

Acute haemolysis by cold antibody during SARS-CoV-2 infection in a patient with Evans syndrome: a case report and literature review

Nicola Osti¹, Jacopo Ceolan¹, Pierluigi Piccoli², Filippo Mazzi¹, Rachele Montemezzi³, Francesco Dima³, Simonetta Friso¹, Francesca Pizzolo¹, Nicola Martinelli¹, Monica Rizzi³, Sara Moruzzi¹, Oliviero Olivieri¹, Lucia De Franceschi¹



¹Department of Medicine, University of Verona & AOUI Verona, Policlinico "GB Rossi", Verona, Italy;

²Department of Transfusion Medicine, University Hospital, Verona, Italy;

³Clinical Biochemistry, University of Verona & AOUI Verona, Policlinico "GB Rossi", Verona, Italy

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic is a global health emergency. Clinical presentation can range from mild to severe lower respiratory tract symptoms, resulting in life-threatening complications such as acute respiratory distress syndrome (ARDS). So far, autoimmune diseases, such as autoimmune haemolytic anaemia (AIHA), immune thrombocytopenic purpura (ITP) and antiphospholipid antibody syndrome have been reported in patients with COVID-19 infection¹. Similar to other viral infections such as cytomegalovirus (CMV) or Epstein-Barr virus (EBV), it has been suggested that a mechanism of molecular mimicry related to SARS-CoV-2 infection could be involved in the pathogenesis of these autoimmune complications¹.

Here, we report a case of a patient with prior diagnosis of AIHA due to warm antibody who developed cold agglutinin syndrome (CAS) associated with SARS-CoV-2 infection.

CASE DESCRIPTION

In August 2020, a 77-year old woman was referred to the Department of Medicine of our University Hospital for severe isolated thrombocytopenia. She presented mild macrocytic anaemia (haemoglobin [Hb] 10.8 g/dL, mean corpuscular volume [MCV] 103 fL, red blood cell distribution width [RDW] 16.6%) and thrombocytopenia (platelets [PLT] $15 \times 10^9/L$). She had a clinical history of essential hypertension, anterior mitral leaflet prolapse and lower limb venous insufficiency. Medication was prescribed including amlodipine (5 mg/day) and lorazepam (1 mg/day). Anti-platelet antibody test was positive for anti-Gp IIb-IIIa antibodies. Direct and indirect antiglobulin tests were also positive (**Figure 1A**). We identified warm, complement-activating, IgG antibodies, reacting against all tested anti-red blood cell (RBC) panels (polyclonal pan-agglutinin antibody) (**Figure 1A**). This was associated with increased reticulocyte count ($125 \times 10^9/L$), reduced haptoglobin, and a slight increase in lactate dehydrogenase (**Figure 1A**). Results of urine analysis were not reported. Bone marrow examination revealed the presence of a small clonal B-cell population, representing 0.1% of total circulating leukocytes (immunophenotypically characterised as: CD19⁺/CD5⁺/CD10⁻/CD20⁺/CD38⁻/kappa cells). Abdominal CT scan revealed normal spleen size.

Serum immunoglobulins (IgA, IgG and IgM) were normal. Anti-nuclear antibodies (ANA) were slightly positive (1: 80, homogeneous pattern) with isolated

