Case Report

Autoanti-C in a patient with primary sclerosing cholangitis and autoimmune hemolytic anemia: a rare presentation

M. Bajpai, A. Maheshwari, S. Gupta, and C. Bihari

Primary sclerosing cholangitis (PSC) is rarely associated with autoimmune hemolytic anemia (AIHA), and the presence of specific autoantibodies has not been reported previously. We present a unique case report of PSC associated with AIHA implicating autoanti-C. A 17-year-old girl was admitted to our hospital with PSC along with AIHA. Her blood sample demonstrated a positive direct antiglobulin test and a positive autocontrol in the antihuman globulin phase, confirming the patient had warm-reactive AIHA. Further testing showed the possibility of anti-C. The patient's Rh phenotype was C+D+E−c−e+. Further testing with select cells, serial alloadsorption, and an elution confirmed anti-C specificity. The patient was transfused with two C−, crossmatch-compatible packed red blood cell units. The patient's hemoglobin level and general condition showed improvement. This unique case report shows PSC associated with AIHA caused by autoanti-C. Usually, warm AIHA presents with a panreactive pattern, and it is difficult to find compatible blood. In this rare case, we could determine the specific antibody; efforts should always be made in cases of AIHA to identify the specificity of autoantibody. ***Immunohematology* 2016;32:104–107.**

Key Words: autoimmune hemolytic anemia, primary sclerosing cholangitis, autoantibody

Autoimmune hemolytic anemia (AIHA) is an uncommon condition, with prevalence of approximately 1.7 per 100,000 individuals.[1] Usually, these antibodies are panreactive, but they may rarely exhibit specific reactivity against red blood cell (RBC) antigens. Diagnosis of AIHA is based mainly on the direct antiglobulin test (DAT). AIHA can be classified as warm type (optimal reactivity at 37°C), cold type (optimal reactivity at 4°C), or both, classified as mixed-type AIHA.[2] On the basis of the presence or absence of underlying etiology, AIHA is further divided into primary (idiopathic) or secondary (secondary to lymphoproliferative disorder, autoimmune diseases, drugs, and non-hematological malignancies).[1] Primary sclerosing cholangitis (PSC) is rarely associated with AIHA, and the presence of a specific autoantibody has not been reported previously.[3,4] We present a unique case report of PSC associated with AIHA caused by autoanti-C.

Case Report

A 17-year-old girl was admitted to our hospital with chief complaints of abdominal pain in the upper-right quadrant, breathlessness, jaundice, fatigue, weakness, and falling hemoglobin for the past 2 weeks. She had similar episodes in the past; the first at the age of 5 years (which resolved within 10–12 days), and again, 16 months later, for which she was treated with steroids and supportive therapy. There was no history of RBC transfusions, a fact that was reconfirmed in view of the long history of the disease. Her family stated that previous episodes had been mild. Family history was not relevant to the current disorder. She had no history of pregnancy or transplantation. On physical examination, she had mild hepatosplenomegaly. Lab tests revealed a hemoglobin (Hb) of 5.8 g/dL (normal range 12–16 g/dL) and a hematocrit of 18.6% (normal range 36–48%). Liver function tests revealed a total bilirubin of 2.3 g/dL (normal range 0.3–1.9 mg/dL) and increased serum alkaline phosphatase, up to 152 IU/L (normal range 44–147 IU/L).

On immunofluorescence, her anti-nuclear antibody test was positive; tests for anti-neutrophilc cytoplasmic antibodies, anti-smooth muscle antibodies, and anti-mitochondrial antibodies were all negative. Serum IgG4 levels were within the normal range. Computed tomography of the abdomen showed diffuse fusiform dilatation and narrowing of the common bile duct with multiple areas of focal fusiform dilatation in intrahepatic biliary radicles, with attenuation in both lobes of the liver. Imaging findings were indicative of PSC. Colonic biopsies were done from multiple sites to rule out ulcerative colitis; on pathological examination, these were largely unremarkable, with no evidence of inflammatory bowel disease.

Materials and Methods

The patient's blood type was group O, D+, her DAT was positive with 4+ reactivity using column agglutination

technique (CAT) with an antihuman globulin (AHG) gel card that is IgG-specific (DiaMed, Cressiers/Morat, Switzerland). Her autocontrol was negative at 4°C, room temperature, and 37°C, but showed 2+ reactivity in the AHG phase on CAT gel card, thus confirming this patient as having warm-reactive AIHA. Further testing was performed to identify the autoantibody.

