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CASE REPORT

Mixed autoimmune haemolytic anaemia in a COVID-19 patient

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Abstract

Introduction: Mixed warm and cold autoimmune haemolytic anaemia (AIHA) secondary to COVID-19 is rarely reported. *Case Report:* We present a case of a 65-year-old Malay lady with no known medical illness, who was admitted for COVID-19 category 3 and mixed warm and cold AIHA. She presented with lethargy, productive cough and on and off fever. Blood investigations showed severe anaemia with spurious macrocytosis, increased lactate dehydrogenase (LDH) and total bilirubin with indirect bilirubin predominance. On full blood picture (FBP), there was normocytic normochromic anaemia with reticulocytosis, red blood cells clumping and NRBC's were seen. Both anti-IgG and anti-C3d were positive for monospecific Coombs test. For indirect Coombs test, auto-IgG and cold agglutinin were detected. *Discussion:* These findings were consistent with mixed warm and cold AIHA. She was treated with intravenous methylprednisolone, before being changed to high dose oral prednisolone. A total of 3 units packed cells were transfused.

Keywords: COVID-19, autoimmune haemolytic anaemia, warm AIHA, cold AIHA

INTRODUCTION

COVID-19 or novel coronavirus is an infectious disease caused by SAR-CoV2 virus. It was first reported in Wuhan City, China on 31st December 2019 and was declared a pandemic by World Health Organisation (WHO) on 11th March 2020. As of the time of writing, approximately 480 million people have been infected with 6 million reported deaths.[1]

Though primarily affecting the pulmonary system, reports have emerged of its effects on various organ systems such as renal, gastrointestinal, cardiology, neurology and haematopoietic systems.[2] COVID-19 is known to have profound effects on the haematopoietic system, causing cytopenia, hypercoagulability and disseminated intravascular coagulation (DIC).[3] In addition, several case reports have implicated an association between COVID-19 and antibody-mediated autoimmune haemolytic anaemia (AIHA), either warm, cold or mixed.

Antibody-mediated autoimmune haemolytic anaemia associated with COVID-19 is rarely reported. To our knowledge, there was only 1 case report of cold AIHA induced by COVID-19 in our Malaysian setting.[4] Here, we are reporting a case of mixed warm and cold AIHA first appearing during COVID-19 infection in an otherwise healthy woman.

CASE REPORT

A 65-year-old Malay lady, with no underlying medical illness, medium-built, presented with lethargy, productive cough, on and off fever and loss of appetite for 6 days duration (symptoms onset was on 7th March 2022). Otherwise, there were no palpitation/lightheadedness/chest pain/abdominal pain/GI losses/alopecia.

On examination, she was alert, conscious, pale, not jaundiced, not tachypneic but had fever 38°C on initial presentation. Otherwise, blood pressure and heart rate were normal. There was no desaturation episode. Lymph nodes were not palpable and there was no splenomegaly.

On chest X-ray there was bilateral lower zones opacity. Electrocardiogram showed sinus rhythm. RTK Antigen for COVID-19 was positive on 12th March 2022 (day 7 of illness). Full blood count (FBC) showed severe anaemia with spurious macrocytosis (Hb 2.7g/dL, MCV 122fL, MCH 65.9pg, MCHC 54g/dL). Direct and indirect Coombs tests were positive. Monospecific

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Coombs test was sent and was positive for both anti-IgG and anti-C3D. Indirect Coombs test identified auto-IgG and cold agglutinin antibody. Cold agglutinin titer was 1:256 dilution. Other parameters of haemolysis were raised such as lactate dehydrogenase (LDH) (437.78IU/L) and total bilirubin (92.53umol/L) with indirect bilirubin predominance (75umol/L). Peripheral blood smear done on day 7 of illness showed normocytic normochromic anaemia with reticulocytosis (15.7%), red blood cells clumping and NRBC's were seen.

She was treated as confirmed COVID-19 category 3 (had pneumonia, but not requiring oxygen) and autoimmune haemolytic anaemia precipitated by COVID-19. A total of 3 units least incompatible packed cells were transfused. Haemoglobin level rose from 2.7g/L to 7.6g/L post transfusion and remained static afterwards. She was initially started on IV methylprednisolone 250mg OD before being changed to high dose oral steroid (tablet prednisolone 1mg/kg).

