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Case Report

CASE REPORT

A case of pernicious anemia requiring differential diagnosis of autoimmune hemolytic anemia complication

Saki Todo[1], Kohei Okamoto[2], Takeshi Sugimoto[1,*], Toshimasa Takahashi[3], Yasushi Nakagawa[3], Takashi Arai[3], Katsuhito Nishiyama[4], Kenta Hara[3], Yoshiro Yasutomo[4] and Koichi Yokono[4]

[1]Department of Hematology and Oncology, Kita-Harima Medical Center, Hyogo, Japan, [2]Department of Nephrology, Kita-Harima Medical Center, Hyogo, Japan, [3]Department of Diabetes and Endocrinology, Kita-Harima Medical Center, Hyogo, Japan, and [4]Department of General and Geriatric Internal Medicine, Kita-Harima Medical Center, Hyogo, Japan

*Correspondence address. Department of Hematology and Oncology, Kita-Harima Medical Center, 926-250 Ichiba-cho, Ono city 675-1392, Hyogo, Japan. Tel: +81-794-88-8800; Fax: +81-794-62-9931; E-mail: takeshi_sugimoto@kitahari-mc.jp

Abstract

An 80-year-old female was admitted to our hospital due to malaise. The initial diagnosis on admission was pernicious anemia (PA), Hashimoto thyroiditis and autoimmune atrophic gastritis. Autoimmune hemolytic anemia was suspected because direct antiglobulin test (DAT) was positive. Treatment with vitamin B12 improved anemia, with the disappearance of hemolysis. In some cases, PA patients with positive DAT may have hemolysis without the involvement of the autoimmune mechanism. Therefore, it is important to carefully assess PA patients with hemolysis and positive DAT for the prevention of unnecessary administration of steroid therapy.

INTRODUCTION

Pernicious anemia (PA) is a megaloblastic anemia caused by the deficiency of vitamin B12 (cobalamin). Vitamin B12 (Vit.B12) is a crucial vitamin for DNA synthesis. Vit.B12 is absorbed in the terminal ileum as a complex with intrinsic factor, secreted by parietal cells of the stomach. In PA, intrinsic factor antibodies inhibit the absorption of Vit.B12 in the ileum by obstructing intrinsic factor binding to Vit.B12. Additionally, parietal cell antibodies, linked to atrophic gastritis, are present in PA. Parietal cell antibodies destroy parietal cells, subsequently resulting in the loss of intrinsic factor produced by parietal cells. Autoimmune atrophic gastritis is caused by this autoimmune reaction by parietal cell antibodies. In some cases, PA is associated with other autoimmune diseases such as Hashimoto thyroiditis, primary biliary cirrhosis or autoimmune hepatitis. Moreover, PA may be associated with the production of red blood cell (RBC) antibodies [1], leading to autoimmune hemolytic anemia (AIHA). Previous studies have reported PA with a positive direct antiglobulin test (DAT). However, a differential diagnosis of AIHA with positive DAT, in which autoimmune hemolysis has not occurred, is difficult in the early

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phases of disease. In this article, a case of PA requiring differential diagnosis of AIHA complication is presented.

CASE PRESENTATION

An 80-year-old female was admitted to the outpatient clinic in our hospital. The patient's chief complaint was malaise lasting ~1 month prior to hospital admission. The patient had a medical history of Hashimoto thyroiditis from the age of 62 years, and osteoporosis was detected at the age of 67 years. No appreciable disease was reported in the patient's familial medical history. The patient did not show an allergic tendency to drugs, nor medical preference about smoking and alcohol drinking. The patient was prescribed with levothyroxine (100 μg/day) and risedronate sodium (2.5 mg/day).

