Autoantibodies mimicking alloantibodies: A case series unveiling the dilemmas of transfusion

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Abstract:

INTRODUCTION: Autoimmune hemolytic anemia is characterized by increased red cell destruction and/or decreased red cell survival due to autoantibodies directed against self-antigens on red cells. Since autoantibodies react with self and nonself red blood cells (RBCs), they tend to mask the underlying clinically significant alloantibodies and many a times mimic a specific pattern like alloantibodies.

MATERIALS AND METHODS: We discuss three immune hematological cases of warm autoantibodies. Antibody screening was performed by solid-phase red cell adherence (SPRCA) technique on a fully automated platform NEO Iris (Immucor Inc., USA). In case of a positive antibody screen, antibody identification was performed using SPRCA, NEO Iris (Immucor Inc., USA). Alloadsorption for adsorbing the autoantibodies was done using in-house prepared allogenic packed RBCs – R1R1, R2R2, and rr.

RESULTS: All cases had warm autoantibody with a broad specificity against self-Rh antigens. Anti "C" and Anti "e" antibodies were identified in case 1 and autoanti "e" antibody in cases 2 and 3. Case 3 had underlying alloanti "E" along with autoanti "e" which posed a transfusion challenge.

CONCLUSION: Our case series highlights the importance of detecting the nature of the antibody whether it is alloantibody or autoantibody with antigen specificity. This would help in selecting appropriate antigen negative blood units for transfusion purpose.

Keywords:

Adsorption, autoantibody, autoimmune hemolytic anemia, Rh antibodies

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Introduction

Autoimmune hemolytic anemia (AIHA) is characterized by increased red cell destruction and/or decreased red cell survival due to autoantibodies directed against self-antigens on red cells.[1] The incidence of the disease has been reported to vary from 1 in 80,000 to 100,000/year.[2] AIHAs are divided into warm, cold, or mixed autoantibody.[1] Warm autoantibodies are more reactive at 37°C, whereas cold autoantibodies react best at 0–5°C.[3]

Since autoantibodies react with all self and nonself RBCs, they tend to mask the underlying clinically significant alloantibodies and many a times mimic a specific pattern like alloantibodies. There are very few studies reporting the frequency and specificity of the autoantibodies with mimicking specificity.[3] We, hereby, discuss three such cases in our setting.

Materials and Methods

The cases were identified and evaluated in the department of transfusion medicine in a tertiary care center in India. As a protocol, all patient samples were subjected for "Type and Screen" policy at our center. Blood grouping was performed on fully automated immunohematology system solid-phase red cell adherence (SPRCA) technology, NEO Iris (Immucor Inc., Norcross, GA, USA) using

commercially available antisera (Immucor Inc., Norcross, GA, USA). Antibody screening was performed on NEO Iris using 4-cell commercial panel (Capture-R ready screen, Immucor Inc., Norcross, GA, USA). Any screen positive was further evaluated for antibody identification. RBC antibody identification was done with a commercial 16-cell identification panel (Ready-Id, NEO Iris, Immucor, USA) and/or with 11-cell panel (ID-DiaPanel, Biorad, Switzerland) in indirect antiglobulin test (IAT) phase. A 10-cell commercial panel by the conventional tube technique (Immucor, USA) was also used wherever necessary. Direct antiglobulin test (DAT) and autocontrol were done by column agglutination technology (CAT) using polyspecific (immunoglobulin G [IgG] and C3d) Coombs' reagent (Biorad, Switzerland) and on SPRCA using monoclonal IgG (NEO Iris, Immucor, USA). Red cell antigen phenotyping for "C," "c," "E," "e," and "K" was done on fully automated immunohematology system SPRCA technology, NEO Iris (Immucor Inc., Norcross, GA, USA) using commercially available antisera (Immucor Inc., Norcross, GA, USA). As per the manufacturer's instruction, these antisera are low protein monoclonal blends manufactured from a blend of IgM antibodies of their respective specificities and they do not enhance agglutination of Ig-coated red blood cells and hence can be used to type DAT positive red cells. Alloadsorption for adsorbing the autoantibodies was performed using in-house prepared allogenic packed RBCs (DCe/DCe (R1R1), DcE/DcE (R2R2), and dce/dce (rr)). Allogenic cell panels (R1R1, R2R2, and rr) are regularly prepared as per the recommendations of the American Association of Blood Banks.[4] Alloadsorption of the serum was done based on the technique described by Issitt *et al*.[5] An equal volume of patient's plasma and packed RBCs R1R1, R2R2, and rr RBCs were mixed and incubated at 37°C for 1 h. Usually, a total of 3 adsorptions were done. After the third adsorption, the adsorbed plasma was tested for the presence of antibody using a commercial 3-cell panel (ID-DiaCell I-II-III, Biorad, Switzerland) along with the necessary controls. If the screen showed a positive reaction, an extended antigen panel of 11 cells (ID-DiaPanel, Biorad, Switzerland) was used to identify the antibody specificity. Both antibody screening and antibody identification on 3-cell and 11-cell panel, respectively, were done by CAT using polyspecific (IgG and C3d) Coombs' reagent (Biorad, Switzerland).

