Cold Agglutinin-Mediated Autoimmune Hemolytic Anemia in Association with Antiphospholipid Syndrome

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Keywords

Cold agglutinin · Hemolytic anemia · Antiphospholipid syndrome · Complement activation

Abstract

Antiphospholipid syndrome and cold agglutinin-mediated autoimmune hemolytic anemia are 2 distinct immune-mediated hematologic disorders. While no clear association exists between these 2 entities, complement activation is known to occur in both of them. Herein, we report a unique case of cold agglutinin hemolytic anemia in a patient with a known primary antiphospholipid syndrome.

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Published by S. Karger AG, Basel

Introduction

Antiphospholipid syndrome (APLS) is an acquired antibody-mediated hypercoagulability state characterized by recurrent vascular thrombotic events and/or obstetric morbidity, in the presence of antiphospholipid antibodies (aPL) [1]. APLS can occur either primarily or in the context of other autoimmune diseases. In systemic

lupus erythematosus (SLE), the prevalence of aPL can reach 30% [2].

Autoimmune hemolytic anemias (AIHA) are a group of rare disorders in which hemolysis occurs due to auto-antibodies directed against red blood cell surface antigens [3]. AIHA can be classified according to the thermal characteristics of the causative antibody into warm and cold types. The majority of patients with AIHA suffer from immunoglobulin G (IgG)-mediated warm-type AIHA [4]. Chronic cold-reactive AIHA accounts for 25% of all AIHA cases and is mediated by cold agglutinin (CA) antibodies, mostly M-type immunoglobulins (IgM). In one cohort, warm-type IgG-mediated AIHA was found to occur in 10% of APLS patients [5].

Here, we present a case of CA IgM-mediated AIHA in a patient with APLS. To the best of our knowledge, such an association has not been described thus far.

Case Presentation

A 53-year-old woman, a habitual heavy smoker, presented to her primary care physician with a 2-week history of gradually worsening fatigue and bilateral knee pain. Laboratory studies revealed severe anemia (hemoglobin [Hb] of 6.4 g/dL). The pa-

tient was referred to the emergency department (ED) and hospitalized to the medical ward between September 13, 2020 and September 22, 2020. During this period the weather was warm with the temperature ranging between 23 and 32°C. She reported a single episode of low-grade fever (37.5°C), and a mild weight loss over the preceding months. No gross external bleeding was noted, and a detailed review of organ systems was otherwise unremarkable.

The patient's past medical record was significant for APLS, diagnosed following 6 miscarriages and an ischemic cerebrovascular accident, combined with positive aPL. She was maintained on warfarin therapy, with therapeutic INR values. Furthermore, 2 years before current hospitalization, she developed pancytopenia, initially identified during routine bloodwork, with white blood cell counts of 3,700–3,800 U/L (normal range 4,500–11,000 U/L), Hb of 9.1–10.0 g/dL (normal range 12.0–16.0 g/dL), and platelet counts of 43,000–88,000 U/L (normal range 150,000–450,000 U/L). Transient hepatocellular enzymes elevations, up to twice the normal values, and mild splenomegaly of 15.5 cm were also reported.

Upon arrival to the ED, the patient was afebrile but slightly tachycardic, with normal blood pressure and oxygen saturation. The spleen was palpated 2 cm below the costal margin, with no other abnormalities on physical examination.

Initial laboratory workup showed pancytopenia, elevated lactate dehydrogenase, and low haptoglobin levels. Coagulation studies revealed a prolonged partial thromboplastin time, with therapeutic INR. Electrolytes, creatinine, liver enzymes, C-reactive protein, iron, and vitamin B12 levels were all within normal limits. A mild folic acid deficiency was found (See Table 1 for summary of the lab results on admission). Urinalysis was unremarkable, and a 24-h urinary protein collection ruled out proteinuria. Blood smear demonstrated spherocytosis and red cell fragments, compatible with hemolysis. Direct antiglobulin test (DAT) was negative for the presence of IgM and IgG, and positive for C₃d [IH-1000/Bio-rad].

A chest X-ray was normal and computed tomography scan of the chest, abdomen, and pelvis demonstrated splenomegaly of 19 cm, with no further abnormalities. Microbiologic, serologic, and polymerase chain reaction tests were negative for cytomegalovirus, Epstein-Barr virus (QS 5/ABI), and *Mycoplasma pneumoniae* (RG Q/Qiagen).