Arrived: 23 April 2021

Revision accepted: 19 July 2021

Correspondence: Lucia De Franceschi
e-mail: lucia.defranceschi@univr.it

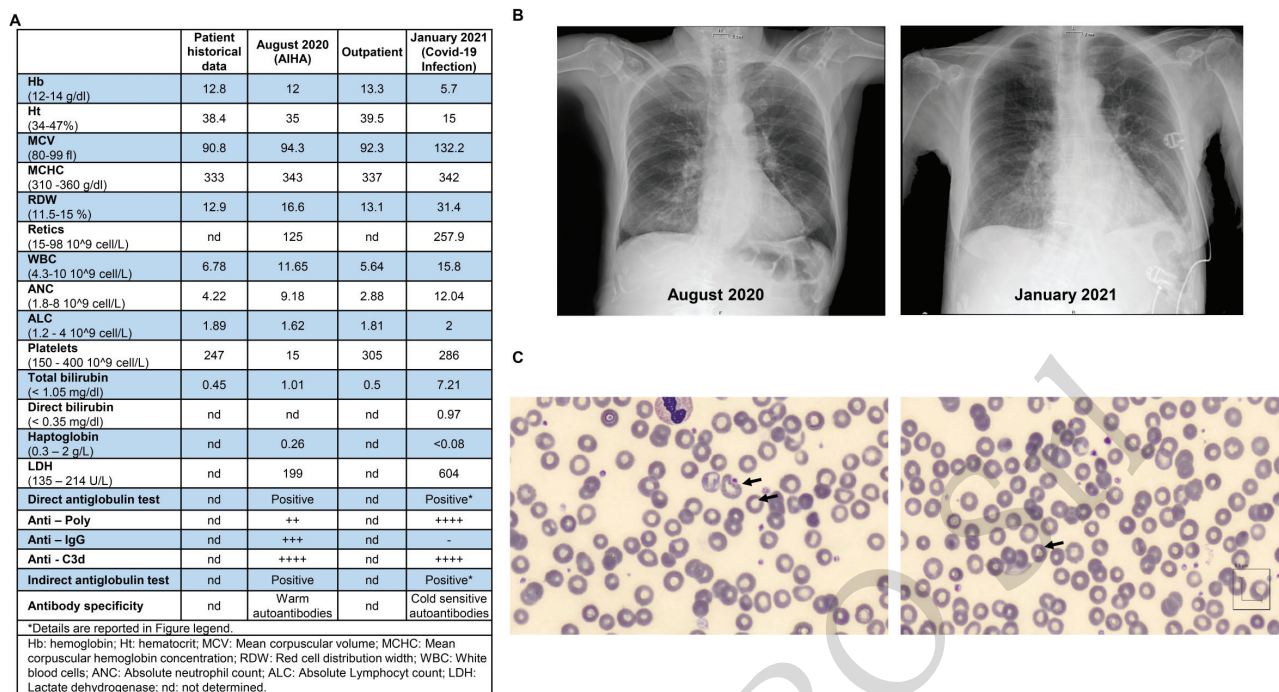


Figure 1 - (A) Haematologic, biochemical parameters and direct/indirect anti-globin tests

*In our case, a dramatic spontaneous agglutination was solved after heating the sample at 37°C for 2 hours. A search for plasma antibodies was positive using saline buffer with 4+ score at 4°C, 2+ score at 20°C, and with a score 0/1 at 37°C. IAT was slightly positive with polyspecific Coombs Igs. Direct antiglobulin test (DAT) was positive with polyspecific Coombs Igs, negative with Coombs anti-IgG but positive with Coombs anti-C3d. Tests with monospecific anti-IgA and anti-IgM serums, normally performed in case of suspected DAT negative autoimmune haemolytic anaemia (AIHA), were not carried out in our case because of the exhaustive data that had been recently obtained.

(B) Patient's chest X-ray in August 2020 when AIHA was diagnosed (left panel) and in January 2021 when SARS-CoV-2-related interstitial pneumonia was diagnosed (right panel)

(C) Patient's peripheral blood smears on admittance to hospital for SARS-CoV-2 infection

Anisopoikilocytosis, red cells with basophilic stippling (black arrows) and knizocytes were observed. Erythrocyte morphology was assessed using May-Grünwald-Giemsa staining; smears were imaged under oil at 100x magnification using a PanFluor objective with 1.30 numeric aperture on a Nikon Eclipse DS-5M camera and processed with Nikon Digital Slide (DS-L1).

anti-Ro-60 (31 CU), suggesting a possible loss of immune tolerance with generation of low titre auto-antibodies. Antiphospholipid (APL) and anti-neutrophil cytoplasm antibodies (ANCA) were negative; C3 and C4 levels were normal.

Serology for HIV, HBV, HCV and parvovirus B19 was negative, whereas serology for EBV and CMV indicated a previous infection. Total body CT scan showed multiple low-density hepatic lesions in the right lobe of the liver (maximum diameter 30 mm). Tumour markers such as CEA, CA19.9, CA15.3, CA125 and alpha-fetoprotein were all negative as was 18-FDG PET. The superior abdominal magnetic resonance imaging allowed us to define the hepatic lesions as previous idiopathic hepatic infarctions. Thus, Evans syndrome was diagnosed, and

oral prednisone 1 mg/kg/day combined with intravenous immunoglobulins (IVIg 0.4 g/kg/d for 5 consecutive days) was started. Since folate plasma level was low (2.5 ng/mL; normal range 10-42 ng/mL), folic acid supplementation was introduced. Both Hb and PLT normalised within 5 days.

