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【 CASE REPORT 】

IgM-mediated Warm Autoimmune Hemolytic Anemia: An Autopsy Report

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Abstract:
A 79-year-old man with Sjögren's syndrome and systemic lupus erythematosus developed acute impaired consciousness and hemolytic anemia. The patient's red blood cells agglutinated spontaneously at 25-37℃. The treatment of red blood cells with 2-mercaptoethanol resulted in the loss of spontaneous agglutination. A diagnosis of IgM-mediated warm autoimmune hemolytic anemia was made. The patient received steroid pulse and plasma exchange therapies. Rituximab was also administered. However, the patient died from multiple organ failure at six days from the symptom onset. The clinical progress of the patient and autopsy findings suggested that complement activation might have been associated with the pathology.

Key words: autoimmune hemolytic anemia, warm antibody, immunoglobulin M

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Introduction

Autoimmune hemolytic anemia (AIHA) is a disease that occurs when anti-red blood cell autoantibodies (anti-RBC autoantibodies) destroy one's own RBCs by attacking molecules expressed on their surface. AIHA is classified into two types: warm AIHA and cold AIHA. In warm AIHA, the optimal binding temperature of anti-RBC autoantibodies to RBCs is approximately the same as the body temperature, and in cold AIHA, it is approximately 4℃. In Japan, the estimated prevalence of AIHA is 3-10 cases per 1,000,000 person-years, and the incidence is 1-5 per 1,000,000 person-years. Approximately 90% of patients present with warm AIHA (1). Autoantibodies associated with warm AIHA are generally from the polyclonal IgG class, and the prognosis is relatively favorable when intervention is performed (2).

We herein report the autopsy findings of a patient with Sjögren's syndrome and systemic lupus erythematosus (SLE) who suddenly presented with impaired consciousness and AIHA caused by warm reactive IgM antibodies.

Case Report

A 79-year-old man was urgently admitted to our hospital with general malaise, diarrhea, and abdominal pain. He had been observed for Sjögren's syndrome and interstitial pneumonia for the past five years but had not received any particular treatment. He had smoked 15 cigarettes a day for 45 years. On admission, the following information was obtained: height, 159.4 cm; weight, 57.1 kg; Glasgow coma scale (GCS) score, E4V5M6; body temperature, 36.3℃; blood pressure, 114/63 mmHg; respiration rate, 78 bpm; and oxygen saturation, 96% (room air). He was diagnosed with mild ischemic enteritis and underwent conservative treatment, and his symptoms improved after several days.

On day 10 of admission, the patient developed a sudden fever (38.7℃), low blood pressure (80/49 mmHg), and impaired consciousness (GCS score, E3V1M3). A head computed tomography (CT) scan did not show any abnormalities. The patient's hemoglobin level on admission was 11.1 g/dL. However, the laboratory findings on day 10 were consistent with hemolytic anemia (hemoglobin level: 5.0 g/dL; total bilirubin level, 4.5 mg/dL; direct bilirubin level, 0.8

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Table 1. Laboratory Findings at the Onset (Day 10).

