CASE REPORT

Anti-S Alloantibody Underlying Pan Agglutinating Autoantibody in a Patient of Systemic Lupus Erythematosus

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INTRODUCTION

Autoimmune haemolytic anaemia (AIHA) is seen in about 13% of Systemic Lupus erythematosus (SLE) cases (1). Autoantibodies make transfusion management of these patients very difficult. Some of these patients have associated underlying alloantibodies which get masked due to pan agglutinating nature of autoantibodies. Furthermore, difficulties are encountered in grouping, cross matching and selection of appropriate blood for transfusion in these patients. Pan agglutinating nature of autoantibodies causes incompatibility with all the donor units selected for transfusion. In such cases, the least incompatible blood may be transfused, if required (2). Presence of underlying alloantibodies, if not detected, will increase the haemolysis of the transfused red cells. Also the titres of alloantibodies increase with subsequent transfusions. Hence, it is imperative that extensive immunohematological workup be done to identify the nature of autoantibodies (if possible) and alloantibodies, including their thermal amplitude, titres and specificities. Here we present a case of underlying anti-S alloantibody along with autoantibody in a case of SLE.

CASE REPORT

A 25 year old female (Gravida 2, Abortion 1) with complaints of easy fatigability, dyspnoea, fever, chills, rigor and vomiting was admitted to our hospital. She was a known case of dimorphic nutritional anaemia and had already received two blood transfusions within last six months. She had no history of oral ulcers, photosensitivity or skin rashes. She had pallor and palpable axillary lymph nodes. Her laboratory tests showed anaemia (31g/l), leucopoenia (2×10^9/l) and thrombocytopenia (19×10^9/l). She was further investigated to find out the cause of pancytopenia. Her reticulocyte

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count was normal (1%) and ESR was markedly raised (140 mm in 1st hr by Westergren method). Serumelectrolytes and total serum bilirubin were normal. Serum iron (50 μg/dl)
and serum transferrin saturation (13.3%) were slightly reduced. Peripheral blood film revealed pancytopenia with normocytic normochromic anaemia. Bone marrow biopsy showed mild erythroid hyperplasia, hence ruling out primary bone marrow causes of pancytopenia, metastasis, malignancy, megaloblastic anaemia, storage diseases, etc.
Radiological investigations revealed mild hepatosplenomegaly, cholelithiasis and minimal pericardial effusion. RA factor, C reactive protein (CRP) and Anti-nuclear antigen were negative. No clinical features, history and laboratory investigations suggestive of chronic infectious etiology were found. FNAC of lymph node showed reactive lymphadenopathy ruling out lymphoproliferative disorders. Gel card test for paroxysmal nocturnal hemoglobinuria (PNH) was negative.
Two units of packed red cells were requested for transfusion. On blood grouping, discrepancy was encountered as forward grouping showed AB Rhm (D) positivity, while reverse blood grouping showed weak (1+) reaction with A, B and O cells at 22°C. However, reaction decreased at 4°C. Auto control (A/C) was also weakly positive (1+) (Table 1).

Table 1. Forward and reverse blood grouping.

	Anti A	Anti B	Anti D1	Anti D2	A/C	A Cell	B Cell	O Cell	Blood Group
22°C	3+	3+	2+	2+	Neg	1+	1+	2+	
4°C	4+	4+	2+	2+	1+	1+	1+	1+	AB +ve
37 °C	4+	4+	2+	2+	1+	+/-	+/-	Weak 1+	

Possibility of autoantibodies was considered due to A/C positivity and pan reactivity in reverse grouping. Dithiothreitol (DTT) treatment of serum as well as diminution of reaction at 4°C ruled out presence of IgM antibodies. Blood group was confirmed to be AB positive.
The sample was referred for immunohaematological workup. Direct antiglobulin test (DAT) was strongly positive (4^+) and DAT profiling using non specific coomb's sera cards showed coating of patient's RBC with IgG as well as C_3d. Possibility of Warm autoimmune haemolytic anaemia (WAIHA) or Mixed AIHA (WAIHA+CAIHA) was considered.
Antibody screening was put up by 3 cells panel which showed pan agglutination. For identification of antibody, a 11-cells panel in LISS/coombs and enzyme phase (by *Diamed microtyping system*) was used. Serum showed pan agglutination reaction of varying strength with all cells of the panel (Figure 1).
Gradation in strength of reaction led to the possibility of underlying alloantibodies. Also in enzyme phase, some cells showed enhancement of reaction whereas some other cells showed diminution of reaction pointing at presence of multiple antibodies. Adsorption studies were not possible due to history of recent transfusions.