Physical examination revealed normal vital signs, and height of 158 cm and weight of 52.5 kg. The patient presented with generalized pallor including anemic conjunctiva, jaundice on the face and bulbar conjunctiva, slightly swollen thyroid gland, jugular venous distention and leg edema. Blood count test revealed the following: hemoglobin (Hb) 3.7 g/dl; mean corpuscular volume (MCV) 125.5 fL; platelet count 94 × 10^9/μl; and macrocytic anemia. Serological test revealed the following abnormalities: lactate dehydrogenase (LD) 3612 IU/l (normal range 119–229) with a high percentage of LD1 and LD2 isozyme; indirect bilirubin (I-Bil) 2.48 mg/dl; and haptoglobin (Hpt) < 10 mg/dl, indicating hemolysis. Vit.B12 was below the detection sensitivity level. Immunological test revealed the elevation of thyroglobulin antibodies (Tg Ab) and thyroid peroxidase antibodies (TPO Ab) to >4000 mg/dl and 397 IU/ml, respectively. Gastric parietal cell antibodies and intrinsic factor antibodies were positive. DAT (direct Coombs test) was positive [Table 1]. Monospecific DAT test with anti-IgG was positive (1+), and same test with anti-C3d was negative. Indirect antiglobulin test (IAT) was negative. Chest X-ray revealed 60% of cardiothoracic ratio and bilateral pleural effusions. Examination with upper gastrointestinal endoscopy revealed atrophic gastritis. Bone marrow aspiration testing identified hypercellular bone marrow and abnormal erythroblast carrying megaloblastic change and abnormality of nuclear division [Fig. 1A and B]. Based on the presence of Vit.B12 deficiency, and positivity of gastric parietal cell antibodies and intrinsic factor antibodies this macrocytic anemia case was diagnosed as PA [2, 3]. This case fulfills the domestic (Japanese) diagnostic criteria for hemolytic anemia, i.e. anemia, jaundice, decrease in Hpt level, increase in indirect bilirubin and urinary urobilinogen levels, and increased erythroblast (immature RBC) count in the bone marrow [4]. In addition, this DAT positive case has the possibility of complicating AIHA by domestic criteria [4]. The initial diagnosis of this patient included PA, Hashimoto thyroiditis, autoimmune atrophic gastritis and suspected AIHA at the time of admission to the hospital.

Figure 2 indicates the clinical course of this case. Shortly after admission (Day 1), the patient received 2 units of RBC. Muscle injection of 1 mg of Vit.B12 (methylcobalamin) was initiated thrice weekly for the first 3 weeks, followed by once weekly up to 2 months from the time of admission. The cause of hemolytic anemia was unclear, and therefore, immunosuppressive therapy using steroids was not administered. Hb levels were monitored and the time of steroid treatment initiation, in case of ineffective Vit.B12 therapy, was recorded. Sodium ferrous citrate (100 mg/day) was orally administered from Days 7 to 28 to prevent iron insufficiency. This intervention resulted in a gradual improvement of Hb levels. Bone marrow reexamination, performed on Day 22, indicated the disappearance of megaloblastic change in erythroblast [Fig. 1C]. The patient was discharged and followed the outpatient clinic after treatment Day 23. The hemolytic signs including the decrease in Hpt level or the abnormality of MCV had disappeared. DAT was negative on treatment Day 7, and additional DAT test on Days 11 and 25 remained negative. Therefore, it was concluded that this hemolytic anemia was not the result of an autoimmune hemolytic

Table 1: Laboratory data on admission

White blood cell	56	×10^2/μl	Total bilirubin	2.60	mg/dl	Thyroglobulin Ab	>4000	IU/ml
Neutrophil	73.5	%	Direct bilirubin	0.12	mg/dl	TPO Ab	397	IU/ml
Monocyte	3.7	%	BUN	14.9	mg/dl	Helicobacter pylori Ab	<3	U/ml
Lymphocyte	21.6	%	Creatinine	0.77	mg/dl	Gastric parietal cell Ab	×40	Titer
Eosinophil	1.2	%	Na	139.7	mEq/l	Intrinsic factor Ab	(–)	
Basophil	0.0	%	K	4.21	mEq/l	Anti-nuclear antibodies	×40	Titer
Red blood cell	90	×10^4/μl	Cl	107	mEq/l	PAIgG	121	ng/10^7 cells
Hemoglobin	3.6	g/dl	CRP	0.05	mg/dl			
MCV	125.6	fL	Vitamin B12	<50	pg/ml	Direct anti-globulin test	(1+)	
MCH	40.0	pg	Folic acid	10.1	ng/ml	Anti IgG	(1+)	
MCHC	31.9	%	Gastrin	2900	pg/ml	Anti C3d	(–)	
Platelet	9.4	× 10^4/μl	Hpt	<10	mg/dl	Indirect anti-globulin test	(–)	
Reticulocyte	3.1	× 10^4/μl				Cold agglutination test	×8	Titer
						ADAMTS-13 activity	52	%
AST	97	IU/l	IgG	1802	mg/dl	HS-PNH cells	(–)	
ALT	62	IU/l	IgA	128	mg/dl			
LD	3612	IU/l	IgM	162	mg/dl			
LD1	51	%	C3	45	mg/dl			
LD2	37	%	C4	14	mg/dl			
LD3	10	%	CH50	29	U/ml			
LD4	1	%						
LD5	1	%						