[CBC]		[Chemistry]		[Serology]	
WBC	196 $\times10^2$/μL	CRP	5.91 mg/dL	IgG	4,790 mg/dL
Neut.	50 %	TP	9.1 g/dL	IgA	301 mg/dL
Lymph.	4.5 %	Alb	2.7 g/dL	IgM	453 mg/dL
Mono.	11.5 %	CK	144 U/L	KL-6	1,295 U/mL
Eos.	2.5 %	LDH	761 U/L	ANA	×1,280
Bas.	0.0 %	T-Bil	4.8 mg/dL	anti-SS-A	>1,200 U/mL
RBC	152 $\times10^4$/μL	D-Bil	0.8 mg/dL	anti-SS-B	2.6 U/mL
Hb	5.0 g/dL	AST	145 U/L	C3	33 mg/dL
Ht	13.2 %	ALT	59 U/L	C4	3 mg/dL
PLT	27.2 $\times10^4$/μL	ALP	251 U/L	C1q	1.6 μg/mL
Ret	108 ‰	γ-GTP	27 U/L	anti-Scl-70 Ab	<1.0 U/mL
spontaneous agglutination 4+		BUN	17.9 mg/dL	anti-centromere Ab	<5.0
		Cre	1.35 mg/dL	anti-ARS Ab	<5.0
[Coagulation]		Na	121 mEq/L	anti-Sm Ab	<1.0 U/mL
PT(sec)	18.5 sec	K	4.3 mEq/L	anti-RNP Ab	18.2 U/mL
PT(%)	44 %	Cl	94 mEq/L	anti-dsDNA Ab	104 IU/mL
PT-INR	1.56	Ca	8.1 mg/dL	anti-CLβ2GPI Ab	1.5 U/mL
APTT	44.6 sec	NH_3	91 μg/dL	MPO-ANCA	1.0 U/mL
D-dimer	44.1 μg/mL	ferritin	34,380 ng/mL	PR3-ANCA	1.0 U/mL
		haptoglobin	<10 mg/dL	anti-cardiolipin Ab	9 U/mL
				anti-CCP Ab	<0.6 U/mL

ANA: anti-nuclear antibody, ANCA: anti-neutrophil cytoplasmic antibody, ARS: aminoacyl tRNA synthetase, CCP: cyclic citrullinated peptide, CLβ2 GPI: cardiolipin β2-glycoprotein I complex, dsDNA: double-stranded DNA, MPO: myeloperoxidase, PLT: platelet, PR3: proteinase 3, RBC: red blood cell, Ret: reticulocyte, WBC: white blood cell

Table 2a. Direct Anti-globulin Test Results.

	AHG	IgG	C3d	CTL
Untreated	4+	4+	4+	4+
2ME-treated	3+	2+	2+	0

2ME-treated: Red blood cells were treated with 2-mercaptoethanol (2-ME) at 37 °C for 30 min before the anti-globulin test.

Table 2b. Agglutinin Titers in Thermal Amplitude Tests against Group O RBCs.

Thermal amplitude	Agglutinaion
37°C	4+
25°C	2+
4°C	1+
25°C+2ME	0

The patient's serum was incubated with group O RBCs for 15 min at 4 °C, 25 °C, and 37 °C.
+2ME: pretreatment of serum with 2-mercaptoethanol.

mg/dL; and lactate dehydrogenase level, 761 U/L) (Table 1). High IgG levels and hypocomplementemia had been observed in the patient several years before the latest admission. However, an increase in IgM levels was observed on admission.

Following the onset of hemolytic anemia, spontaneous agglutination of RBCs at 25-37℃ was observed but was reversed at 4℃. After being washed with saline at 37℃, the RBCs were still agglutinated. The treatment of RBCs with 2-mercaptoethanol (2ME) resulted in the loss of agglutination. A direct antiglobulin test was performed after the treatment of RBCs with 2ME, after which weak reactions with anti-IgG and anti-C3d were observed (Table 2a). In addition, when the patient's serum was incubated with group O RBCs for 15 minutes at 4℃, 20℃, and 37℃, hemagglutination occurred even in saline, and the reaction was strongest at 37℃. The hemagglutination was abolished by 2ME treatment of the serum (Table 2b), suggesting that the responsible autoantibody was that to IgM, which is optimally reactive at 25-37℃.

The patient received RBC transfusion combined with steroid pulse therapy (methyl-prednisolone at 1 g/day for 3 days) on days 10-12 and simple plasma exchange therapy [fresh-frozen plasma (FFP) 36 units, 80 mL/kg] on days 11, 12, and 15, and he underwent continuous hemodiafiltration on days 12-15. In addition, rituximab (583 mg, 375 mg/m^2) was administered on day 12 (Figure). The level of consciousness improved slightly (GCS score, E3V1M6) after anemia was resolved and the blood pressure rose. After each of three plasma exchanges, intravascular hemolysis was enhanced. The patient died of acute respiratory failure and cardiac arrest on day 16.