Immune serologies demonstrated elevated aPL and IgM levels (Sysmex c5100; Alegria/Orgentec; BN ProSpec/Siemens). ANA, rheumatoid factor, anti-neutrophil cytoplasmic antibodies, and cryoglobulins were negative (IF/Biorad; Atellica/Siemens; Alegria/Orgentec; manual). Complement levels were low (BN ProSpec/Siemens) (See Table 2 for summary of the patient's immune workup during hospitalization). Serum protein electrophoresis was positive for monoclonal IgM, with mildly elevated levels of both κ and λ free light chains and a normal light chain ratio (Hydrasys/Sebia). Serum protein immunofixation was positive for IgM/ κ . Peripheral blood flow cytometry was unremarkable (Navios EX/Beckman), and a bone marrow biopsy revealed abundance of red cell precursors with a shift to the left and normal myeloid maturation; several clusters of small lymphoid cells, some staining positive for CD20+ and some for CD3+ were also present. Polymerase chain reaction for monoclonal B-cell gene rearrangement was negative (TC 2720/ABI), effectively ruling out the presence of a lymphoproliferative process.

Table 1. The patient's laboratory workup upon admission to the hospital

Lab parameter	Patient	Normal range
WBC count	2,400 U/L	4,050–11,840 U/L
Hb	6.9 g/dL	12.4–16.1 g/dL
PLT count	94,000 U/L	203,000–445,000 U/L
Potassium	3.6 mmol/L	3.5–5.1 mmol/L
Sodium	138 mmol/L	136–145 mmol/L
Creatinine	66 μ mol/L	49–90 μ mol/L
LDH	294 U/L	120–246 U/L
CRP	0.75 mg/dL	<0.5 mg/dL
Albumin	42 g/L	32–48 g/L
ALT	14 U/L	10–49 U/L
Alk. P	68 U/L	46–116 U/L
T. Bilirubin	23.8 μ mol/L	5–21 μ mol/L
INR	2.66 ratio	0.9–1.2 ratio
PTT	53.5 s	22.4–36.3 s
Iron	66.0 μ g/L	50–170 μ g/L
Transferrin	196.2 mg/dL	250–380 mg/dL
Ferritin	83 ng/mL	10–291 ng/mL
Total iron binding capacity	249.17 mg/L	250–380 mg/L
Vitamin B12	503 pg/mL	211–911 pg/mL
Folic acid	3.59 ng/mL	>5.4 ng/mL
Haptoglobin	3 mg/L	30–200 mg/L

Normal ranges refer to females. WBC, white blood cell; Hb, hemoglobin; PLT, platelet count; LDH, lactate dehydrogenase; CRP, C-reactive protein; PTT, partial thromboplastin time.

Thus, the patient was diagnosed as suffering from IgM-mediated AIHA in association with APLS. While a short corticosteroid course has produced no benefit, her Hb levels remained stable throughout the hospitalization. The patient was discharged in a good condition for outpatient follow-up with supportive treatment including folate supplementation, and anticoagulation. Over the next 2 months, she was treated with 2 units of packed red blood cells on monthly outpatient follow-up visits, which led to gradual improvement in clinical symptoms and laboratory parameters. On her third outpatient visit, 3 months following discharge she had a white blood cell count of 4,400 U/L, Hb of 11.3 g/dL, and a platelet count of 70,000 U/L.

Discussion

In this case report we describe a unique combination of APLS and CA IgM-mediated AIHA, presenting as pancytopenia, DAT-positive hemolytic anemia, splenomegaly and low complement levels, without lymphadenopathy or evidence of clonal lympho-proliferation on bone marrow biopsy. CA IgM-mediated AIHA is a well-defined entity, where the relatively high thermal amplitude (28–30°C) of CA allows erythrocyte binding by the anti-

