



## Letter to the Editor

# Red cell autoantibody mimicking anti-C specificity: a rare manifestation



Dear Editor,

The association of systemic lupus erythematosus (SLE) with autoimmune hemolytic anemia (AIHA) is a known phenomenon. In many cases of AIHA, no autoantibody specificity is present. The patient's serum reacts with all of the red blood cell (RBC) samples tested and the autoantibody appears to have broad specificity in the Rh blood group system. Occasionally, RBC autoantibodies demonstrate apparent specificity for simple Rh antigens but autoantibodies to Wr<sub>b</sub>, K<sub>pb</sub>, J<sub>ka</sub>, and U antigens have also been reported.<sup>1,2</sup> We hereby report a rare example of an autoantibody mimicking anti-C specificity in a patient with lupus nephritis.

### Case description

A 26-year-old lady was admitted to our tertiary care center with a three-week history of pedal edema. She also had a two-week prior history of still birth (at 32 weeks of gestation). At the time of admission, she was not on any medication. There was no previous history of transfusion. The patient was hemodynamically stable. The complete blood count revealed hemoglobin: 7.5 g/dL, total leukocyte count:  $16.4 \times 10^3/\mu\text{L}$  and platelet count:  $142 \times 10^9/\text{L}$ . RBC indices were as follows: mean corpuscular volume (MCV) = 119.8 fL, mean corpuscular hemoglobin (MCH) = 97.0 pg, and mean corpuscular hemoglobin concentration (MCHC) = 81.0 g/dL. Peripheral smear revealed normocytic normochromic anemia with occasional nucleated RBCs. The corrected reticulocyte count was 3.4%. However, no spherocytes were evident. Other pertinent investigations were as follows: total bilirubin – indirect fraction = 7.2 mg/dL and direct fraction = 1.2 mg/dL, low complement C3 = 58.5 mg/dL (normal range: 90–180 mg/dL), low complement C4 = 9 mg/dL (normal range: 10–40 mg/dL), elevated lactate dehydrogenase (LDH) = 1226 U/L (normal range: up to 225 U/L), creatinine = 0.7 mg/dL (range: 0.7–1.2 mg/dL) and albumin = 1.2 mg/dL (range: 3.4–5.2 mg/dL). Renal biopsy was performed and the diagnosis was consistent with lupus nephritis – Class V.

We received a request for blood grouping and a direct antiglobulin test (DAT). On visual inspection, the plasma and serum revealed evidence of hemolysis. Blood grouping was B RhD positive. DAT was positive for immunoglobulin G(3+) and C3d(2+) in column agglutination technology (CAT) using monospecific Coombs' reagent (Biorad, Switzerland). RBC antibody screening was positive (3+) in an indirect antiglobulin test (IAT) phase using a commercial 3-cell panel (ID-Diacell I-II-III, Biorad, Switzerland) with a positive auto control. RBC antibody identification was achieved with a commercial 11-cell identification panel (ID DiaPanel, Biorad, Switzerland) in IAT phase. The antibody was identified as anti-C as it reacted (3+ grade of agglutination) with all C-positive cells and showed no reaction with all C-negative cells. Antibodies eluted from the patient's RBCs using an acid elution kit (Diacidel, Biorad, Switzerland) exhibited anti-C specificity. Heat elution (carried out at 56 °C for 10 min) of the patient's RBCs was performed and extended Rh antigen phenotyping of the RBCs was attained using monoclonal antibodies in CAT (Diaclon Rh-Subgroups+K, Biorad, Switzerland). Her Rh antigen phenotyping was C<sup>+</sup>, c<sup>-</sup>, E<sup>-</sup>, e<sup>+</sup>. The presumed Rh haplotype was D<sub>Ce</sub>/D<sub>Ce</sub> (R1R1). Rh genotyping facilities are not available at our center.

Adsorption of the serum was done based on the technique described by Issit et al.<sup>3</sup> An equal volume of patient's serum and papain treated packed RBCs (D<sub>Ce</sub>/D<sub>Ce</sub>, R1R1 and dce/dce, rr RBCs) were mixed and incubated at 37 °C for one hour. After single adsorption, the adsorbed serum was tested for the presence of anti-C using an RBC antibody screening panel. Antibodies were completely adsorbed from the serum with C-negative as well as C-positive cells. The mimicking specificity was confirmed by: (1) antibody reactivity was consistent with anti-C, (2) patient's RBCs were positive for C antigen and (3) adsorbed serum did not retain the anti-C activity. The presence of underlying antibodies was tested based on the dilution technique described by Jang et al.<sup>4</sup> The patient's serum was diluted in normal saline using a 2-fold serial dilution technique. Titration was carried out by CAT using a LISS/Coombs' card; 1+ reaction was achieved at 1:16 dilution. The diluted serum was tested against an 11-cell

identification panel (ID DiaPanel, Biorad, Switzerland). Alloantibodies were not detected. One unit of B RhD<sup>+</sup> C negative Kell negative packed RBCs was crossmatched for this patient and found compatible, although she did not require a transfusion during this admission. Initially, the patient was administered a pulse dose of intravenous methylprednisolone which was later tapered to oral prednisolone. Over a period of one week, the patient's condition improved gradually and she was discharged. The patient at present is being followed-up regularly.

