

## CASE REPORT

# Coombs-negative hemolytic anemia in a male with COVID-19

Ju Young Bae<sup>1</sup>  | June Evelyn Jeon<sup>1</sup> | Khalil Ian Hussein<sup>1</sup>  | Merlin Sung Lee<sup>2</sup>

<sup>1</sup>Department of Medicine, Greenwich Hospital, Yale-New Haven Health System, Greenwich, CT, USA

<sup>2</sup>Division of Hematology and Medical Oncology, Greenwich Hospital, Yale-New Haven Health System, Greenwich, CT, USA

**Correspondence**

Ju Young Bae, 5 Perryridge Road, Greenwich, CT 06830.  
Email: Ju.Young.Bae@greenwichhospital.org

**Abstract**

This case illustrates the need to consider SARS-CoV-2 infection as a catalyst for Coombs-negative hemolytic anemia as well as the potential for IVIG to serve as an effective treatment for the condition.

**KEYWORDS**

acute medicine, emergency medicine, hematology, infectious diseases

## 1 | INTRODUCTION

Clinical manifestations of SARS-CoV-2 infection include hematologic complications such as coagulopathies, anemia, and platelet disorders. However, Coombs-negative hemolytic anemia associated with COVID-19 has rarely been reported. We describe an atypical presentation of Coombs-negative hemolytic anemia in a COVID-19 patient with no significant past medical history who responded well to IVIG.

The clinical manifestations of SARS-CoV-2 infection include various hematologic complications, such as coagulopathies, anemia, and platelet disorders.<sup>1</sup> Hematologic biomarkers such as D-dimer and lactate dehydrogenase (LDH) correlate with disease severity and thus have prognostic utility.<sup>2</sup> Anemia is a relatively common finding in COVID-19 patients and may be associated with disease severity.<sup>2,3</sup> A study of hospitalized COVID-19 patients found that those diagnosed with autoimmune hemolytic anemia (AIHA) required significantly longer hospital stays than anemic COVID-19 patients with negative direct agglutination tests (DAT).<sup>4</sup> Multiple case reports discuss COVID-19 patients presenting with AIHA or cold agglutinin syndrome.<sup>5-7</sup> However, Coombs-negative hemolytic anemia associated with COVID-19 has rarely been reported.<sup>8</sup> We describe an

atypical presentation of Coombs-negative hemolytic anemia in a COVID-19 patient with no prior history of hematological disorders who responded well to intravenous immunoglobulin (IVIG) therapy.

## 2 | CASE HISTORY/EXAMINATION

A 51-year-old African-American male presented with a six-day history of fever, chills, myalgia, and progressive shortness of breath and one day of brown urine. He had no significant past medical history or family history. His only home medications were over-the-counter vitamin supplements. Vital signs were temperature 99°F, blood pressure 135/75 mm Hg, heart rate 82 beats/minute, respiratory rate 18 breaths/minute, and oxygen saturation 85% on room air that normalized with 100% fraction of inspired oxygen via non-rebreather mask. Physical examination was unremarkable. Initial labs included white blood cell (WBC) count of  $14.3 \times 10^3 \mu\text{l}$  with 83% neutrophils and 7% lymphocytes, hemoglobin (Hb) 11.2 g/dl, hematocrit (Hct) 36.4%, mean corpuscular volume (MCV) of 89.4 fl, platelets  $200 \times 10^3 \mu\text{l}$ , nucleated red blood cells (RBC) 1.7%, total bilirubin 3.8 mg/dl, direct bilirubin 0.3 mg/dl, haptoglobin <8 mg/dl, total