Cross-matches between patient's serum and packed red cell (PRC) units were performed between patient's adsorbed plasma and PRC units by CAT using polyspecific Coombs' reagent.

Informed consent was taken to authorize the use of patient's information who were included in this case series.

Results

Case 1

An 11-year-old girl was admitted to our center with a 1½-month history of jaundice in September 2019. She had a history of urinary tract infection at the age of 6 months. During admission, she informed of no history of transfusion. The patient was hemodynamically stable. The complete blood count revealed hemoglobin: 7.4 g/dL, total leukocyte count: 11.3×10^3/L, and platelet count: 403×10^9/L. RBC indices were as follows: mean corpuscular volume 113.2 fL, mean corpuscular hemoglobin (MCH) 36.2 pg, and MCH concentration 32.0 g/dL. The corrected reticulocyte count was 12% (range: 0.5%–2.5%). Other pertinent investigations were as follows: total bilirubin was 6 mg/dL and direct fraction was 0.5 mg/dL, normal complement C3 level 101.9 mg/dL (normal range: 90–180 mg/dL), elevated lactate dehydrogenase 935 U/L (normal range: up to 225 U/L), creatinine 0.36 mg/dL (range: 0.3–0.7 mg/dL), and albumin 4.34 mg/dL (range: 3.4–5.2 mg/dL).

Anticipating a need of transfusion, we received sample for blood grouping, antibody screening, and DAT. On visual inspection, the plasma revealed evidence of hemolysis. Blood group of the patient was O Rh D positive. DAT was positive (4+) with both the techniques (CAT and SPRCA).

RBC antibody screening was pan positive (3+) on SPRCA with a positive autocontrol. RBC antibody identification on 16-cell identification panel in IAT phase was pan positive (3+).

Her Rh antigen phenotyping was D+, C+, c+, E−, e+, and K−. Autoadsorption was not attempted owing to the limited patient sample volume. Alloadsorption was done using in-house prepared allogenic packed RBCs R1R1, R2R2, and rr. After the third adsorption, the adsorbed plasma was tested for the presence of antibody using a commercial 3-cell panel. The 3-cell panel was negative with R1R1 and was positive with R2R2 and rr, indicating the possible presence of anti-C and anti-e antibodies. Adsorbed plasma of R2R2 and rr was tested with 10-cell commercial panel by the conventional tube technique, which showed pattern suggestive of anti-C and anti-e. The patient's phenotype (positive for "C" antigen and "e" antigen) and no history of transfusion in the presence of positive autocontrol further indicated the presence of autoantibody. The antibodies reacted only at 37°C and in AHG phases. Thus, the overall workup confirmed the presence of warm autoantibody mimicking anti-C and anti-e.