Table 2. The patient's immune workup during hospitalization

Lab parameter	Patient	Normal range
IgA	130.00 mg/dL	70–400 mg/dL
IgM	658.00 mg/dL	40–230 mg/dL
IgG	1,200.00 mg/dL	700–1,700 mg/dL
ANA	Negative	
C ₃	72.80 mg/dL	90–180 mg/dL
C ₄	4.46 mg/dL	10–40 mg/dL
C-ANCA	1.5 U/mL	<10 U/mL
P-ANCA	1.4 U/mL	<5 U/mL
Rheumatoid factor	9.8 IU/mL	<15 IU/mL
Serum protein electrophoresis	Positive for para-protein	
Serum protein immunofixation	IgM/κ	
Free chain kappa	52.5 mg/L	6.7–22.4 mg/L
Free chain lambda	41.1 mg/L	8.3–27 mg/L
Kappa/lambda ratio	1.28	0.31–1.56
Circulating anticoagulants	Positive	
Circulating anticoagulants ratio	3.15	≤1.4
Dillute Russell's viper venom time ratio	2.62	≤1.35
Anti-cardiolipin IgM	74.8 U/mL	<7 U/mL
Anti-cardiolipin IgG	67.9 U/mL	<10 U/mL
Anti-b2-glycoprotein IgM	60.9 U/mL	<5 U/mL
Anti-b2-glycoprotein IgG	47.2 U/mL	<5 U/mL
Cryoglobulin	Negative	

Normal ranges refer to females. IgM, immunoglobulin M; IgG, immunoglobulin G; ANCA, anti-neutrophil cytoplasmic antibodies.

bodies, and consequent agglutination during passage through the peripheral circulation [6]. IgM binds C₁ on the red cell membrane, causing activation of the classical complement pathway. Once back to the body core, CA disassociates from the erythrocytes, while C₃b (further cleaved to C₃d) remains bound, resulting in a positive DAT for C₃d and, in the majority of cases, negative for IgM and IgG [7]. CA-AIHA may appear as 1 of 2 entities – a primary disorder called cold agglutinin disease (CAD) or a secondary syndrome [7]. CAD has been established as a clonal lymphoproliferative disorder of the bone marrow [6]. The secondary CA syndrome (CAS) is a CA-mediated disorder, occasionally arising as a complication of certain infections, and possibly malignancies. Most commonly, CAS has been described in association with *M. pneumoniae*, Epstein-Barr virus and cytomegalovirus infections. While CA-AIHA has been reported in SLE [7], the prevalence of autoimmune diseases in CA-AIHA remains low [8].

While various hematologic abnormalities may occur in association with APLS, the incidence of warm-type AIHA in patients with APLS, is relatively low [9]. In the largest cohort of APLS patients, AIHA was seen in only

6.6% of the cases [10]. A higher incidence of 10.4% was described in a later cohort of 308 patients [5]. Leukopenia, on the other hand, appears more frequently in APLS patients with SLE [10].

Hypocomplementemia is common in patients with primary APLS, reflecting complement activation and consumption [11]. Several mechanisms by which complement activation in APLS patients contributes to thrombotic events have been suggested [12]. The hemolytic process in CA-AIHA is completely complement-dependent [13]. The primary mechanism is activation of the classical complement pathway and extravascular hemolysis [6], though terminal complement cascade activation and intravascular hemolysis also exist in certain cases. The persistent complement activation and consumption cause low levels of C₃ and C₄ in CAD patients [14]. Novel complement-targeted therapeutics are currently under investigation for the treatment of both CA-AIHA and APLS [6, 12, 13, 15].

While splenic infarcts remain a known complication of thrombotic events in APLS, splenomegaly is rare and tends to be secondary to other disease manifestations, such as portal vein thrombosis [8]. The prevalence of he-

patic pathologies in APLS is very low (<1%). These include Budd-Chiari syndrome and thrombosis of smaller hepatic venules [10]. Erythrocyte sequestration was found to occur in the liver and spleen in CA-AIHA [16]. To the best of our knowledge, no hepatic or splenic disturbances were yet associated with primary CAD.

Besides the choice of a therapeutic strategy, an important concern in our patient remains a possible presence of a lymphoproliferative disease, despite a negative bone marrow biopsy. While some reports have suggested an association between APLS and hematological malignancies, the relation remains elusive [17]. CAD, on the other hand, is highly associated with bone marrow lymphoproliferative disorders, confirmed by immunohistochemistry in as many as 75% of the patients [18]. While spleen biopsy was not undertaken due to the procedural risk [17], the likelihood of a spleen-limited lymphoproliferative process remains exceedingly low. With a limited follow-up period on an outpatient basis so far, the patient has remained stable, without evidence of progression and without clinical symptoms requiring intervention.

In summary, we present here a previously unreported combination of 2 immune-mediated diseases, CA-AIHA and APLS. Further reports are required to discern a possible etiological association between these 2 entities, and to consider therapies targeting the common underlying process.

Statement of Ethics

Written informed consent was obtained from the patient for publication of this case report.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Author Contributions

All authors made a substantial contribution to the case report and approved the final version of the manuscript.

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