There was no evidence of thrombotic event or acute bleeding. Screenings for other infective aetiologies of cold AIHA such as Epstein-Barr virus (EBV), cytomegalovirus (CMV) and *Mycoplasma Pneumoniae,* however, were not taken. Screening for autoimmune diseases was also not done. Clinically, she had no signs and symptoms which were suggestive of autoimmune diseases. There was no constitutional symptom that could suggest underlying lymphoid malignancy. Infectious mononucleosis or atypical pneumonia secondary to *Mycoplasma Pneumoniae* were also clinically unlikely.

She was discharged home well on day 11 of illness (17th March 2022) with tablet prednisolone 40mg OD and folic acid supplementation. An appointment was given to monitor haemoglobin level and to taper down prednisolone accordingly. Unfortunately, she did not turn up.

DISCUSSION

Gregory Lazarian *et al*. reported that the median age of AIHA associated with COVID-19 was 62 years old (range 61-89 years). The median time of AIHA onset from COVID-19 symptoms was 9 days (range 4-13 days), a timeframe compatible with that of cytokine storm. The median haemoglobin level was 7g/dL (range 3.8-10.8), with haemoglobin level drop by more than 3g/dL. These findings match with our patient's presentation, although she presented with a more severe anaemia and had no baseline haemoglobin level previously. However, unlike other studies, this patient did not have any comorbidity that predisposed her to a severe form of COVID-19.[5]

In warm AIHA, erythrocytes opsonised with IgG are destroyed by the mononuclear phagocytic system in the spleen (extravascular haemolysis). On the other hand, haemolysis of cold AIHA is complement-dependent. The autoantibody, cold agglutinin antibody is usually of IgM class that binds the 'I/i' antigen on the red cell surfaces, thus activating the classical complement pathway. Phagocytosis of C3-labelled cells takes place in the liver (extravascular haemolysis) and occasionally intravascular through membrane attack complex. Mixed warm and cold AIHA is specified by the existence of warm IgG autoantibody combined with high-titre cold agglutinins.[6,13,14]

Warm AIHA cases can be classified as primary, or secondary to disorders such as chronic lymphocytic leukaemia (CLL), systemic lupus erythematosus (SLE) or common variable immunodeficiency. Recently, several cases have been described in COVID-19. Cold agglutinin disease (CAD) is primary AIHA without underlying clinical disorder. However, newer studies have suggested that a clonal lymphoproliferative bone marrow disorder could possibly be the underlying condition. Meanwhile, secondary cold agglutinin syndrome is secondary to either infections like *Mycoplasma pneumoniae*, Epstein–Barr virus, cytomegalovirus and SARS-CoV-2, or cancers like aggressive B-cell lymphoma. The mixed form of AIHA usually presents with a lower haemoglobin level, has a poorer prognosis, and frequently needs a more aggressive treatment.[6]

The pathogenesis of AIHA in COVID-19 could possibly be molecular mimicry between Ankyrin 1 (ANK-1) and SARS-CoV2 surface glycoprotein called Spike protein. ANK-1 is an erythrocyte membrane protein that shares 100% identity with the Spike protein. Thus, it is postulated that a potential immunological cross-reactivity could occur.[7]

Our patient fulfilled the diagnostic criteria of mixed warm and cold AIHA. There was severe anaemia with an increased in reticulocytes count. Other haemolytic workups were also suggestive such as increased in indirect bilirubin, increased LDH and positive Coombs test. On peripheral blood smear, RBC agglutination was seen which suggests cold AIHA, along with polychromasia and NRBCs that might indicate stress erythropoiesis. In warm AIHA,

microspherocytes are formed when IgG-bound erythrocytes had part of their membrane removed before being further destructed in spleen.[6,15,16]

Monospecific Coombs test was positive for both anti-IgG (usually warm) and anti-C3D (usually cold). Both autologous IgG (warm AIHA) and cold agglutinin autoantibodies (cold AIHA) were detected with cold agglutinin titres 1:256 dilution. Cold agglutinin titre more than 1:64 dilution is considered significant.[6] The big 'I' antigen specificity is seen in 90% of cases and in *M. Pneumoniae* infection. The small 'i' antigen is seen in infectious mononucleosis and Hodgkin's lymphoma.[14,15] Pathological cold agglutinins have wide thermal amplitude exceeding 30°C.[15]

The first line of treatment in primary and secondary warm AIHA is prednisolone at a dose of 1mg/kg body weight. For emergencies, high-dose intravenous methylprednisolone, intravenous immune globulin and plasma exchange are possible treatment choices. The addition of rituximab to first-line therapy can be considered in patients with a severe disease.[6,16]

