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Case Reports and Series

Cold agglutinin syndrome as a complication of Covid-19 in two cases

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ABSTRACT

Background: Cold agglutinins are autoantibodies against RBC antigens, leading to hemolysis at less-than-physiological temperatures through complement fixation. Production can be triggered by infections, resulting in secondary cold agglutinin syndrome (CAS). This syndrome has been classically described in the setting of *Mycoplasma pneumoniae* infection, as well as with several viral pathogens.
Cases: Here, we present two cases of cold agglutinins identified in the context of Covid-19 in critically ill patients treated at our institution. Each case was characterized by little *in-vivo* hemolysis, but these antibodies complicated laboratory assessment and renal replacement therapy. Management included anticoagulation and warming of dialysis circuit.
Conclusions: Despite minimal *in-vivo* hemolysis, these antibodies are of clinical significance given their implications for laboratory assessment and renal replacement therapy, particularly with the frequency of multi-organ system dysfunction associated with severe Covid-19.

Introduction

Cold agglutinins are autoantibodies against RBC antigens, leading to hemolysis at less-than-physiological temperatures through complement fixation [1]. Production can be triggered by infections, resulting in secondary cold agglutinin syndrome (CAS), and has been classically described in the setting of *Mycoplasma pneumoniae* infection, as well as with viral infections such as Epstein–Barr virus, varicella, and influenza [1]. Here, we present two cases of cold agglutinins identified in the context of Covid-19, a clinical syndrome that was recently concurrently described at another institution [2].

Case 1

A 70-year-old man presented with fevers and cough. Chest imaging demonstrated bilateral infiltrates, and he was intubated for hypoxia before transfer to our medical center. Nasopharyngeal PCR was positive for SARS-CoV-2. He received vasopressors, ceftriaxone, and hydroxychloroquine. He developed renal failure requiring continuous renal replacement therapy (CRRT). On hospital day 5, providers noted repeated clotting of his CRRT circuit, prompting hematology consultation.

Testing included platelets 823 × 10^9/L, D-dimer 3799 ng/mL (upper limit 230), and fibrinogen greater than 1000 mg/dL. Protime (PT) and activated partial thromboplastin time (aPTT) were within reference ranges. Evaluation from day 2 included a negative antibody screen, but samples from days 5 and 6 demonstrated a cold-reactive autoantibody and direct antiglobulin test (DAT) positive for C3b/C3d and negative for IgG. This antibody did not react at physiological temperature but reacted with patient and donor red blood cells (RBCs) at cold temperatures. Peripheral smear demonstrated RBC agglutination. Serum protein electrophoresis (SPEP) with immunofixation was performed which showed an IgG kappa monoclonal protein, with an M-spike of 1.1 g/dL, against a background oligoclonal banding characteristic of multiple clonality. Serum kappa and lambda free light chains were elevated with a normal kappa/lambda ratio. Despite elevated lactate dehydrogenase (LDH) at 2151 U/L (upper limit 610), there was little active *in-vivo* hemolysis as evidenced by normal haptoglobin and stable hemoglobin on serial measurements. However, repeated instances of clotted specimens made laboratory monitoring challenging. He was managed with warming of CRRT circuit *via* integrated warming unit, running tubing under a Bair hugger warmer, and heparin infusion with improvement in CRRT function.

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Table 1
Direct antiglobulin and drug dependent RBC antibody testing.

Test	Case 1 Result	Case 2 Result
ABO Rh	O Rh Positive	O Rh Negative
DAT Polyspecific	Positive (2+)	Negative
DAT Anti-IgG	Negative	Negative
DAT Anti-C3d	Positive (1+)	Negative
DAT Anti-C3b, C3d	Positive (4+)	Negative
DAT Saline Control	Negative	Negative
New Antibodies in Serum	Autoanti-I reactive at IS, RT, 37 °C	Autoanti-I reactive at 18 °C, 4 °C
Ceftriaxone dependent RBC antibody	Negative	Negative
Hydroxychloroquine dependent RBC antibody	Negative	Negative

Initial testing performed by University of North Carolina McClendon Laboratories in Chapel Hill, NC. Confirmatory testing and drug-dependent antibody testing performed by Versiti in Milwaukee, WI.
DAT: Direct antiglobulin test. RBC: Red blood cell. IS: Immediate spin. RT: Room temperature.

His course was complicated by progressive thrombocytopenia, with nadir of 62×10^9/L. Testing confirmed heparin-induced thrombocytopenia. He was transitioned to argatroban without further CRRT failure.

Case 2

A 67-year-old man developed dyspnea upon returning from New York, prompting hospital presentation. Nasopharyngeal PCR was positive for SARS-CoV-2. He was managed with azithromycin, hydroxychloroquine, and ceftriaxone. He was intubated on hospital day 5 and developed renal failure requiring CRRT on day 10.

Laboratory evaluation was notable for D-dimer 3050 ng/mL, fibrinogen greater than 1000 mg/dL, with normal PT/aPTT. In patient's sample from day 10, a cold-reacting antibody was identified. This antibody reacted with all patient and donor RBCs at cold temperatures but did not react at physiological temperature. DAT was negative. There was no significant hemolysis, with stable blood counts, minimal hyperbilirubinemia (1.3 mg/dL), LDH 1051 U/L, and normal haptoglobin. A serum protein electrophoresis demonstrated no evidence of monoclonal protein.

His course was complicated by refractory septic shock and hypoxic respiratory failure, and the patient died after his family elected for comfort-oriented care.

Discussion

Additional antibody testing was performed (Table 1). Drug-dependent RBC antibody studies were negative for ceftriaxone and hydroxychloroquine for both patients. Both autoantibodies were found to be anti-I. Weiner et al. described this near-universal RBC antigen in 1956 after encountering a patient with a cold agglutinin who was unable to be transfused without severe hemolysis [3]. Subsequent studies revealed that anti-I antibodies are often seen as a post-infectious complication in patients with *M. pneumoniae* infection. The trigger for the formation of this specific autoantibody after *M. pneumoniae* infection is not known, as there does not appear to be an I antigen on this organism [4]. Interestingly, cold agglutinin syndrome with anti-I was also reported in a patient infected with the influenza A H1N1 during the 2009 pandemic [5]. However, anti-I antibodies have not been associated with coronavirus infection prior to the Covid-19 pandemic to our knowledge.

The first patient was also found to have evidence of a monoclonal gammopathy on SPEP. While monoclonal gammopathies have been associated with CAS [1], the presence of a monoclonal gammopathy does not explain the acute development of CAS noted in this patient. His conversion from initially negative to subsequently positive antibody testing at our facility provides strong evidence that this antibody developed during his infection. The oligoclonality noted in the background of his SPEP likely reflected a pro-inflammatory state in the setting of his infection.

The presence of these autoantibodies presented clinical challenges. For Case 1, lab specimens needed to be kept warm as clotting of specimens resulted in difficulties in laboratory testing and discrepant values on several occasions. Additionally, we hypothesized that Case 1's repeated CRRT circuit failure reflected in-circuit agglutination at room temperature rather than thrombosis, as CRRT performance improved with warming. The greater difficulties experienced for Case 1 may reflect differences in the thermal amplitude of the two antibodies. Case 2's antibody reacted only at less-than-room temperatures, while Case 1's antibody reacted at room temperature (Table 1). Consequently, despite minimal *in-vivo* hemolysis, these antibodies are of clinical significance given their implications for laboratory assessment and renal replacement therapy, particularly with the frequency of multi-organ system dysfunction associated with severe Covid-19.

Declaration of competing interest

None.

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