R1R1, R2R2, and rr PRC units were cross-matched with the patient serum and adsorbed plasma. Adsorbed

plasma with R2R2 and rr was found compatible with PRC units lacking C antigen and e antigen (i.e., R2R2 phenotype). However, the patient was managed conservatively and transfusion was not needed.

Case 2

A 51-year-old female was admitted elsewhere with stroke in the later part of October 2019. Her blood samples were sent to our department for blood grouping and antibody screening, anticipating blood transfusion. She was never been transfused previously. She had two living children (P2L2). No other lab reports or patient details were available to us.

She was typed as group "O" having Rh and Kell phenotype of D+, C+, c+, E−, e+, and K−. RBC antibody screening was pan positive (3+) in IAT phase on SPRCA with a positive autocontrol. DAT was positive (3+) with both (CAT and SPRCA) the techniques.

RBC antibody identification was done with a commercial 16-cell identification panel in IAT phase and was positive with varying strengths (1+, 2+, and 3+) with all the e + cells except for one which was negative for e antigen, suggesting the presence of autoantibody with anti-e specificity. To reconfirm, 11-cell panel on CAT was also tested with the sample, which revealed a clear pattern for the presence of autoantibody with anti-e specificity. The antibodies reacted only at 37°C and in AHG phases. Alloadsorption was performed as described previously.[5] The antibody screening was negative with adsorbed plasma on 3-cell panel on CAT. The PRC units with R2R2 phenotype were compatible after AHG cross-match using the patient's neat and adsorbed plasma. Thus, the overall workup confirmed the presence of autoantibody against "e" antigen. The patient was managed conservatively and did not require any transfusion.

Case 3

A 67-year-old male was admitted at our tertiary care center in November 2019 for chemotherapy for B-cell chronic lymphocytic leukemia. He was a known hypertensive with coronary artery disease and Type II diabetes mellitus. His blood samples were sent to our department for blood grouping, antibody screening, and identification anticipating future blood transfusion.

He had received one unit of PRC a year ago. He was typed Group "O" having Rh and Kell phenotype of D+, C+, c+, E−, e+, and K−. DAT by CAT and SPRCA was positive (4+). RBC antibody screening was pan positive with variable strengths (3 + and 4+) on SPRCA with a positive autocontrol (4+). RBC antibody identification in IAT phase was positive with varying strengths (2+, 3+, and 4+).

Autoadsorption was not attempted owing to the limited patient sample volume. Alloadsorptions of the plasma were done. Antibody screening with adsorbed plasma was positive. After crossing out all other antigens, adsorbed plasma from R1R1 and rr cells revealed a pattern confirming the presence of alloantibody against "E" antigen when tested with 11-cell panel. However, the R2R2 adsorbed plasma showed a pattern suggesting the presence of autoantibody with "anti-e" specificity on 11-cell panel. The antibody reacted only at 37°C and in AHG phase.

The baseline hemoglobin level was 6.7 g/dL. He received 2 units of "E" negative PRC and the posttransfusion hemoglobin was 7.2 g/dL. He was given dexamethasone and rituximab 600 mg after the PRC transfusion. He was subsequently transfused with another unit of "E" negative PRC and was discharged with a hemoglobin of 7.8 g/dL. He was advised to continue the steroid treatment in a tapering dose for the next 4 days in addition to his regular medications for his underlying disease conditions. All the three transfusions were uneventful. Thereafter, he was advised to get admitted after 3 weeks for the second dose of rituximab.

Discussion

AIHA is a rare clinical disorder characterized by shortening of red cell survival due to of the presence of autoimmune antibodies and requires efficient immune hematological support.[6] Transfusing AIHA patient is a challenge to the immune hematologist.