In cold AIHA, patients can be monitored first without treatment in compensated haemolysis and if the symptoms are mild. Nonpharmacologic management includes thermal protection. Rituximab monotherapy is the most commonly used first-line therapy. Nevertheless, in secondary warm or cold AIHA, the underlying causes should be managed first.[6]

In mixed AIHA, glucocorticoids are given in high doses, and rituximab should be considered early.[6] However, according to several studies, rituximab if given during COVID-19 infection can lead to a higher possibility of serious illness and mortality.[8,9] Our patient showed promising response to glucocorticoid therapy.

Transfusion should be commenced in life-threatening anaemia. Pretransfusion antibody screening will be positive and cross matching will show incompatibility due to the autoantibody present.[6]

COVID-19 has been noted to predispose patients to thrombotic events.[3] Both warm and cold AIHA are also associated with a risk of thrombosis.[6,10] Fortunately, our patient had no apparent sign and symptom of thromboembolic events.

This patient was discharged without morbidity or mortality. In other case reports, the patients were discharged home well[4,5,11], while in another, the patient perished.[12] Further research is warranted to study the prognosis of AIHA in SARS-CoV2 patients.

CONCLUSION

Autoantibody-mediated autoimmune haemolytic anaemia in COVID-19 is rare, but several case reports have been published. Further studies are required to establish the relationship between AIHA and COVID-19, the course of the disease and optimal management.

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REFERENCES

1. https://covid19.who.int/
2. Jain U (August 03, 2020) Effect of COVID-19 on the Organs. Cureus 12(8): e9540.
3. Terpos E, Ntanasis-Stathopoulos I, Elalamy I, *et al*. Hematological findings and complications of COVID-19. Am J Hematol. 2020;95(7):834-47.
4. Chang CY, Chin HH, Chin PW, Zaid M. Cold agglutinin-mediated autoimmune haemolytic anaemia associated with COVID-19 infection: a case report. Hong Kong Med J. 2022;28(3):257-259.
5. Lazarian G, Quinquenel A, Bellal M, *et al*. Autoimmune haemolytic anaemia associated with COVID-19 infection. Br J Haematol. 2020 Jul;190(1):29-31.
6. Berentsen S, Barcellini W. Autoimmune Hemolytic Anemias. N Engl J Med. 2021 Oct 7;385(15):1407-19.
7. Angileri F, Légaré S, Marino Gammazza A, Conway de Macario E, Macario AJL, Cappello F. Is molecular mimicry the culprit in the autoimmune haemolytic anaemia affecting patients with COVID-19? Br J Haematol. 2020 Jul;190(2):e92-e93.
8. Esmaeili S, Abbasi MH, Abolmaali M, *et al*. Rituximab and risk of COVID-19 infection and its severity in patients with MS and NMOSD. BMC Neurol. 2021;21(1):183.
9. Melong Pianta Taleng CM, Lauper K, Gilbert B, *et al*. Incidence of COVID-19 in patients treated with infliximab compared with patients treated with rituximab. RMD Open. 2021;7(3):e001711.
10. Broome CM, Cunningham JM, Mullins M, *et al*. Increased risk of thrombotic events in cold agglutinin disease: A 10-year retrospective analysis. Res Pract Thromb Haemost. 2020;4(4):628-35.
11. Brazel D, Eid T, Harding C. Warm and Cold Autoimmune Hemolytic Anemia in the Setting of COVID-19 Disease. Cureus. 2021;13(9):e18127.

12. Maslov DV, Simenson V, Jain S, Badari A. COVID-19 and Cold Agglutinin Hemolytic Anemia. TH Open. 2020;4(3):e175-e177.
13. Berentsen S. Cold agglutinin disease. Hematology Am Soc Hematol Educ Program. 2016 Dec 2;2016(1):226-31.
14. Das SS, Bhattacharya S, Bhartia S. Clinical and serological characterization of cold agglutinin syndrome in a Tertiary Care Hospital in Eastern India. Asian J Transfus Sci. 2015;9(2):173-6.
15. Brugnara C, Berentsen S. Cold agglutinin disease. UpToDate. 2022 April.
16. Kalfa TA. Warm antibody autoimmune hemolytic anemia. Hematology Am Soc Hematol Educ Program. 2016;2016(1):690-7.