FIGURE 1

	Rh-hr		Rh-hr						Kell						Duffy		Kidd		Lewis		P	MNS				Luth		Xg	Result	
			D	C	E	c	e	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	Xg^a	LISS/ Coombs	Enzyme
1	$C^wCD.ee$	$R_1{}^wR_1$	+	+	0	0	+	+	0	+	0	+	0	+	+	0	+	+	0	+	0	+	+	0	+	0	+	+	2 +	1 +
2	CCD.ee	R_1R_1	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	+	0	0	+	0	+	+	+	0	+	0	2 +	2 +
3	ccD.EE	R_2R_2	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	0	0	+	0	0	+	+	0	0	+	+	4 +	4 +
4	Ccddee	r'r	0	+	0	+	+	0	0	+	0	+	0	+	0	+	+	0	0	+	+	+	0	0	+	0	+	+	2 +	3 +
5	ccddEe	r''r	0	0	+	+	+	0	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	0	+	+	2 +	4 +
6	ccddee	rr	0	0	0	+	+	0	+	+	0	+	0	+	+	0	0	+	0	+	+	+	0	+	+	0	+	+	2 +	4 +
7	ccddee	rr	0	0	0	+	+	0	0	+	+	+	0	+	0	+	+	0	0	0	+	+	+	0	+	0	+	0	4 +	4 +
8	ccD.ee	R_0r	+	0	0	+	+	0	0	+	0	+	0	+	0	0	+	0	0	0	+	+	0	+	+	0	+	0	2 +	4 +
9	ccddee	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	+	0	0	0	+	+	+	+	+	+	2 +	3 +
10	ccddee	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	+	0	+	0	+	+	+	0	+	0	0	+	+	4 +	4 +
11	ccddee	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	0	+	0	0	+	+	4 +	4 +
																											AUTO CONTROL	2 +	2 +

Figure 1. Antigram of 11 cells panel showing panagglutination.

FIGURE 2

	Rh-hr		Rh-hr						Kell						Duffy		Kidd		Lewis		P	MNS				Luth		Xg	Result	
			D	C	E	c	e	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	Xg^a	LISS/ Coombs	Enzyme
1	$C^wCD.ee$	$R_1{}^wR_1$	+	+	0	0	+	+	0	+	0	+	0	+	+	0	+	+	0	+	0	+	+	0	+	0	+	+	Neg	Neg
2	CCD.ee	R_1R_1	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	+	0	0	+	0	+	+	+	0	+	0	2 +	Neg
3	ccD.EE	R_2R_2	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	0	0	+	0	0	+	+	0	0	+	+	3+	Neg
4	Ccddee	r'r	0	+	0	+	+	0	0	+	0	+	0	+	0	+	+	0	0	+	+	+	0	0	+	0	+	+	Neg	Neg
5	ccddEe	r''r	0	0	+	+	+	0	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	0	+	+	Neg	2+
6	ccddee	rr	0	0	0	+	+	0	+	+	0	+	0	+	+	0	0	+	0	+	+	+	0	+	+	0	+	+	3+	2+
7	ccddee	rr	0	0	0	+	+	0	0	+	+	+	0	+	0	+	+	0	0	0	+	+	+	0	+	0	+	0	Neg	2+
8	ccD.ee	R_0r	+	0	0	+	+	0	0	+	0	+	0	+	0	0	+	0	0	0	+	+	0	+	+	0	+	0	3+	Neg
9	ccddee	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	+	0	0	0	+	+	+	+	+	+	3+	2+
10	ccddee	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	+	0	+	0	+	+	+	0	+	0	0	+	+	4 +	2+
11	ccddee	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	0	+	0	0	+	+	4 +	Neg
																											AUTO CONTROL	2 +	Neg

Figure 2. Antigram of 11 cells panel used in antibody identification (anti S Antibody).