Warm autoantibodies react with self-antigen optimally at 37°C and are present in serum of about 80% of patients with warm AIHA.[7] In AIHA, most of the warm antibodies are pan-agglutinins in nature and lack any apparent specificity (based on weak/negative reaction with Rh null red cells).[4] However, it has been observed that a significant proportion of these antibodies are directed against antigens of Rh system.[8,9] Further, it was established that absent or weak reactivity of autoantibodies with Rh null red cells does not indicate specificity to Rh complex because Rh null cells might have other membrane abnormalities.[6] Race and Sanger in 1954 were the first to report autoantibodies having a clearly defined Rh specificity, for example, anti "e".[6,10] The autoantibodies having an apparent and relative specificity for a single antigen (e.g., anti "e") along with an evidence of hemolysis required compatible antigen negative unit for transfusion.[6]

In the first case, we found autoantibodies mimicking against two Rh antigen specificities, i.e., anti-"C" and

anti-"e." The prevalence of "C" and "e" antigens is very high in Indian population as reported previously. In an erstwhile study at our center, the Indian donors ($n = 3073$) were typed and prevalence of "C" and "e" antigens was found to be 87% and 98% respectively.[11] After extensive literature search, we did not come across any autoantibodies with specificity against both anti-"e" and anti-"C". In a patient diagnosed with primary sclerosing cholangitis associated with AIHA, autoantibody with anti-C specificity was identified.[12] The said patient was transfused with two units negative for C antigen which were crossmatch compatible. Issitt *et al.* reported specificity of autoantibodies among 87 patients with AIHA warm autoantibodies; only 4 cases were reported to have anti-"e" or anti-"c" antibodies.[5]

Procuring "e" and "C" negative unit (R2R2) usually is a difficult task in a country like ours wherein the resources are not widely available. The importance becomes high in situations with time constraints. The risk–benefit ratio needs to be weighed when comparing the time required to search for such antigen negative units and time the patient can sustain without transfusion support. Anticipating such situations and many others, our center has a set protocol to perform the extended Rh and Kell phenotyping of all the blood donors as a routine to find compatible antigen negative units during immediate/urgent requirements.

Although we detected the autoantibody specificity against Rh, the presence of anti-Ce (anti-RH7) could not be ruled out. Ce (RH7) is considered to be a cis gene product. This compound antigen can form antibody and few reports of this alloantibody causing hemolytic disease of the new born and delayed type hemolytic transfusion reactions have been mentioned.[13]

The second case which came was for the presence of autoantibody with anti-e specificity. A similar case was discussed by Pahuja and Verma, in a 2½-year-old boy diagnosed of AIHA with anti "e" specificity.[6] In most patients with warm AIHA, RBC autoantibodies react with all RBCs (pan-reactive). Infrequently, these autoantibodies do have apparent specificity (patient's RBCs may or may not contain the antigen) which disappears following adsorption with antigen positive or negative cells.[4] Subramaniyan and Veerasamy also explained a case where in they had an autoantibody mimicking anti-C specificity.[14] After adsorption with R1R1 and rr RBCs, the adsorbed plasma was tested for the presence of anti-C using an RBC antibody screening panel. Antibodies were completely adsorbed from the plasma with C-negative as well as C-positive cells.[14] This was similar to the case in discussion, where adsorption with R1R1, R2R2, and rr RBCs revealed complete adsorption of anti-e antibody, suggesting that the autoantibodies were completely adsorbed by e-positive and e-negative cells equally.

Blood transfusion for patients with AIHA presents a unique set of potential problems because of the masking effect of the presence of RBC alloantibodies by autoantibodies, the need for complex pretransfusion immune hematological workup, and the challenge to find a compatible unit.[15]

Selecting the red cell unit for transfusion was highly challenging for the third case due to the presence of alloantibody against 'E' antigen with concomitant presence of autoantibody of anti-e specificity. This posed a dilemma in selecting antigen negative unit for the patient. In case series report by Yürek *et al.*, there were no cases that definitively demonstrated significant exacerbation of hemolysis in patients with true AIHA.[16] Since our patient had transfusion requirement, appropriate "E" negative PRC units were transfused. All the three transfusions went uneventful. The patient was also started on corticosteroids and rituximab medication. More recently, rituximab is being used as a second-line therapeutic approach following corticosteroid therapy, instead of the traditional secondary approach of splenectomy.[15] Patients demonstrated higher initial clinical response rates and relapse-free survival over a 3-year time period, when compared to glucocorticoid therapy alone.[15] Our patient was discharged with a stable hemoglobin of 7.8 g/dL and was advised for admission after 3 weeks for the second dose of rituximab.