In January 2021, the patient was readmitted to our unit because of intense asthenia and jaundice. The PCR nasopharyngeal swab for SARS-CoV-2 was positive and bilateral interstitial pneumonia was confirmed; this required oxygen supplementation (**Figure 1B**). Severe haemolytic anaemia (Hb 5.5 g/dL, LDH >600 U/L) associated with anisopoikilocytosis, red cells with basophilic stippling and knizocytes was observed on the blood smear (**Figure 1A and C**)². We

observed haemoglobinuria (0.2 mg/dL) along with dark urine, suggesting intravascular haemolysis. No imaging of the spleen was obtained at the second hospital admission. Acrocyanosis, livedo reticularis, and Raynaud phenomenon were all absent. Direct antiglobulin test identified haemoagglutinins activating complement with optimal reactivity of the autoantibodies detected at core temperature of 4°C as well as at body temperature; specificity could not be determined. The cold haemagglutinins were detected respectively at high titre when tested against autologous RBC (1: 1,024) and at low titre when tested against a pool of RBC from either blood donors with O blood group antigen (1: 16) or from umbilical cord blood (1: 16). Taken together, these data supported the diagnosis of CAS associated with SARS-CoV-2 infection (**Figure 1A**). After consideration of the related risks and benefits of immunosuppression during an acute viral infection, oral prednisone 1 mg/kg/d associated with IVIg (0.4 g/kg/d for 5 consecutive days) was started instead of rituximab, which represents the first-choice treatment for CAS³. Haemolysis decreased after two weeks of specific treatment, associated to patient recovery from COVID-19 infection. Cold agglutinins were undetectable when our patient was discharged after 18 days of hospitalisation with Hb 9 g/dL and PLT $211 \times 10^9/L$.

DISCUSSION

A review of the literature revealed several case reports and case series connecting SARS-CoV-2 to AIHA or Evans syndrome (**Table I**). Similarly to our patient, CAS activating complement was present in 50% of the papers analysed (**Table I**). In our patient, the presence of a small B-cell clone in the bone marrow might have been the only factor other than SARS-CoV-2 infection responsible for the relapse of AIHA sustained by CAS⁴; this is of interest since such B-cell clonal involvement is less often seen in CAS (20%) than in warm AIHA (24%)³ response to treatment, and occurrence of acute complications were retrospectively investigated in 308 primary AIHA. In addition, in our patient, the presence of immune dysregulation is suggested by previously diagnosed Evans syndrome, which may favour a shift from warm to cold auto-antibody-mediated AIHA during COVID-19 infection.

Angileri *et al.* have recently proposed a mechanism of molecular mimicry linking COVID-19 and AIHA, which

involves the immunogenic epitope ankyrin-1 (ANK-1), a RBC membrane protein, that shares peptide sequences with SARS-CoV-2 Spike protein⁵. This might initiate the generation of autoantibodies, which bind to the RBC membrane promoting RBC removal. Another important player in autoimmune response is the complement system. Activation of alternative complement pathway has been described during SARS-CoV-2 infection⁶. In both warm AIHA and CAS, complement pathway is generally initiated on the erythrocyte surface by autoantibodies directed against RBC antigens⁷. Complement deposition on the RBC surface leads to phosphatidylserine (PS) exposure due to Ca^{2+} influx and membrane deformation⁸. On the other hand, sustained inflammatory response, such as in sepsis, is associated with RBC membrane oxidative stress, promoting RBC membrane PS exposure⁹. Recent studies on RBC during SARS-CoV-2 infection have shown reduction of RBC membrane deformability, increased membrane protein oxidation, and abnormal membrane lipid composition¹⁰. Taken together, these factors might increase the number of RBC exposing PS during SARS-CoV-2 infection¹¹. This in turn might activate the alternative complement pathway with complement deposition on the RBC membrane¹², ending in immune-mediated acute haemolysis mainly characterised by intravascular haemolysis. Indeed, abnormality of RBC membrane in patients with COVID19 is also supported by the recent observation by Berzuini *et al.* of a crossreaction of eluates from DAT-positive COVID19 patients with RBC of DAT-negative COVID19 subjects¹³. Worthy of note, another peculiarity of SARS-CoV-2 infection is the association of AIHA and thrombocytopenia (Evans syndrome) or the presence of thrombocytopenia preceding AIHA. This latter may be due to an epidemiologic effect since response to treatment is more frequent in ITP than in AIHA³; the occurrence of acute complications were retrospectively investigated in 308 primary AIHA. This suggests a possible involvement of PS exposure for both platelet removal and complement activation¹⁴.