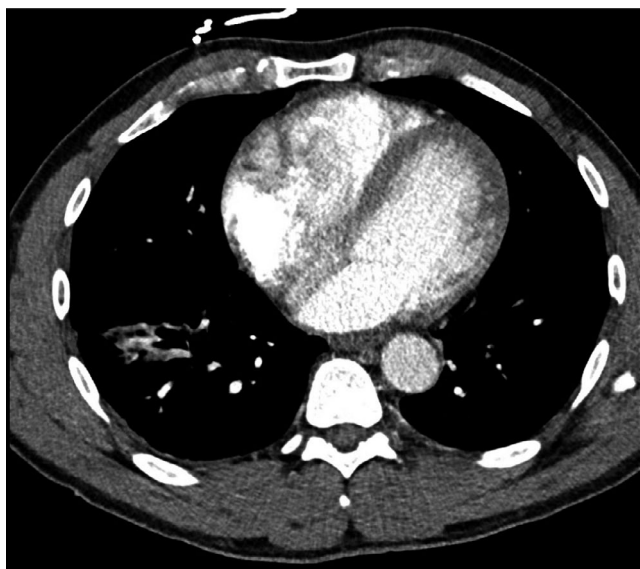
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creatinine kinase (CK) 480 U/L, aspartate aminotransferase (AST) 92 U/L, lipase 1066 U/L, prothrombin time (PT) 10.2 seconds, PT-International Normalized Ratio (INR) 0.96, lactate dehydrogenase (LDH) 997 U/L, ferritin 3527 ng/ml, D-dimer 3.94 mg/L FEU, and positive SARS-CoV-2 polymerase chain reaction (PCR). Influenza A/B PCR and urine antigen testing for legionella and strep pneumoniae were negative. Urinalysis was significant for 2+ protein, 3+ blood, positive leukocytes, many epithelial cells, 9 WBC per high powered field (HPF), and 100 RBC/HPF. Bilateral pulmonary infiltrates were seen on chest X-ray and computed tomography (CT) angiogram of the chest, the latter of which found no evidence of pulmonary embolism (Figure 1). On hospital day 2, Hb fell to 7.2 g/dL (Hct 24.7%), while absolute reticulocytes increased to  $0.1774 \times 10^6$  cells/ $\mu$ l and reticulocyte percentage rose to 6.57%. LDH (1652 U/L), AST (111 U/L), and lipase (1593 U/L) remained elevated.

## 2.1 | Differential diagnosis, investigations, and treatment

In addition to acute COVID-19 pneumonia, the patient's low haptoglobin, acute hemoglobin drop coupled, elevated reticulocyte count, and high indirect bilirubin raised concern for a separate disease process, such as hemolytic anemia, which led to extensive diagnostic workup summarized in Table 1. Acute hemorrhage was considered a possibility, but this was unlikely due to absence of melena, absence of bright red blood per rectum, and no evidence of internal bleeding found on CT of the abdomen and pelvis, which also found no evidence of intrahepatic or hepatobiliary disease. Common etiologies of hemolytic anemias were considered, including autoimmune



**FIGURE 1** CTA chest performed as per pulmonary embolism protocol

hemolytic anemia, cold agglutinin disease, and glucose-6-phosphate dehydrogenase (G6PD) deficiency. However, G6PD, cold agglutinin titer, and immunoglobulin A levels were all unrevealing. Antibody screen was negative as was direct coombs testing. A total of three tests were performed before we accepted this was a case of “Coombs-negative” hemolytic anemia, and they were I—direct antiglobulin test (DAT) polyspecific, which is capable of detecting IgG and complement; II—DAT IgG, which detects only IgG; and III—DAT C3, which detects only complement.

Plasma cell dyscrasia and thrombotic microangiopathy (TMA) were additional diagnostic considerations. However, serum protein electrophoresis, immune-electrophoresis, and free light chain assays were all unremarkable. A peripheral blood smear showed mild anisocytosis and rouleaux with occasional nucleated red blood cells, rare spherocytes, and polychromasia, but no schistocytes. Additionally, left shift was evident with myelocytes, occasional metamyelocyte and band forms, with no evidence of blasts or atypical lymphocytes. C3 was within normal range. The two most common forms of TMA among adults are thrombotic thrombocytopenia purpura (TTP) and hemolytic uremic syndrome (HUS). Evidence against TTP and HUS as potential diagnoses includes the patient's peripheral smear not showing schistocytes and the patient's renal function and platelet count remaining stable throughout his illness. Additionally, the patient had no purpura and no gastrointestinal symptoms. As for other, less common forms of TMA, the patient's complement testing was within normal limits.

Common causes of megaloblastic anemia, such as folate or vitamin B12 deficiency, were considered, but this was deemed unlikely due to the patient's normal MCV and serum testing that ruled out deficiency of either vitamin. Paroxysmal nocturnal hemoglobinuria (PNH) was an important diagnosis to consider. However, flow cytometry results were normal, and this would typically show reduced levels of GPI-anchored proteins on peripheral blood cells in cases of PNH. Hematologic malignancies were considered unlikely due to benign blood smear and flow cytometry results. A chronic bone marrow process such as myelodysplastic syndrome was considered unlikely for three reasons. First, this patient presented with near normal cell counts before his anemia dramatically worsened over 48 hours. Secondly, our patient's MCV was normal, whereas MCV tends to be high in MDS. Third, while our patient's age of 51 does not exclude MDS, the fact that MDS does typically affect the elderly makes this diagnosis less likely. After comprehensive hematologic work up, the patient was diagnosed with Coombs-negative hemolytic anemia from SARS-CoV-2 infection.

