


CASE REPORT

Cold agglutinin anti-I antibodies in two patients with COVID-19

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Abstract

Background: Cold agglutinin syndrome (CAS) is associated with various diseases. Several studies of CAS associated with coronavirus disease 2019 (COVID-19) reported hemolytic anemia and thrombosis; however, the clinical significance of cold agglutinins (CA) in patients with COVID-19 is unclear. Here, we present two cases of CA identified in the context of COVID-19 without hemolytic anemia and clotting.

Case report and Discussion: Two patients with no known risk factors for CA were diagnosed with COVID-19; peripheral blood smears reveal red blood cells (RBCs) agglutination. These patients showed a high CA titer. We confirmed retrospectively that the CA was an anti-I antibody. The two COVID-19 cases with a high CA titer showed no hemolysis or thrombosis. *Mycoplasma pneumoniae* is known to cause CAS, but not all patients who have a high CA titer show hemolysis. Coagulation abnormalities are documented in severe COVID-19 cases. Although CA increases the risk of thrombosis in those with lymphoproliferative diseases, the role of anti-I antibodies in COVID-19 is unclear. The impact of CAS on clinical presentations in COVID-19 remains a matter of verification.

Conclusions: A high CA titer was identified in COVID-19 patients without hemolytic anemia and clotting. Anti-I antibodies were identified. Further studies are required to clarify the pathophysiology of CA in COVID-19.

KEYWORDS

anti-I antibody, cold agglutinin, COVID-19, red blood cells

1 | BACKGROUND

Cold agglutinin syndrome (CAS) is associated with infection, autoimmune disorders, and lymphoproliferative disorders. The vast majority of cold agglutinin (CA) is IgM, a class of immunoglobulin that triggers agglutination, hemolytic anemia, and thrombosis.¹ CAS associated with coronavirus disease 2019 (COVID-19) has been reported in several patients, with most cases having hemolytic anemia² and anti-I antibodies during continuous renal replacement

therapy (CRRT).³ The present report describes two patients with COVID-19 who developed a high titer of CA without hemolytic anemia or thrombosis.

2 | MATERIALS AND METHODS

All assays were performed as described in the American Association of Blood Banks Technical Manual.⁴ Serum samples were obtained

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from patient 1 on Day 11 post-admission and cryopreserved at -20°C , whereas plasma samples were obtained from patient 2 on Day 32. To ascertain the presence of CAs, serum/plasma samples were processed using rabbit erythrocyte stroma (RESt®, Immucor), which absorbs anti-I antibodies.⁵ Adult O red blood cell (RBC) reagents (Ortho® BioVue™ Screen J; RBC-I, RBC-II, RBC-III) were purchased from Ortho Clinical Diagnostics (Raritan). Newborn baby O RBCs were collected from residual blood samples. Autologous RBC was available in only patient 2.

RBCs agglutination assay was performed as follows: A 100 μl aliquot of serum/plasma with or without adsorption with RESt® was mixed with 50 μl of 3% adult or newborn baby RBCs. The mixture was incubated at 4°C or 25°C for 10 minutes. Saline-indirect anti-globulin (saline-IAT) was performed as follows: The above mixture of RBCs and serum/plasma was incubated at 37°C for 60 min, and 100 μl of anti-human globulin (Immucor) added to the mixture after washing three times with normal saline. These procedures were performed in test tube method. RBCs agglutination was evaluated after centrifugation at 1180g for 15 s in all the assay.

3 | CASE PRESENTATION

3.1 | Patient 1

A 64-year-old man presented with fever for 5 days. His medical history included a myocardial infarction 7 years earlier. Real-time reverse transcription polymerase chain reaction (rRT-PCR) of a nasopharyngeal swab confirmed COVID-19 infection. Chest imaging demonstrated bilateral infiltrates. He was admitted to the hospital due to hypoxia in January, which is winter time in Japan, but the room temperature kept warm (between 20 and 24°C) by air conditioner.