Reti, Reticulocyte; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; CRP, C-reactive protein; Ab, Antibodies; TPO, thyroid peroxidase; PAIgG, platelet associated immunoglobulin G; ADAMTS-13, a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13; HS-PNH cells, high sensitivity paroxymal nocturnal hemoglobinuria type cells.

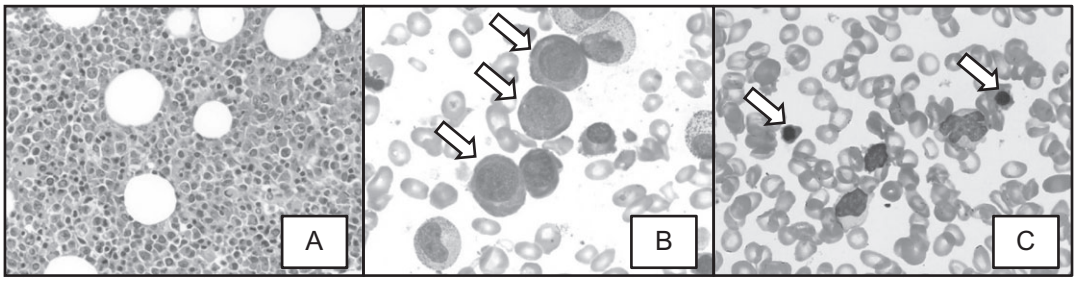

Figure 1: Bone marrow aspiration test at admission. (**A**) (×40) Hypercellular marrow. (**B**) (×400) Erythroblast revealed megaloblastic change and abnormality of nuclear division (arrow). (**C**) Bone marrow aspiration test 3 weeks after initiating Vit.B12 administration. Erythroblast decreased and attained its normal size (arrow).

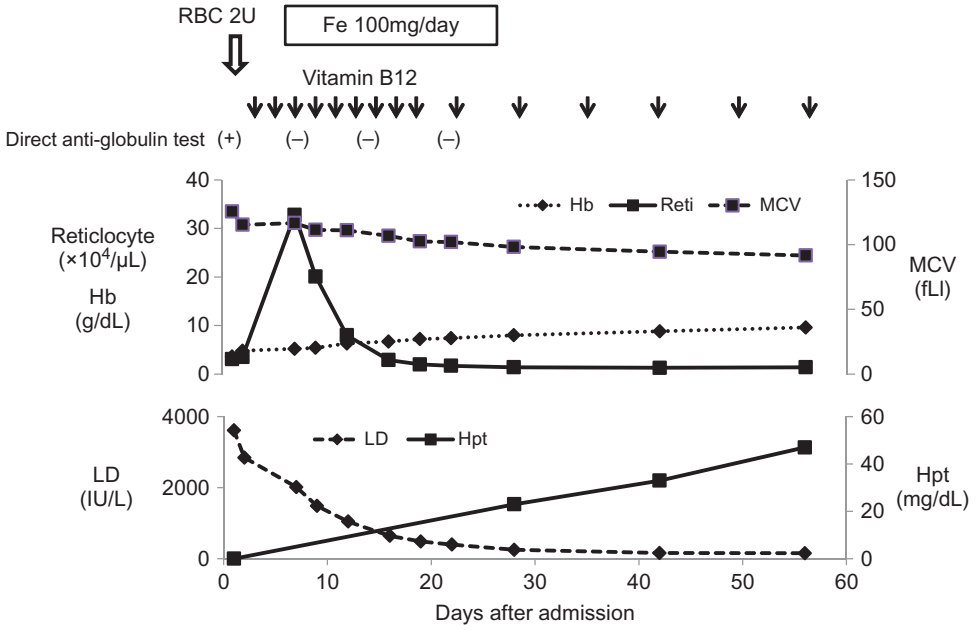

Figure 2: Clinical course.

condition, but rather caused by PA. Five months after starting therapy, the Hb level increased to 10.5 mg/dl.