## Discussion

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 negative cells. This concept was first described by Fudenberg as "wrong antibodies"; these are said to be antibodies with mimicking specificity, usually directed against Rh antigens (e, E and c), although their true specificity is mostly anti-Hr or anti-Hr0.<sup>5</sup> However, apparent anti-C in African descendants may be anti-Rh31 or anti-Rh34.<sup>2</sup> Autoantibodies may also mimic the specificities of anti-K, anti-Jka and anti-Kpb.<sup>5</sup> To date, only little is known about the autoantibodies with mimicking specificity in patients with warm AIHA. Their frequency is reported to range from 12% to 27%.<sup>4</sup> However, their exact prevalence in our patient population is not known. To the best of our knowledge, there are no unequivocal reports of autoantibodies with mimicking specificity from the Indian subcontinent.

Multiple mechanisms have been proposed for their occurrence. Autoantibodies with mimicking specificity can be induced by drugs such as alpha-methyl dopa or autoantibodies unfolding to alloantibodies at a later stage.<sup>6</sup> Occasionally, autoantibodies (anti-D, -C and -e) mimic alloantibodies, wherein the patient's RBCs show marked depression of Rh antigen expression during the hemolytic episode. Antibodies can be eluted from RBCs despite a negative DAT.<sup>1,2</sup> The reason for the occurrence of such antibodies in our patient could not be established. In patients with warm autoantibodies, the presence of alloantibodies can be detected by adsorption techniques using ZZAP (a mixture of 0.1 mol/L dithiothreitol plus 0.1% cysteine-activated papain or 0.1% ficin), PEG (Polyethylene glycol) or by diluting patient's serum. The dilution technique of patient's serum is found to be a good alternative to ZZAP or PEG adsorption for detecting underlying alloantibodies in patients with autoantibodies with mimicking specificity because the adsorption techniques are time-consuming and such facilities may not be available in all centers.<sup>4</sup> Antibodies with mimicking specificity have been reported to cause clinically significant hemolysis.<sup>6</sup> In contrast, Jang et al. suggested that these antibodies do not result in hemolytic transfusion reactions when such patients are transfused with "least incompatible units".<sup>4</sup> Our patient had evidence of hemolysis at the time of admission, probably mediated by autoantibodies. Patients having autoantibodies with mimicking specificity in their serum should be transfused with antigen negative units.

To conclude, autoantibodies with mimicking specificity are rarely encountered in the routine practice. They are confirmed by adsorption of patient's serum with antigen-negative RBCs. Dilution of the patient's serum can be used as an alternative to exhaustive techniques such as ZZAP adsorption to identify the underlying alloantibodies in patients with warm autoantibodies especially when the facilities to perform adsorptions are not available. However, it should be borne in mind that only the alloantibodies whose titer is higher than that of the autoantibodies' titer will be detected. Given the clinical implication, greater emphasis should be paid on establishing an accurate rapid diagnosis and transfusing antigen-negative RBC units.

## Conflicts of interest

The authors declare no conflicts of interest.

## REFERENCES

1. Fung MK. Technical manual. 18th ed. Bethesda: American Association of Blood Banks; 2014, 435 pp.
2. Petz LD, Garratty G. Immune hemolytic anemias. 2nd ed. Philadelphia, PA: Churchill Livingstone; 2004. p. 231-42.
3. 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(6):481-6.
4. Jang M-J, Cho D, Park K-U, Yazer MH, Shin M-G, Shin J-H, et al. Autoantibodies with mimicking specificity detected by the dilution technique in patients with warm autoantibodies. *Ann Lab Med*. 2013;33(5):343-8.
5. Issitt PD, Pavone BG. Critical re-examination of the specificity of auto-anti-Rh antibodies in patients with a positive direct antiglobulin test. *Br J Haematol*. 1978;38(1):63-74.
6. Dwyre DM, Clapper A, Heintz M, Elbert C, Strauss RG. A red blood cell autoantibody with mimicking anti-E specificity. *Transfusion*. 2004;44(9):1287-92.

Rajeswari Subramaniyan\*, Mangalakumar Veerasamy  
Kovai Medical Center and Hospital, Coimbatore, India

\* Corresponding author at: Department of Transfusion Medicine, Kovai Medical Center and Hospital, Avinashi Road, Coimbatore 641 014, India.

E-mail address: [arthisoundarya@gmail.com](mailto:arthisoundarya@gmail.com)  
(R. Subramaniyan).

Received 20 August 2016

Accepted 22 November 2016

Available online 23 December 2016

1516-8484/

© 2016 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).  
<http://dx.doi.org/10.1016/j.bjhh.2016.11.002>