On the sixth day of admission, the hypoxia worsened and he required mechanical ventilation. We started treatment with dexamethasone, tocilizumab, and piperacillin-tazobactam. A peripheral blood smear was checked because the mean corpuscular hemoglobin concentration (MCHC) was high; the smear revealed agglutination of red blood cells (RBC). Laboratory testing revealed macrocytic anemia and a low level of zinc, with no evidence of hemolytic anemia. A direct antiglobulin test (DAT) was positive for C3b/C3d and negative for IgG. His CA titer was 1:512 (reference range 0–1:63), and Donath–Landsteiner antibodies were negative. The results of the laboratory tests are shown in Table 1. A bone marrow biopsy showed no evidence of malignant lymphoma cells. His ventilator support was tapered off after treatment. Zinc supplementation improved the hemoglobin level to 10.6 mg/dl. He was discharged home on Day 38 after showing clinical improvement; the CA titer dropped to 1:64.

Serum from patient 1 strongly agglutinated adult RBCs at 4°C , which was impaired at 25°C . Saline-IAT showed was negative. When anti-I bodies were adsorbed using RESt®, agglutination was impaired. Newborn RBCs did not agglutinate at any temperature

(Table 2). These findings suggested the presence of anti-I IgM antibody was existed.

3.2 | Patient 2

A 76-year-old woman diagnosed with COVID-19 by rRT-PCR 6 days earlier was admitted to our hospital with hypoxia in May which is spring time. Her pneumonia improved following treatment with dexamethasone and remdesivir, but she got a urinary tract infection. A peripheral blood smear taken on Day 25 post-hospital admission showed RBC agglutination. The hemoglobin concentration was 15.0 g/dl, with DATs showing positive for C3b/C3d but negative for IgG. The results of other laboratory tests are shown in Table 1. The CA titer was 1:2048. On her first visit to the outpatient clinic after discharge, her CA titer had fallen to 1:512.

Plasma from the patient 2 strongly agglutinated adult RBCs at 4°C as observed in the serum from the patient 1. However, reduced effect of agglutination by warming and adsorption with RESt® was weaker than observed in the patient 1 serum. In the analysis using autologous RBCs, the reduced effect by warming was weak but strong by adsorption with RESt® (Table 2). Accordingly, the evaluation of agglutination using ficin-treated RBCs was required so as to examine the possibility of anti-Pr antibody. Patient 2' plasma also agglutinated ficin-treated RBC reagents (data not shown). These results indicated that patient 2' cold antibody was not anti-Pr antibody but anti-I antibody.

4 | DISCUSSION

Previous COVID-19 with CAS (COVID-19/CAS) cases showed hemolytic anemia and, albeit rarely, clotting.^{2,3} COVID-19/CAS is usually detected by a drop in hemoglobin levels during hospital administration. In this case report, RBC agglutination was checked visually because the MCHC of both patients was high, and a high CA titer and agglutination of RBCs were present in the absence of hemolysis and thrombosis. Some COVID-19/CAS cases do not show clinical manifestations, such as hemolysis and/or thrombosis.³ Therefore, sub-clinical COVID-19/CAS might be overlooked.

Cold agglutinins have been reported in the setting of other infections such as Mycoplasma, Epstein Barr virus, and Rubella virus.⁶ Not all patients with virus infections who develop CA will have clinically significant hemolysis.⁷ The old literature reported that the occurrence of hemolytic anemia was only 11 out of 200 patients with atypical pneumonia of unknown etiology who had high CA titer.⁸ Clinical manifestations might not be present because the two patients had no exposure of cold stimulate. Most cases of secondary CAS associated with a viral infection are polyclonal,⁹ and they are less pathogenic than monoclonal cases.^{7,10}

Previous COVID-19/CAS cases revealed that the CA was an anti-I antibody as well as mycoplasma.³ Anti-I antibodies, which