Cross matching with multiple donor units showed varying degrees of incompatibility (1^+ to 4^+) by IAT and the least incompatible unit was issued with consent of treating physician.
After this, steroid treatment was started. Repeated laboratory investigations showed pancytopenia and increased reticulocyte count (10%) with increase in haemoglobin (80 gm/l) Repeat antibody profiling by 3 cells, 6 cells and 11 cells (Figure 2) panels showed negative auto control and presence of Anti-S alloantibody as autoantibodies masking it had been suppressed by steroids.

Reaction strength in cells with S antigen showed diminution in enzyme phase, hence confirming anti-S antibody. Extended antigenic phenotyping of the patient's red blood cells revealed negativity for S antigen confirming the allo nature of anti-S antibody.
Steroid was substituted by Cyclosporin due to deterioration in patient condition. Indirect Immunoflorescence showed presence of ds-DNA, ANA and Ro positivity. Hence, according to the updated criteria of SLE (3), four out of 11 features i.e. Serositis (pericardial effusion), haematological disorders (haemolytic anaemia, leucopoenia, thrombocytopenia in the absence of offending drug), immunological disorder (anti-ds DNA) and presence of antinuclear antibodies (ANAs in the absence of drugs known to induce ANA's) were present. Patient was hence diagnosed as Systemic lupus erythematosus (SLE) with Warm Autoimmune haemolytic anaemia having autoantibodies with underlying Anti-S alloantibody.

DISCUSSION

SLE is a multisystem autoimmune disease of unknown aetiology in which tissues and cells are damaged by pathogenic autoantibodies and immune complexes. Diagnostic criteria of SLE have been updated by Hochberg et al. (3).
Anaemia is present in about 50% of SLE cases (1) which may be due to anaemia of chronic diseases, Fe deficiency, CRF or haemolytic anaemia. AIHA in SLE is seen in about 13% of cases. Secondary AIHA is associated with autoimmune condition (connective tissue disorders) in about 18.5% of cases (4). AIHA shows varied spectrum of signs and symptoms. Diagnosis of AIHA is based on clinical presentation as well as serological evaluation of autoantibodies. Our patient presented with weakness, dizziness, fever, splenomegaly, hepatomegaly and lymphadenopathy, suggestive of AIHA (5). While most case of AIHA show increased reticulocyte count, 25% of patients with AIHA have normal reticulocyte count (4).
The association of coexisting alloantibodies with autoantibodies in patients with SLE is well established; however, there are very few reports showing presence of Anti-S alloantibody in these patients (6). It is important to detect this alloantibody because it causes HTRs and HDFNs (13).
The pathogenesis of AIHA is based on the development of autoantibodies. Because of A/C positivity and pan reactivity in reverse grouping, presence of autoantibodies was considered in this case. It is imperative to look for concomitant underlying alloantibodies which are masked by pan agglutinating autoantibody. These autoantibodies are mostly of IgG type, some are IgM type. IgG antibodies are

incomplete and are not picked up by normal saline cross matching, but are picked up in Coombs phase (7). Boorman et al. (8) and Mollison et al. (9) applied the antiglobulin technique to detect autoantibodies present on the red cells of patients with acquired haemolytic anaemia. In today's practice also, antiglobulin test via advanced techniques are used for detection of incomplete IgG antibodies. In various reports of WAIHA, incidence of RBC's coated with IgG and C_3d ranges from 24% (10) to 63% (11) while those coated with IgG is 20% (11) to 66% (10) and with only C_3d is 7% (10) to 14% (11). In our case, patient's RBC were coated with IgG and C3d. Possibility of Cold AIHA was least considered due to diminution of reaction at 4°C.

There are several studies and case reports showing the presence of pan agglutinating autoantibodies with underlying alloantibodies in patients of AIHA (12-14). Incidence of alloantibodies in patients with WAIHA who have been transfused varies from 32-75% (13-15). Maley et al studied about 126 cases (12) in which 31% showed the presence of alloantibodies while a study of 71 cases by Sudipta Shekhar Das et al. found it to be 30.4% (13).

The development of red cell alloantibodies depend on many factors such as the frequency of antigen in the population, whether or not it is an effective immunogen, the presence of nonhuman sources of antigen similar to the blood group antigen and the amount of antigen exposure (14). Various auto adsorption, alloadsorption and elution techniques have been described for the detection of underlying alloantibodies. Autoadsorption as well as adsorption with phenotypically matched red cells was not possible in this case as patient had received transfusions before admission.