For management of SARS-CoV-2 infection, the patient was started on hydroxychloroquine and azithromycin as this was consistent with our health system's COVID-19 treatment protocols at the time, and he received ceftriaxone due to

TABLE 1 Summary of Clinical Laboratory Results

Variable	Reference Range, Adults	Day of Admission	Hospital Day 1	Hospital Day 2	Hospital Day 3	Hospital Day 4	Hospital Day 5	2-month Follow-up
Hemoglobin (g/dL)	13.7–18.0	11.2	9.0	7.2	7.3	7.2	7.4	15.1
Hematocrit (%)	41.0–54.0	36.4	29.4	24.7	24.2	24.3	24.4	47.0
Red Cell count (M/ $\mu$ l)	4.6–6.1	4.1	3.2	2.6	2.6	2.6	2.6	5.70
MCV (fl)	80.0–99.0	89.4	90.7	93.9	93.4	93.1	92.4	82.5
RDW-CV (%)	11.5–14.5	13.4	13.2	13.0	12.6	12.8	13	13.4
Platelets ( $\times$ 1000/ $\mu$ l)	140–446	200	187	191	197	209	215	180
Reticulocyte percentage (%)	0.5–1.80		3.79	6.57				
Absolute Reticulocyte count ( $10^6$ cells/ $\mu$ l)	0.023–0.140		0.1239	0.1774				
White blood count ( $\times$ 1000/ $\mu$ l)	3.8–10.6	14.3	15.3	13.7	12.9	10.4	9.5	4.9
Differential count								
Neutrophils (%)	38.0–74.0	83.0	79.0	65.4	63.0	59.0		63.4
Lymphocytes (%)	14.0–43.0	7.0	7.0	14.1	18.0	15.0		26.1
D-dimer (mg/L FEU)	<0.49	3.94	5.41	7.91	6.62	6.35	7.56	
Fibrinogen (mg/dl)	187–446	497						
Ferritin (ng/ml)	26–388	3527	2716	2324	1979	1733	1402	
Prothrombin Time (sec)	9.4–12.0	10.2	10.4	10.5	10.9	11.1	11.3	
PTT (sec)	23.0–31.0		22.5	22.3	23.3	22.2	23.9	
INR	0.88–1.14	0.96	0.98	0.99	1.03	1.05	1.07	
C-Reactive Protein (mg/dl)	0.0–1.0	0.5	0.4	0.6	0.5	<0.3	<0.3	
Aspartate Aminotransferase (U/L)	5–37	92	110	111	71	41	35	35
Alkaline phosphatase (U/L)	20–135	124	109	104	94	84	79	101
Lipase (U/L)	73–393	1066	1258	1593	962	751	782	
Total CK (U/L)	20–232	480	491	475	293	169	188	
Lactate Dehydrogenase (U/L)	35–190	997	1284	1652	1489	1123	908	153
Haptoglobin (mg/dl)	43–212	<8			<8			101
Total Bilirubin (mg/dl)	0.0–1.0	3.8	3.8	3.7	2.2	0.6	0.3	0.5
Direct Bilirubin (mg/dl)	0.0–0.3	0.3						
Vitamin B12 (pg/ml)	211–911		1169					
Folate (ng/ml)	>5.4		22.3					
Direct Antiglobulin Test			Negative	Negative				
Cold Agglutinins	<1:32		<1:32					
C3 Complement (mg/dl)	90–180			118				
Antibody Screen				Negative				
G6PD (U/g Hgb)	7.0–20.5			8.7				

Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; HD, Hospital Day; INR, international normalized ratio; MCV, mean corpuscular volume.

concern for superimposed bacterial pneumonia. On hospital day 2, he received one unit packed red blood cell transfusion and began a 3-dose course of IVIG therapy. Because the patient's procalcitonin peaked at 0.26 ng/mL, and the patient's blood cultures from admission had no growth, ceftriaxone was stopped after two doses.

## 2.2 | Outcome and follow-up

The patient's symptoms rapidly improved with IVIG initiation, and he was discharged home without any supplemental oxygen requirement on hospital day 5 with Hb of 7.4 g/dL and LDH 908 U/L. Repeat bloodwork one week after hospital discharge showed improvement in Hb to 10.9 g/dL and LDH to 432 U/L and normalization of haptoglobin to 78 mg/dl. At two-month follow-up testing, the patient's Hb was 15.1 g/dl and his LDH had normalized to 153 U/L.