Rh genotyping facilities were not available at our center and therefore could not be performed in any of the cases.

Conclusion

The autoantibodies present in a patient may seldom mimic a specific antibody. It is worthwhile to perform advanced immune-hematological workup to determine the exact specificity of this antibody. This would not only help in providing antigen negative blood to the patient but also help to determine the exact nature of the antibody.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Gehrs BC, Friedberg RC. Autoimmune hemolytic anemia. Am J Hematol 2002;69:258-71.

2. Duffy TP. Autoimmune hemolytic anemia and paroxysmal nocturnal hemoglobinuria. In: Simon TL, Dzik WH, Synder EL, Stowell CP, Strauss RG, editors. Rossi's Principles of Transfusion Medicine. 3rd ed. Philadelphia, USA: Lippincott Williams and Wilkins Publication; 2002.

3. Jang MJ, Cho D, Park KU, Yazer MH, Shin MG, Shin JH, *et al.* Autoantibodies with mimicking specificity detected by the dilution technique in patients with warm autoantibodies. Ann Lab Med 2013;33:343-8.

4. Fung MK. Technical Manual. 18th ed. Bethesda: American Association of Blood Banks; 2014. p. 435.

5. Issitt PD, Combs MR, Bumgarner DJ, Allen J, Kirkland A, Melroy-Carawan H. Studies of antibodies in the sera of patients who have made red cell autoantibodies. Transfusion 1996;36:481-6.

6. Pahuja S, Verma D. Autoimmune hemolytic anemia caused by anti "e": A challenge: A case report with review of literature. Asian J Transfus Sci 2017;11:195-8.

7. Petz LD, Garratty G. Acquired Immune Hemolytic Anemias. New York: Churchill Livingstone; 1980.

8. Weiner W, Battey DA, Cleghorn TE, Marson FG, Meynell MJ. Serological findings in a case of haemolytic anaemia, with some general observations on the pathogenesis of this syndrome. Br Med J 1953;2:125-8.

9. Garratty G. What do you do when all units are incompatible? Rev Med Inst Mex Seguro Soc 2005;43:107-11.

10. Race RR, Sanger R. Blood Groups in Man. 2nd ed. Oxford: Blackwell Scientific Publications; 1954. p. 361.

11. Makroo RN, Bhatia A, Gupta R, Phillip J. Prevalence of Rh, Duffy, Kell, Kidd & MNSs blood group antigens in the Indian blood donor population. Indian J Med Res 2013;137:521-6.

12. Bajpai M, Maheshwari A, Gupta S, Bihari C. Autoanti-C in a patient with primary sclerosing cholangitis and autoimmune hemolytic anemia: A rare presentation. Immunohematology 2016;32:104-7.

13. Molthan L, Matulewicz TJ, Bansal-Carver B, Benz EJ. An immediate hemolytic transfusion reaction due to anti-C and a delayed transfusion reaction due to anti-Ce+e: Hemoglobinemia, hemoglobinuria and transient impaired renalfunction. Vox Sang 1984;47:348-53.

14. Subramaniyan R, Veerasamy M. Red cell autoantibody mimicking anti-C specificity: A rare manifestation. Rev Bras Hematol Hemoter 2017;39:91-2.

15. Simon TL, McCullough J, Snyder EL, Solheim BG, Strauss RG. Rossi's Principles of Transfusion Medicine. 5th ed. West Sussex, UK: Wiley & Blackwell; 2016. p. 148.

16. Yürek S, Mayer B, Almahallawi M, Pruss A, Salama A. Precautions surrounding blood transfusion in autoimmune haemolytic anaemias are overestimated. Blood Transfus 2015;13:616-21.