After his death, we obtained consent for an autopsy from

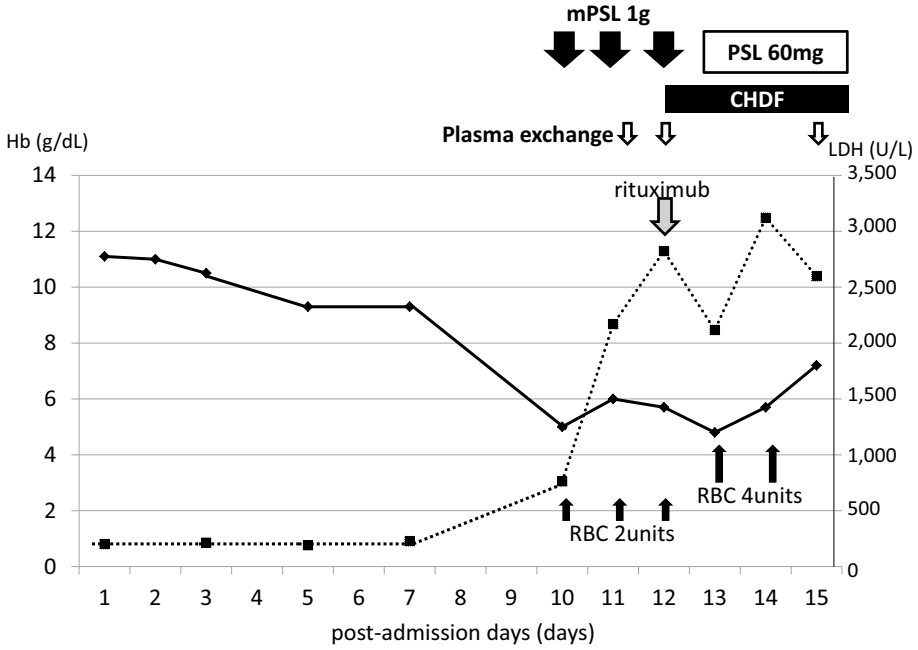

Figure. **Clinical course. CHDF: continuous hemodiafiltration, Hb: hemoglobin, LDH: lactate dehydrogenase, mPSL: methylprednisolone, PSL: prednisolone, RBC: red blood cell transfusion**

his family, and a pathological autopsy was performed. The autopsy showed hyperplastic bone marrow due to hemolytic anemia, necrosis in the central region of the hepatic lobules, cholestasis, multiple bile plugs, and an enlarged spleen. There were multiple enlarged lymph nodes in the periaortic and tracheal regions and hilum of both lungs, the kidneys, and the region surrounding the spleen. In addition, extremely fibrous intimal thickening of the pulmonary arterioles, suggesting secondary pulmonary hypertension, and mesangial proliferative glomerulonephritis as lupus nephritis class IV-G (A) were observed; these findings indicated SLE. Sclerotic interstitial nephritis was observed in the renal cortex, which was attributed to Sjögren's syndrome. The cause of death was diagnosed as respiratory failure due to pulmonary hypertension caused by SLE and hepatic failure due to severe hemolytic anemia.

Discussion

A patient with Sjögren's syndrome and SLE developed warm AIHA. Approximately 90% of warm AIHA cases are associated with IgG, and IgM-mediated warm AIHA is an extremely rare disease (3). The patient showed spontaneous agglutination of RBCs, which occurred at 25-37℃ and was abolished with 2ME. In addition, warm agglutinins against untreated RBCs were present in the serum, and this hemagglutination was also treated with 2ME. These findings indicated that IgM autoantibodies play a crucial role in the development of AIHA in this patient. On the other hand, a direct antiglobulin test on the patient's RBCs was performed after treatment with 2ME because of the spontaneous agglutination, and weak reactions with anti-IgG and anti-C3d were observed. This suggests that IgG autoantibodies were also present. Arndt et al. reported that, in 49 cases of IgM-warm AIHA, 94% of serum samples had warm autoagglutinins, and 78% of cases showed spontaneous agglutination (3). Furthermore, routine direct antiglobulin tests detected RBC-bound C3 and IgG in 90% and 24% of cases in their study, respectively. Patients who develop IgM-mediated warm AIHA commonly have an underlying disease that causes several immune abnormalities, and some of the underlying diseases associated with IgM-mediated warm AIHA are Sjögren's syndrome (4), severe combined immunodeficiency (5), idiopathic thrombocytopenic purpura (6), and eosinophilic granulomatosis with polyangiitis (7).