First, the patient's serum was tested against a three-cell antibody screening panel, which showed reactivity in one cell in the AHG phase, and the autocontrol was positive. An 11-cell identification panel was used that showed the possibility of the antibody being of anti-C specificity (Table 1). The patient's Rh phenotype was performed showing C+D+E–/c–e+. For confirmation, three samples of C+ RBCs and three samples of C– RBCs were tested against the patient's serum (rule of 3). The C– cells were compatible and the C+ cells were incompatible with the patient's serum, confirming the specificity as anti-C. For further confirmation, we performed serial alloadsorptions with C+ RBCs; the adsorbed patient serum showed no agglutination with the panel cells, which included C+ RBCs. Furthermore, before alloadsorption, the C+ allogeneic RBCs were DAT– (control), and after alloadsorption, they became DAT+, thus confirming adsorption of anti-C (Table 2). We performed an acid elution (Gamma ELU-KIT, Immucor Medizinische Diagnostik, Rödermark, Germany) of these alloadsorbed RBCs, and the eluate showed agglutination with C+ cells and no agglutination with C– cells (control). This testing confirmed the presence of autoanti-C of warm type reacting at the AHG phase only. An eluate of the patient's RBCs was made and tested against the identification panel. The results showed a nonspecific pattern: stronger reactivity with all C+ RBCs, but weak reactivity with some C– RBCs.

As the patient's hemoglobin was continuously falling, she was treated with immunosuppressants (azathioprine and steroids); the next day, she was transfused with two C–, crossmatch-compatible units of packed RBCs with no untoward reactions. Rh-phenotype matching was not done, since we have a small inventory, and it would not have been possible. The patient's Hb showed an immediate rise from 5.8 to 9.6 g/dL and then dropped to 8.5 g/dL the next day; her general condition improved. Her Hb was maintained at this

Table 2. Reactivity of patient's serum with RBCs before and after alloadsorption

RBC phenotype	Before alloadsorption	After alloadsorption with R_1R_1 RBCs	After alloadsorption with rr RBCs
R_1R_1 (D+C+E–c–e+)	2+	Negative	2+
R_2R_2 (D+C–E+c+e–)	Negative	Negative	Negative
rr (D–C–E–c+e+)	Negative	Negative	Negative
DAT of alloadsorbed RBCs	NT	2+	Negative

RBC = red blood cell; DAT = direct antiglobulin test; NT = not tested.

Table 1. Reactivity of patient's serum with screening cells and identification panel cells

		Screening cells																		
		Rh-hr					Lewis		MNS				P	Kell		Duffy		Kidd		Results
Cell No.	RhType	D	C	c	E	e	Le^a	Le^b	M	N	S	s	P1	K	k	Fy^a	Fy^b	Jk^a	Jk^b	LISS/AHG
1	R_1wR_1	+	+	0	0	+	+	0	+	0	+	+	+	0	+	0	+	+	+	2+
2	R_2R_2	+	0	+	+	0	0	+	+	+	+	0	+	0	+	+	0	0	+	Neg
3	rr	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	+	0	Neg
		Identification panel cells																		
1	R_1wR_1	+	+	0	0	+	0	+	+	+	0	+	0	0	+	+	0	+	0	2+
2	R_1R_1	+	+	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	+	2+
3	R_2R_2	+	0	+	+	0	0	+	+	0	+	0	+	0	+	+	+	0	+	Neg
4	r′r	0	+	+	0	+	0	+	+	+	+	0	+	0	+	+	0	+	0	2+
5	r″r	0	0	+	+	+	0	+	0	+	0	+	+	0	+	+	0	+	+	Neg
6	rr	0	0	+	0	+	+	0	+	0	+	0	0	+	+	0	+	+	0	Neg
7	rr	0	0	+	0	+	0	0	0	+	0	+	+	0	+	+	+	+	0	Neg
8	R_0r	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	0	+	0	Neg
9	rr	0	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	Neg
10	rr	0	0	+	0	+	+	0	0	+	0	+	+	0	+	+	0	+	0	Neg
11	rr	0	0	+	0	+	0	0	+	0	+	+	0	0	+	+	0	0	+	Neg
Autocontrol																				2+

LISS = low-ionic-strength saline; AHG = antihuman globulin; Neg = negative (no agglutination).

level with minor fluctuations for 1 month. The patient was lost to follow-up beyond that. No more transfusions were required during the rest of her hospital stay.

Discussion

PSC is an uncommon condition characterized by chronic progressive inflammatory fibrosis of the biliary tract, which can lead to biliary cirrhosis, portal hypertension, and liver failure.[5,6] The recommendation for patients younger than 25 years of age with PSC is to perform additional testing for autoimmune hepatitis. The prevalence of autoimmune hepatitis in PSC patients ranges from 1.4 percent to 17 percent.[7] Patients with PSC should also be tested for serum levels of IgG4, since a subset of these patients have markedly high levels of IgG4 (>140 mg/dL).[8] Corticosteroids and other immunosuppressant agents are more effective in patients with IgG4-related PSC than in those with non–IgG4-related PSC. In our reported case, the patient's IgG4 levels were in the normal range, but because of the autoimmune association, she was started on immunosuppressive therapy.