## 3 | DISCUSSION

Multiple viral infections can cause hemolytic anemia, including influenza virus, hepatitis A virus, hepatitis E virus, Epstein-Barr Virus, cytomegalovirus, and human parvovirus B19.<sup>9</sup> In the case of Coombs-negative autoimmune hemolytic anemias associated with viral infections, one hypothesis attributes the condition to either relatively low numbers of antibodies on red cell membranes or the low sensitivity of the conventional tube method used for performing DAT.<sup>10</sup> Immunosuppressant therapies including corticosteroids remain the mainstay of treatment for these conditions.<sup>10</sup> Parvovirus B19 can also cause anemia secondary to pure red cell aplasia that is responsive to IVIG therapy.<sup>11</sup> To our knowledge, only one study on coombs-negative hemolytic anemia associated with COVID-19 has been published, and it describes the results of plasma-Hb testing for intravascular hemolysis involving 38 consecutive COVID-19 patients.<sup>8</sup> Most were noted to have elevated plasma-Hb (defined as >5 mg/dl), but only 24% of patients had pathogenic levels, defined as >30 mg/dl.<sup>8</sup>

Our patient presented with findings concerning for hemolytic anemia at the time of SARS-CoV-2 diagnosis. Given multiple negative DAT results, non-autoimmune causes of hemolytic anemia were investigated. Cephalosporins and hydroxychloroquines are known to cause hemolytic anemia, but the patient had presented with laboratory findings consistent with hemolytic anemia prior to initiation of treatment. Hydroxychloroquine can be a trigger for hemolytic anemia in patients with G6PD deficiency, but our patient tested negative for this condition. There was no evidence of parasitic disease on the blood smear. Other viral causes were not explored, but

based on the onset of hemolytic anemia coinciding with newly diagnosed COVID-19 and the anemia's subsequent resolution with improvement in symptoms related to COVID-19, we suspect that our patient had Coombs-negative hemolytic anemia associated with COVID-19. Bone marrow biopsy was considered for this patient but ultimately deferred to the overall clinical picture. The patient had presented with near normal counts of white blood cell ( $14.3 \times 1000/\mu\text{l}$ ), hemoglobin (11.2 g/dl), and hematocrit (36.4%), and a normal platelet count of  $200 \times 1000/\mu\text{l}$ . Within 48 hours, his hemoglobin fell to 7.2 g/dl and failed to improve with 1 unit of packed red blood cell transfusion as his mildly elevated white blood cell count trended downward toward normal range and his platelet count remained normal. With treatment of his condition, he experienced very rapid clinical improvement with stabilization of his hemoglobin, normalization of his white blood cell count, and continued normality of his platelet count. By the time of his outpatient follow-up with hematology within days after his hospital discharge, his hemoglobin had recovered to 10.9 g/dl without any interim treatment. Due to the rapidity of onset of the patient's anemia with no apparent effects on other cell types as well as the rapid recovery, the hematologist deemed that bone marrow biopsy was unlikely to reveal evidence of bone marrow disorder or failure. The patient's rapid improvement may be due to IVIG therapy or reflective of spontaneous recovery.

We hypothesize that COVID-19 may trigger a Coombs-negative hemolytic anemia generally after the viral infection with two possible mechanisms. First, there is always the possibility of low-affinity antibodies that eluded the assays described above. Prior research into DAT-negative autoimmune hemolytic anemias have shown they can result from RBC-bound IgG, RBC-bound IgA, RBC-bound IgM, and low-affinity IgG that are undetected by commonly used assays.<sup>12</sup> A non-autoimmune hypothesis for the mechanism of DAT-negative hemolytic anemia specifically for COVID-19 patients speculates that intravascular hemolysis may be a complication of COVID-19 as a result of the virus' ability to invade erythrocytes via potential SARS-CoV-2 spike protein-CD147 interaction.<sup>8</sup> This route of invasion via CD147 was first discovered by Wang et. al., and CD147 is a blood group antigen previously found to be essential for invasion of erythrocytes by *P. falciparum*.<sup>13,14</sup>

In conclusion, this case illustrates the need to consider SARS-CoV-2 infection as a potential catalyst for Coombs-negative hemolytic anemia as well as the potential for IVIG to serve as an effective treatment for the condition. Further study is required to determine the true mechanism for this clinical entity as well as the ideal treatment.

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Published with written consent of the patient.

## AUTHOR CONTRIBUTIONS

Ju Young Bae: As first author, JYB was responsible for initial conception of this work, reviewed all cited literature, wrote initial drafts of each section of the manuscript and made revisions. June Evelyn Jeon: As second author, JJ revised initial drafts, acquired relevant clinical data, and created table. Khalil Ian Hussein: As third author, KIH revised drafts of all sections for clarity, accuracy, and for inclusion of additional intellectual content; KIH also reviewed cited literature to contribute to the analysis. Merlin Sung Lee: As final author, MSL made the final diagnosis, was responsible for the initial design of this work, reviewed cited literature, and revised drafts for important intellectual content.

## ETHICAL APPROVAL

Our institutional review board (IRB) does not require that case reports be submitted for IRB approval. The subject of this case report gave written consent to submit this case report for publication.

## DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions.

## ORCID

Ju Young Bae  <https://orcid.org/0000-0001-8509-7099>

Khalil Ian Hussein  <https://orcid.org/0000-0001-9095-0103>

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