For the treatment of IgG-mediated warm AIHA, corticosteroids are generally used, with a success rate of 70-85%, and with some interventions, such as steroid therapy, the 1-year mortality rate ranges from 8.5% to 9.1%, which is relatively favorable (8, 9). In contrast, a therapy for IgM-mediated warm AIHA has not been established, and standard therapies for IgG-mediated warm AIHA are commonly unsuccessful (9, 10). In previous case reports, treatment with steroids and/or transfusion was mainly performed in patients with IgM-mediated warm AIHA. However, in several cases, high-dose intravenous immunoglobulin and plasma exchange therapies were provided to the patients, or rituximab was administered (7, 11). Recently, a randomized and double-blind controlled trial evaluating the safety and efficacy of rituximab for warm AIHA in adults (the RAIHA study) showed that the administration of rituximab combined with steroids for newly developed warm AIHA in adults significantly improved the complete remission and mortality rates compared to placebo (12). However, based on the participants' clinical progress, the AIHA in that study was mediated by IgG antibodies. In our patient, rituximab was administered, and

plasma exchange therapy was provided in addition to steroid pulse treatment. However, the treatment did not improve the disease condition, and the patient died six days after the acute exacerbation of hemolytic anemia.

During the autopsy of the patient, imaging findings of numerous bile plugs in the liver, splenomegaly, and bone marrow hyperplasia were observed, and these findings were consistent with severe AIHA. Cold agglutinin disease (CAD) is characterized by the IgM-mediated agglutination of RBCs, which was noted in our patient as well. IgM autoantibodies in CAD react at 4℃ and then cause Raynaud's phenomenon, acrocyanosis, and thrombosis in the microcirculation when subjected to cold stimulation. In contrast, although IgM autoantibodies reacted at 37℃ in the present patient, no ischemic changes due to thrombi were observed in the major organs, including the liver, kidney, and intestinal tract on the autopsy. Therefore, there is likely a pathological cause for the severe outcome in the present case, aside from thrombosis.

IgM antibodies are present in the form of flat pentametric and hexametric structures in the blood and cause stronger complement activation than other autoantibodies. Since C3, which is generated by complement activation, functions as an opsonin that causes extravascular hemolysis, reduced C3 levels are used as a predictive index for hemolysis (13). If there is strong complement activation, the formation of C5 convertase and the insertion of the membrane attack complex into the RBC membrane occurs, causing intravascular hemolysis. In addition, eculizumab, which is an antibody against C5, has been reported to be effective for treating refractory IgM-mediated warm AIHA (8, 14). Our patient had severe hypocomplementemia on admission, and his intravascular hemolysis exacerbated after plasma exchange with normal FFP. The fact that the complement, which had been consumed in the patient's serum, was replenished with FFP and reacted with IgM antibodies might have enhanced the intravascular hemolytic reaction. The clinical progress of the patient and autopsy findings suggest that complement activation may be associated with the pathology. Simple plasma exchange or double-filtration plasmapheresis with a solution of albumin rather than FFP, in which the complement is not replenished, may be appropriate for the treatment of IgM-warm AIHA. This merits further investigation.

Conclusion

We encountered a 79-year-old man with Sjögren's syndrome and SLE who developed IgM-mediated warm AIHA, which is an extremely rare disease with severe outcomes. Further studies must be conducted in order to validate the pathology and determine the optimal treatments for this disease.

The authors state that they have no Conflict of Interest (COI).

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