Clinical management of AIHA with or without thrombocytopenia associated with SARS-CoV-2 infection was mainly based on the combination of steroids and IVIg (**Table I**). Jacobs *et al.* reported a case of AIHA treated with prednisone 1 mg/kg/d followed by rituximab (600 mg at day 10 after hospitalisation) due to

Table I - Review of the literature on autoimmune haemolytic anaemia and SARS-CoV-2 infection

Supplementary references*	DAT	Cold agglutinins	Antibody specificity	Thrombocytopenia ¹	Treatment ²	Pts
Capes A, <i>et al.</i> 2020 ^{s1}	C3d	Present	Anti-i	Absent	None	1
Demire N, <i>et al.</i> 2020 ^{s2}	IgG + C3d	Absent	n.r.	Present (Evans syndrome)	Steroids (methylprednisolone 1 mg/kg), hydroxychloroquine, favirapir, plasmapheresis (2 consecutive days), IVIg	1
Georgy J, <i>et al.</i> 2021 ^{s3}	Positive	Absent	n.r.	Present (Evans syndrome)	Steroids (dexamethasone 40 mg)	1
Hindilerden F, <i>et al.</i> 2020 ^{s4}	IgG + C3d	Absent	n.r.	Absent	Steroids (prednisolone 1 mg/kg), favirapir, IVIg	1
Hiseh T, Sostier O, 2021 ^{s5}	Positive	Absent	IgG panagglutinins + Anti-k	Absent	Steroids (dexamethasone), remdesivir, convalescent plasma	1
Huscenot T, <i>et al.</i> 2020 ^{s6}	IgG + C3 positive	Present Present	n.r. n.r.	Absent Absent	None	2
Jacobs J, Eichbaum Q, 2020 ^{s7}	IgG + C3	Present	Anti-i IgG panagglutinins	Absent	Steroids (prednisone 1 mg/kg), rituximab, tocilizumab	1
Jawed M, <i>et al.</i> 2020 ^{s8}	C3d	Absent	n.r.	Absent	None	1
Jensen C, <i>et al.</i> 2020 ^{s9}	C3 negative	Present Present	Anti-i Anti-i	Absent Absent	Hydroxychloroquine Hydroxychloroquine	2
Lazarian G, <i>et al.</i> 2020 ^{s10}	IgG + C3d	Absent	n.r.	n.r.	Steroids	7
	IgG + C3d	Absent	n.r.	n.r.	Steroids	
	C3d	Present	n.r.	n.r.	Steroids, rituximab	
	IgG + C3d	Present	n.r.	n.r.	Steroids	
	C3d	Present	n.r.	n.r.	None	
	IgG	Absent	n.r.	n.r.	Steroids, rituximab	
	IgG	Absent	n.r.	n.r.	None	
Li M, <i>et al.</i> 2020 ^{s11}	Positive	Absent	n.r.	Present (Evans syndrome)	IVIg, heparin (for DVT)	1
Lopez C, <i>et al.</i> 2020 ^{s12}	IgG + C3d	Absent	n.r.	Present	Steroids (prednisone 60 mg) hydroxychloroquine, IVIg	1
Maslov D, <i>et al.</i> 2020 ^{s13}	Not reported	Present	n.r.	Absent	None	1
Patil N, <i>et al.</i> 2020 ^{s14}	C3	Present	n.r.	Absent	Steroids (methylprednisolone 120 mg), hydroxychloroquine	1
Whalster L, <i>et al.</i> 2020 ^{s15}	IgG + C3d	Absent	n.r.	Present	Steroids (not specified)	1
Zagorsky E, <i>et al.</i> 2020 ^{s16}	IgG + C3	Present	n.r.	Present	None	1

* See Online Supplementary Content; ¹Prior diagnosis or concomitant to AIHA; ²Other than red blood cell transfusions, antibiotics and anticoagulants. DAT: direct antiglobulin test; IVIg: intravenous immunoglobulins; AIHA: autoimmune haemolytic anaemia; DVT: deep venous thrombosis; Pts: number of patient; n.r.: not reported.

worsening of the haemolytic anaemia (Table I and Online Supplementary Content). However, due to the severity of anaemia, Demire *et al.* used plasmapheresis followed by prednisone 1 mg/kg/d in combination with IVIg (Table I and Online Supplementary Content). In our case, we combined prednisone 1 mg/kg/d and IVIg. Since recent evidence in patients with inflammatory rheumatic diseases indicates that rituximab therapy is

associated with a more severe clinical presentation of SARS-CoV-2 infection¹⁵, we suggest a cautious approach when considering rituximab as a therapeutic strategy for AIHA. Thus, supportive care with rEPO and IVIg is crucial in the clinical management of AIHA during COVID-19 infection, as in septic patients¹⁶.

