advice to be followed up in hematology day-care.

DISCUSSION

Blood transfusion is a common lifesaving procedure required in different clinical situations. The safe, effective and quality blood product transfusion ensures safe transfusion to the patient[8]. In this case report, the challenges in pretransfusion testing due to high titer cold agglutinin interference are described. Here, DTT treatment of the RBC was able to destroy the IgM cold autoantibody coating of the patient's RBC and thus, able

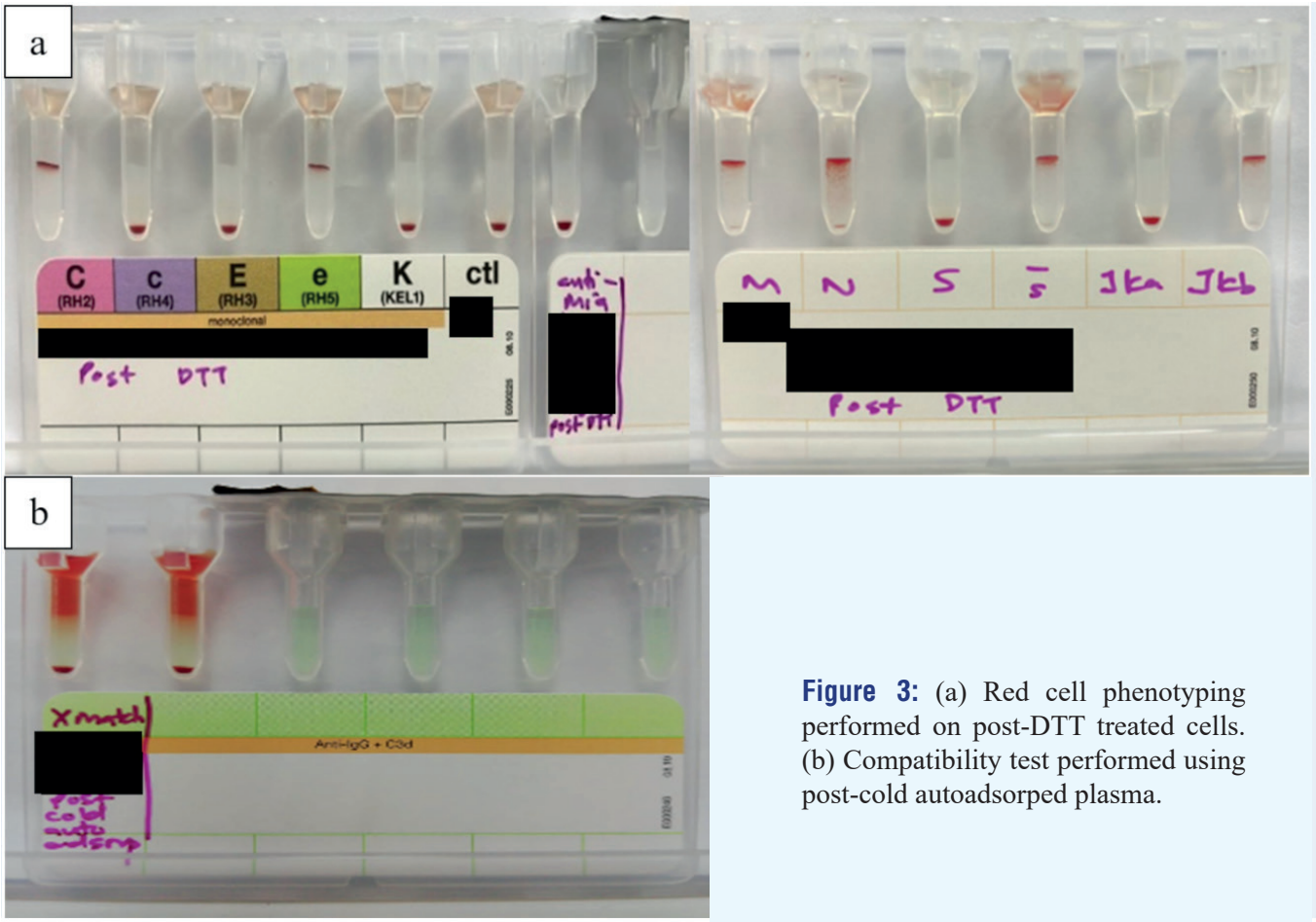

Figure 3: (a) Red cell phenotyping performed on post-DTT treated cells. (b) Compatibility test performed using post-cold autoadsorped plasma.

to determine the ABO, RhD, DAT and red cell phenotype correctly. On the other hand, cold autoadsorption on the patient's plasma, removes the cold autoantibody and subsequently no alloantibody was detected; and also confirms the reverse grouping. Although we have applied the pre-warm technique, which is the least complex method to remove the interference of cold autoantibodies, the discrepancy was not resolved. It was only resolved with DTT treatment of the RBC and cold autoadsorption on the patient's plasma. The specificity of the cold autoantibody identified in this patient was anti-I, which was high in titer and had a wide thermal amplitude up to 37°C.

Cold agglutinins are capable of spontaneous in vitro agglutination at cold temperatures because large-sized IgM molecules are able to span the distance between RBCs and overcome the natural repulsive forces between cells[9]. Thus, cold autoantibodies having high titer and wide thermal amplitude cause discrepancies in ABO/RhD grouping and incompatible crossmatching[10] as it happens to our patient. Moreover, high titer antibodies with a wide thermal amplitude, exceeding the room temperature, can agglutinate RBC and cause complement activation and hemolysis[10]. Transfusion in these patients should be given slowly with close monitoring and maintaining the warmth of the patient. Transfusion should be restricted to cases of severe anaemia or hemolytic crisis cases[5, 10].

In patients with autoantibodies, underlying alloantibodies could be masked and failed to be recognized, posing a challenge in immunohematology testing. Adsorption techniques, such as autoadsorption and alloadsorption, using reagents such as polyethylene glycol or low ionic strength saline, are required to detect underlying alloantibodies[11]. In our patient, cold autoadsorption has been done and subsequently, no underlying alloantibody was detected. Detection of any clinically significant alloantibody requires the transfusion of corresponding antigen negative blood to prevent hemolysis [12].

Dithiothreitol (DTT) is a reducing reagent used to disperse the cold IgM autoantibody coating RBC by reducing the disulfide bonds of IgM molecules, decreasing their polyvalency and ability to directly agglutinate RBC. It also denatures or modifies some blood group antigens, namely Kell, Lutheran, YT, JMH, LW, Cromer, Indian, Dombrock, and Knops systems and subsequently may not be recognized by the corresponding antibodies. Therefore, need to take precautions while doing phenotyping for these antigens on DTT-treated cells[13].

CONCLUSION

This case report highlights the difficulty in pretransfusion testing due to a high titer and wide thermal amplitude cold autoantibody interference, which was resolved with DTT treatment and cold autoadsorption method. Blood bank staff need to be highly aware of such cold antibody cases and should resolve them before issuing the blood to the patient for patient safety and transfusion efficacy.

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