DISCUSSION

Autoimmune polyglandular syndrome (APS) is characterized by numerous features of autoimmune diseases. APS is defined as sequential or simultaneous deficiencies in the function of several endocrine organs, and it is classified into three types based on the combination of endocrine deficiency [5, 6]. APS Type III is characterized by autoimmune thyroiditis and other types of autoimmune disease without Addison's disease. This patient was classified as having APS Type IIIb, in which Hashimoto thyroiditis, autoimmune atrophic gastritis, and PA are combined. Complication of AIHA usually observed in APS IIIc was also suspected in the initial stage of the clinical course due to positive DAT.

PA with positive DAT status is associated with the appearance of hemolysis [1, 7–9]. The autoantibody produced in PA patients may usually have non-specific reaction with erythrocyte. However, in some cases, autoantibody affects hemolytic reaction to erythrocyte [7, 8]; in which anemia will be persist with Vit.B12 treatment itself. PA has the status of ineffective erythropoiesis, where most of erythroblasts are dying in the intramedullary area by apoptosis, and release LDH [10]. The 'hemolysis' in PA is not the same status as that of AIHA; where hemolysis occurs on intra-vascular or extra-vascular area. Because the feature or laboratory data is similar between PA and AIHA, we express the hemolytic feature of PA as 'hemolysis' in this article. In this connection, serum LD level is known to be elevated generally [11], and one study showed LD was elevated in 80.4% of PA cases [12]. In this condition, it is clinically important to determine the involvement of AIHA in the occurrence of hemolysis. Previous reports were divided into the following three groups: (a) positive DAT status changed to negative after Vit.B12 therapy with improving anemia, (b) positive DAT status changed to negative after Vit.B12 and steroid therapy with improving anemia, (c) positive DAT status was maintained after Vit.B12 and steroid therapy with improving anemia. PA cases with positive DAT status did not receive stereotyped therapy and DAT status after treatment remains positive in some cases, regardless of anemia improvement. Our presenting case is suited to group (a), and similar two cases [1] and another single case [9] are included in this group. Yeruva *et al.* reviewed 14 middle-aged or elderly female PA patients with AIHA complication, involving other autoimmune diseases

such as systemic lupus erythematosus (SLE) [13]. According to the 2012, Systemic Lupus International Collaborating Clinics (SLICC) classification criteria, positive DAT status is an independent factor in the judgment of immunological status irrespective of the existence of hemolytic anemia [14]. In above criteria, SLE patients with positive DAT status do not develop autoimmune hemolytic condition. Because most PA patients have hemolysis owing to ineffective erythropoiesis, PA patients complicated with SLE may have non-autoimmune-mediated hemolysis. Hence, it may be difficult to determine the cause of hemolysis (autoimmune or non-autoimmune mechanism) in PA patients complicated with SLE.