In conclusion, our case highlights the contribution of SARS-CoV-2 infection in triggering CAS in patients with

previously diagnosed Evans syndrome or either isolated AIHA or ITP. The review of the literature points out the importance of abnormalities of the RBC membrane induced by SARS-CoV-2 infection, possibly favouring PS exposure and complement deposition on RBC membrane. Further studies are required to better understand the link between SARS-CoV-2 infection, autoimmune haemolytic anaemia and thrombocytopenia.

Keywords: cold agglutinin syndrome, autoimmune haemolytic anaemia, COVID-19, complement.

The Authors declare no conflicts of interest.

REFERENCES

1. Ehrenfeld M, Tincani A, Andreoli L, et al. Covid-19 and autoimmunity. *Autoimmunity Review* 2020; **19**: 102597.
2. Berzuini A, Bianco C, Migliorini AC, et al. Red blood cell morphology in patients with COVID-19-related anaemia. *Blood Transfus* 2021; **19**: 34-6.
3. Barcellini W, Fattizzo B, Zaninoni A, et al. Clinical heterogeneity and predictors of outcome in primary autoimmune hemolytic anemia: a GIMEMA study of 308 patients. *Blood* 2014; **124**: 2930-6.
4. Randen U, Trøen G, Tierens A, et al. Primary cold agglutinin-associated lymphoproliferative disease: A B-cell lymphoma of the bone marrow distinct from lymphoplasmacytic lymphoma. *Haematologica* 2014; **99**: 497-504.
5. Angileri F, Lègaré S, Marino Gammazza A, et al. Is molecular mimicry the culprit in the autoimmune haemolytic anaemia affecting patients with COVID-19? *Br J Haematol* 2020; **190**: e92-3.
6. Chauhan AJ, Wiffen LJ, Brown TP. COVID-19: A collision of complement, coagulation and inflammatory pathways. *J Thromb Haemost* 2020; **18**: 2110-7.
7. Berentsen S. Role of complement in autoimmune hemolytic anemia. *Transfus Med Hemother* 2015; **42**: 303-10.
8. Balola AHA, Mayer B, Bartolmäs T, Salama A. Sublytic terminal complement components induce eryptosis in autoimmune haemolytic anaemia related to IgM autoantibodies. *Cell Physiol Biochem* 2019; **53**: 453-64.
9. Brugnara C, De Franceschi L. Effect of cell age and phenylhydrazine on the cation transport properties of rabbit erythrocytes. *J Cell Physiol* 1993; **154**: 271-80.
10. Kubánková M, Hohberger B, Hoffmanns J, et al. Physical phenotype of blood cells is altered in COVID-19. *Biophys J* 2021; doi: 10.1016/j.bpj.2021.05.025.
11. De Jong K, Larkin SK, Styles LA, et al. Characterization of the phosphatidylserine-exposing subpopulation of sickle cells. *Blood* 2001; **98**: 860-7.
12. Lombardi E, Matte A, Risitano AM, et al. Factor H interferes with the adhesion of sickle red cells to vascular endothelium: A novel disease-modulating molecule. *Haematologica* 2019; **104**: 919-28.
13. Berzuini A, Bianco C, Paccapelo C, et al. Red cell-bound antibodies and transfusion requirements in hospitalized patients with COVID-19. *Blood* 2020; **136**: 766-8.
14. Singh A, Uzun G, Bakchoul T. Primary immune thrombocytopenia: novel insights into pathophysiology and disease management. *J Clin Med* 2021; **10**: 789.
15. Avouac J, Drumez E, Hachulla E, et al. COVID-19 outcomes in patients with inflammatory rheumatic and musculoskeletal diseases treated with rituximab: a cohort study. *Lancet Rheumatol* 2021; **3**: e419-426.
16. Fattizzo B. Evans syndrome and infections: A dangerous cocktail to manage with caution. *Blood Transfus* 2021; **19**: 5-8.