Most anti-S antibodies are reactive in AHG phase and some at 22°C, 37°C and in saline phase. Effect of proteases on test for detection of S antigen is variable; however papain inhibits its reaction as was seen in this case. Anti-S usually occur following red cell immunisation and is capable of causing haemolytic transfusion reaction (HTRs) and haemolytic disease of foetus and newborn (HDFN) and is clinically significant (14).

Glucocorticoid is initial therapy of choice for WAIHA (1-1.5 mg/kg/day). Steroid treatment causes increase in reticulocyte count as seen in this patient with increase in Hb levels. Transfusion is very problematic in patients with haemolytic anemia. However, if clinical situation warrants, transfusion should not be withheld.

CONCLUSION

This case report reinforces the significance of detection of alloantibodies in the presence of panreactive warm autoantibodies. Adsorption studies (which are the mainstay for immunohaematological work up in these cases) are not possible if patient has received recent blood transfusions. The present case is rare and interesting as gradation in the reaction strength in the Indirect Antiglobulin test pointed to the possibility of underlying alloantibodies. It is a great challenge to provide compatible units to patients who require urgent transfusions in such scenario.

A complete record of the patient should be maintained and corresponding antigen negative component units should be made available for transfusion under strict clinical supervision.

ACKNOWLEDGMENT

The authors declare no conflict of interest.

REFERENCES

1 Nossent JC, Swaak AJ: Prevalence and significance of haematological abnormalities in patients associated with SLE. Q J Med 1991; 80:605-612.
2 Regina M Leger. The positive direct antiglobulin test and immune mediated hemolysis. In: John D Roback. AABB Technical Manual. 16th ed. Bathseda, Maryland: American association of Blood Banks. 2008; 504-15.
3 Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997; 40:1725.
4 Naithani R, Agrawal N, Mahapatra M, Pati H, Kumar R, Choudhary VP. Autoimmune Hemolytic anemia in India: Clinico-hematological spectrum of 79 cases. Hematology. 2006;11:73-6.
5 Pirofsky B. Clinical aspects of autoimmune hemolytic anemia. Semin Hematol. 1976; 13:251–65.
6 Guastafierro S, Sessa F, Cuomo C, Tirelli A. Delayed hemolytic transfusion reaction due to anti-S antibody in patient with anti-Jk(a) autoantibody and multiple alloantibodies. Ann Hematol. 2004; 83:307-8.
7 Coombs RR, Mourant AE, Race RR. In vivo isosensitisation of red cells in babies with haemolytic diseases; Lancet 1946; 1:264-6.
8 Boorman KE, Dodd BE, Loutit JF, Haemolytic icterus (acholric javndice) congenital and acquired.Lancet. 1941;1(6405):812-4.
9 Loutit JF, Mollison PL. Haemolytic icterus (acholric javndice) congenital and acquired. J Pathol Bacteriol. 1946; 58:711-28.
10 Sokol RJ, Hewitt S, Stamps BK. Autoimmune haemolysis: An 18 year study of 865 cases referred to a regional transfusion centre. Br Med J 1981; 282:2023-7.
11 Petz LD, Garratty G. Immune Hemolytic anemias, 2nd ed. Philadelphia: Churchill Livingstone, 2004.
12 Maley M, Bruce DG, Babb RG, Wells AW, Williams M. The incidence of red cell alloantibodies underlying panreactive warm autoantibodies. Immunohematology. 2005; 21(3):122-5.
13 Das SS, Chaudhary R. Utility of adsorption techniques in serological evaluation of warm autoimmune haemolytic anaemia. Blood Transfus. 2009; 7:300-4.
14 Chow MP, Yung CH, Hu HY, Mo LL. Autoantibody complicating multiple alloantibodies in a patient with massive transfusion. J Med Sci. 1989; 9(5):339-44.
15 Walker RH, Lin DT, Hartrick MB. Alloimmunization following blood transfusion. Arch Pathol Lab Med. 1989; 113:254-61.
16 Daniels G. Other Blood groups. In: John D Roback. AABB Technical Manual. 16th ed. Bathseda, Maryland: American association of Blood Banks. 2008; 411-7.