In the literature, a few cases have been reported showing an association between PSC and AIHA, not associated with inflammatory bowel disease, as in our case.[9,10] A study done by Vaglio et al. showed that out of 100 AIHA cases studied, 54 percent were associated with an underlying disorder; of these secondary AIHA cases, autoimmune disorders were most frequent, and the majority (64%) of antibodies detected were of the warm-reactive type.[11] In this case, similar findings were observed, since the autoantibody showed reactivity only at 37°C in the AHG phase.

Detection of the specificity of autoantibody is usually not possible, since most cases show a panreactive pattern with antibody identification cell panels. A few cases of autoantibodies against the Rh blood group system (anti-E, anti-c, anti-D, anti-e) have been reported in the past with a specific autoantibody pattern from cell identification panels.[12] To the best of our knowledge, a confirmed case of autoanti-C has not been reported until now, although it was suspected in two cases in which autoanti-C-mimicking alloantibodies had been reported previously, but phenotyping identified the patients' RBCs as C–.[12,13] Our case is unique because PSC was associated with AIHA caused by autoanti-C.

Blood transfusion support for these patients is a challenging task. Extensive immunohematological workups are required, and even then, the results are often inconclusive. When the antigen panel shows panreactivity and compatible blood is not available, transfusion should not be withheld if the patient is in critical condition and requires an urgent transfusion. Autoadsorption should be done to rule out the presence of alloantibodies in the adsorbed serum; if antibodies are detected, then antigen-negative RBCs may be transfused. In patients who are negative for alloantibodies, phenotype-matched blood may be transfused to prevent the development of alloantibodies.[14] In rare cases, when it is possible to identify the autoantibody, antigen-negative RBCs are the therapy of choice, as illustrated in this case.

Acknowledgments

The authors thank Dr. Pritesh Rajani and Mr. Pankaj Jain for their scientific and technical input in writing this report.

References

1. Eaton WW, Rose NR, Kalaydjian A, Pedersen MG, Mortensen PB. Epidemiology of autoimmune diseases in Denmark. J Autoimmun 2007;29:1–9.
2. Leger RM. The positive direct antiglobulin test and immune mediated hemolysis. In: Fung MK, Grossman BJ, Hillyer CD, Westhoff CM, eds. AABB technical manual. 18th ed. Bethseda, MD: AABB, 2014;439.
3. Moeller DD. Sclerosing cholangitis associated with autoimmune hemolytic anemia and hyperthyroidism. Am J Gastroenterol 1985;80:122–5.
4. Baker SS, Compton CC. A 17-year-old boy with autoimmune hemolytic anemia and abnormal liver function. N Engl J Med 1991;324:180–8.
5. Wiesner RH, LaRusso NF. Clinicopathologic features of the syndrome of primary sclerosing cholangitis. Gastroenterology 1980;79:200–6.
6. Chapman RW, Arborgh BA, Rhodes JM, et al. Primary sclerosing cholangitis: a review of its clinical features, cholangiography, and hepatic histology. Gut 1980;21:870–7.
7. Hirschfield GM, Karlsen TH, Lindor KD, Adams DH. Primary sclerosing cholangitis. Lancet 2013;382:1587–99.
8. Mendes FD, Jorgensen R, Keach J, et al. Elevated serum IgG4 concentration in patients with primary sclerosing cholangitis. Am J Gastroenterol 2006;101:2070–5.

9. Moeller DD. Sclerosing cholangitis associated with autoimmune hemolytic anemia and hyperthyroidism. Am J Gastroenterol 1985;80:122–5.
10. Salagre KD, Sahay RN, Patil A, Joshi A, Shukla A. Leukemoid reaction, a rare manifestation of autoimmune hemolytic anemia in a case of small duct primary sclerosing cholangitis. J Assoc Physicians India 2013;61:494–5.
11. Vaglio S, Arista MC, Perrone MP, et al. Autoimmune hemolytic anemia in childhood: serologic features in 100 cases. Transfusion 2007;47:50–4.
12. 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;38:63–74.
13. Weber J, Caceres VW, Pavone BG, Issitt PD. Allo-anti-C in a patient who had previously made an autoantibody mimicking anti-C. Transfusion 1979;19:216–8.
14. Petz LD. A physician's guide to transfusion in autoimmune haemolytic anaemia. Br J Haematol 2004;124:712–6.

Meenu Bajpai, MD, Additional Professor (corresponding author), Department of Transfusion Medicine, Institute of Liver & Biliary Sciences, Sector D-1, Vasant Kunj, New Delhi 110070, India, meenubajpai@hotmail.com; Ashish Maheshwari, MD, Senior Resident, Department of Transfusion Medicine; Shruti Gupta, MD, Senior Resident, Department of Transfusion Medicine; Chhagan Bihari, MD, Associate Professor, Department of Pathology, Institute of Liver & Biliary Sciences, New Delhi, India.