Rabinowitz *et al.* classified these PA patients with positive DAT status into 2 groups: (a) hemolysis by autoimmune-mediated reaction and (b) hemolysis with non-autoimmune-mediated reaction [15]. The former group shows continuous positive DAT status, whereas the latter group shows inverted negative DAT status after PA treatment. It is speculated that the case discussed in this article can be classified in the latter group due to the change of DAT status from positive to negative with the improvement of anemia by Vit.B12 treatment only. In this case, anemia is mainly caused by the malabsorption of Vit.B12 as a result of the presence of intrinsic and parietal cell antibodies without the complication of AIHA. The deficiency of Vit.B12 levels leads to the inhibition of DNA synthesis, which consequently results in immature and ineffective erythropoiesis. The positive DAT status in this case may be derived from non-specific autoantibody, because it turned negative after Vit.B12 treatment. The mechanism of producing non-specific autoantibody is not clear. It is known that autoantibody classified as IgG is more bound on older erythrocyte than younger erythrocyte [16]. A hypothesis is that the decrease of producing newly erythrocyte by ineffective erythropoiesis on PA patients leads to the increase of older erythrocyte; the erythrocyte will increase the number of binding IgG antibody as a consequence. To conclude, PA patients with positive DAT status may have hemolysis without the involvement of the autoimmune mechanism. Therefore, it is important to carefully assess PA patients with hemolysis and positive DAT status for the prevention of unnecessary administration of steroid therapy.

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CONFLICT OF INTEREST STATEMENT

None declared.

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ETHICAL APPROVAL

No ethical approval required.

CONSENT

Informed consent has been taken from the patient herself.

GUARANTOR

Dr Takeshi Sugimoto.

REFERENCES

1. Pirofsky B, Vaughn M. Addisonian pernicious anemia with positive antiglobulin tests. A multiple autoimmune disease syndrome. *Am J Clin Pathol* 1968;**50**:459–66.
2. Bizzaro N, Antico A. Diagnosis and classification of pernicious anemia. *Autoimmun Rev* 2014;**4–5**:565–8.
3. Annibale B, Lahner E, Fave GD. Diagnosis and management of pernicious anemia. *Curr Gastroenterol Rep* 2011;**13**:518–24.
4. Kamesaki T. Diagnosis and treatment of hemolytic anemia. *Rinsho Ketsueki* 2015;**56**:1894–902. (in Japanese).
5. Betterle C, Dal Pra C, Mantero F, Zanchetta R. Autoimmune adrenal insufficiency and autoimmune polyendocrine syndromes: autoantibodies, autoantigens, and their applicability in diagnosis and disease prediction. *Endocr Rev* 2002;**23**:327–64.
6. Betterle C, Zanchetta R. Update on autoimmune polyendocrine syndromes (APS). *Acta Biomed* 2003;**74**:9–33.
7. Zafad S, Madani A, Harif M, Quessar A, Benchekroun S. Pernicious anemia associated with autoimmune hemolytic anemia and alopecia areata. *Pediatr Blood Cancer* 2007;**49**:1017–8.
8. Vucelić V, Stancić V, Ledinsky M, Getaldić B, Sović D, Dodig J, *et al.* Combined megaloblastic and immunohemolytic anemia associated—a case report. *Acta Clin Croat* 2008;**47**:239–43.
9. Fujii T, Fujinami S, Tagawa Y, Kadota Y, Nakatani S, Takeuchi Y, *et al.* Pernicious anemia associated with autoimmune hemolytic anemia. *Japanese J Transfus Med* 1992;**38**:573–6. (in Japanese).
10. Stabler SP. Vitamin B_{12} deficiency. *N Engl J Med* 2013;**368**:2041–2.
11. Green R. Vitamin B_{12} deficiency from the perspective of a practicing hematologist. *Blood* 2017;**129**:2603–11.
12. Song IC, Lee HJ, Kim HJ, Bae SB, Lee KT, Yang YJ, *et al.* A multicenter retrospective analysis of the clinical features of pernicious anemia in a Korean population. *J Korean Med Sci* 2013;**28**:200–4.
13. Yeruva SL, Manchandani RP, Oneal P. Pernicious Anemia with Autoimmune Hemolytic Anemia: A Case Report and Literature Review. *Case Rep Hematol* 2016;**2016**:7231503.
14. Petri M, Orbai AM, Alarcón GS, Gordon C, Merrill JT, Fortin PR, *et al.* Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum* 2012;**64**:2677–86.
15. Rabinowitz AP, Sacks Y, Carmel R. Autoimmune cytopenias in pernicious anemia: a report of four cases and review of the literature. *Eur J Haematol* 1990;**44**:18–23.
16. Kay MM. Generation of senescent cell antigen on old cells initiates IgG binding to a neoantigen. *Cell Mol Biol* 1993;**39**:131–53.