TABLE 1 Laboratory testing of the two cases

variable	Case 1 on admission (at agglutination)	Case 2 on admission	Case 2 at agglutination
White blood cell count (/μl)	9600	6900	7200
Neutrophils (%)	95.5	76.6	66.5
Lymphocytes (%)	3.5	14.6	22.5
Eosinophils (%)	0.0	0	0.5
Basophils (%)	0.0	0.1	0
Monocytes (%)	1.0	8.7	10.5
Red blood cell count ($\times 10^6/\mu\text{l}$)	228	496	486
Hemoglobin (g/dl)	8.4	15.5	15
Hematocrit (%)	23.8	44.1	44.5
MCV (fl)	104	89	92
MCH (pg)	36.8	31.3	30.9
MCHC (%)	35.3	35.1	33.7
Platelet count ($\times 10^4/\mu\text{l}$)	25.7	15.9	46.3
Total bilirubin (mg/dl)	0.4	0.6	0.3
Total protein (g/dl)	5.2	6.6	6.2
AST (U/L)	128	63	24
ALT (U/L)	38	32	24
LDH (U/L)	345	461	172
ALP (U/L)	233	222	11
C-reactive protein (mg/dl)	3.8	6.95	5.51
Haptoglobin (mg/dl)	2-276	NA	2-2391
Vitamin B12 (pg/ml)	456	NA	766
Folic acid (ng/ml)	6	NA	3.2
Zinc (μg/dl)	24	NA	NA
IgG (mg/dl)	1146	NA	1004
IgA (mg/dl)	374	NA	355
IgM (mg/dl)	162	NA	73
ACPA (U/ml)	<0.5	NA	NA
RF (IU/ml)	<3	NA	NA
Anti-cardiolipin antibody (U/ml)	<8.0	NA	NA
Lupus anticoagulant	0.9	NA	NA
Cryoglobulin	Negative	NA	NA
Immune fixation	Negative	NA	Weak positive for IgG-lambda
Hepatitis B surface antigen	Negative	NA	Positive but negative for HBV-DNA
Hepatitis C antibody	Negative	NA	Negative
HIV antibody	Negative	NA	Negative

Abbreviations: ACPA, anti-cyclic citrullinated peptide antibody; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HIV, human immunodeficiency virus; Ig, immunoglobulin; LDH, lactic acid dehydrogenase; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; NA, not available; RF, rheumatoid factor.

TABLE 2 Results of RBC agglutination assays

Condition	RBC-I			RBC-II			RBC-III			autologous RBCs			newborn baby RBCs		
	4°C	25°C	37°C-saline-IAT	4°C	25°C	37°C-saline-IAT	4°C	25°C	37°C-saline-IAT	4°C	25°C	37°C-saline-IAT	4°C	25°C	37°C-saline-IAT
Case 1 Untreated serum	4+	1+	0	4+	1+	0	3+	1+	0	NA	NA	NA	W+	0	0
Adsorbed serum	0	0	0	0	0	0	0	0	0	NA	NA	NA	0	0	0
Case 2 Untreated serum	4+	2+	0	3+	2+	0	3+	1+	0	4+	3+	0	W+	0	0
Adsorbed serum	3+	0	0	3+	W+	0	3+	W+	0	1+	0	0	0	0	0

Abbreviations: IAT, indirect antiglobulin test; NA, not available; RBC, red blood cell.

bind to the I antigen on the surface of RBCs, are elicited in mycoplasma pneumoniae infection.¹¹ COVID-19 binds to human cells via sialic acid-containing glycolipids on the cell surface.¹² However, the precise mechanism of action of anti-I antibodies in COVID-19 is unclear.

Individuals with COVID-19 may have various coagulation abnormalities that create a hypercoagulable state that can be thought of in terms of Virchow's triad: endothelial injury, stasis, and a hypercoagulable state.^{13,14} The presence of CAS may increase the risk of thrombosis.^{15,16} It is possible that unrecognized mechanisms such as CAS may have an impact on hypercoagulation. Therefore, it is clinically meaningful to identify complications associated with COVID-19/CAS.

5 | CONCLUSIONS

Here, we report a high CA titer and in vitro RBCs agglutination in two COVID-19 patients without hemolytic anemia or clotting due to a high MCHC. This study confirmed that anti-I antibodies were present in both patients. However, in this case report, CA had no clinical significance making it necessary to clarify the pathophysiology of CA in COVID-19.

AUTHOR CONTRIBUTIONS

H.I, A.D., K.H., H.K., T.H., and D.N. were the attending physicians. H.I, A.D., and S.Y. wrote the article. T.K performed the agglutination tests. T.I supervised and helped to write the article. All authors revised and approved the article.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this published article.

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