

A Case of Immune-Mediated Coombs-Negative Hemolytic Anemia in a Patient with Systemic Lupus Erythematosus

W. M. D. A. S. Wanninayake*, Tilan Aponso, Manohari Seneviratne and Dhanapala Dissanayake

Medical Unit, National Hospital of Sri Lanka, Colombo, Sri Lanka

*Corresponding Author

W.M.D.A.S.Wanninayake, Medical Unit, National Hospital of Sri Lanka, Colombo, Sri Lanka.

Submitted: 2024, Apr 29; Accepted: 2024, May 27; Published: 2024, Jun 05

Citation: Wanninayake, W. M. D. A. S., Aponso, T., Seneviratne, M., Dissanayake, D. (2024). A Case of Immune-Mediated Coombs-Negative Hemolytic Anemia in a Patient with Systemic Lupus Erythematosus. *Int Internal Med J*, 2(6), 01-05.

Abstract

Systemic lupus erythematosus (SLE) is an auto-immune connective tissue disorder that can involve any organ in the body. The American College of Rheumatology criteria (ACR) include autoimmune hemolytic anemia with reticulocytosis as one of the criteria in the diagnosis of SLE. Anti-erythrocyte antibodies in SLE are mainly warm-type IgG. We present a case of systemic lupus erythematosus presenting with immune-mediated Coombs-negative hemolytic anemia. This case highlights the possibility of autoimmune hemolysis occurring with a negative Coombs test, where the autoantibody levels are too low to be detected by a conventional direct antiglobulin test. (DAT)

Keywords: Coombs Negative Hemolytic Anemia, Immune-Mediated Intravascular Hemolysis, Systemic Lupus Erythematosus

1. Case Presentation

A 49-year-old previously healthy woman presented with yellowish discoloration of the eyes and the passage of dark-colored urine for 3 days. She denied pale stools, steatorrhea, or body itching. She complained of a mild fever associated with generalized body aches for the last 3 days. Since the third day of the illness, she has complained of shortness of breath on exertion in NYHA class II. Over the last 6 months, she has experienced an inflammatory type of non-deforming symmetrical polyarthritis involving the small joints of the hands and non-scarring alopecia. Examination revealed an averagely built female who was pale with a mild tinge of icterus. She did not have any evidence of active synovitis or lymphadenopathy. She had mild hepatomegaly with a smooth surface measuring 3cm below the subcostal margin, but no splenomegaly. The rest of the examination was normal.

Upon investigation, she was found to have a normochromic normocytic anemia with mild thrombocytopenia and absolute lymphopenia. Her inflammatory markers, including CRP and ESR, were elevated. The blood picture revealed polychromasia and reticulocytosis with spherocytes, which supported ongoing extravascular hemolysis. Other biochemical parameters were also supportive of an ongoing hemolytic anemia. Her abdominal ultrasound showed mild hepatomegaly. The direct Coombs test was negative. Further testing with the gel card method revealed warm IgG-type auto-antibodies. She tested positive for ANA and DS-DNA, and the diagnosis of systemic lupus erythematosus with autoimmune hemolytic anemia was made. She was pulsed with

intravenous methylprednisolone for 3 days and converted to oral prednisolone at 1 mg/kg. She had a dramatic recovery without blood transfusions, along with improvements in her hyperbilirubinemia, hemoglobin, and LDH levels.

2. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune-related connective tissue disorder that can involve any organ in the body. Hematological manifestations of the disease can manifest as leucopenia, lymphopenia, immune thrombocytopenic purpura, thrombotic thrombocytopenic purpura (TTP), autoimmune hemolytic anemia (AIHA), and autoimmune myelofibrosis. Autoimmune hemolytic anemia is mainly caused by warm-type IgG. The direct antiglobulin test (DAT) is usually positive in AIHA, but the literature reports DAT-negative hemolytic anemia in approximately 5–10% of patients. The presence of very low antibody levels to be detected by conventional DAT is the suggested pathophysiology in this category of patients.

This case of Coombs-negative autoimmune hemolytic anemia in a patient with SLE highlights the need to use more sensitive methods for detecting lower antibody titers in patients with higher suspicion for autoimmune-mediated hemolysis.

3. Case report

A 49-year-old previously healthy woman presented with yellowish discoloration of the eyes and the passage of dark-colored urine for 3 days. She denied pale stools, steatorrhea, or body itching.

She complained of a mild fever associated with generalized body aches for the last 3 days. Since the third day of her illness, she has complained of shortness of breath on exertion in NYHA class II. She denied a productive cough, urinary symptoms, diarrhea, or abdominal pain. She did not have a history of blood transfusions or any past history of similar episodes in association with a febrile illness. She was not on any long-term medications. Over the last 6 months, she has experienced small joint pain and swelling, along with early morning stiffness and alopecia as well. The joint involvement was symmetrical, involving both proximal inter-phalangeal joints (PIP) and distal interphalangeal joints (DIP). There were no joint deformities. She did not have any photosensitive skin rashes, Raynaud's phenomenon, frothy urine, hematuria, previous pregnancy losses, or thrombotic events.

Examination revealed an averagely built female who was pale with a mild tinge of icterus. She did not have any evidence of active synovitis or lymphadenopathy. She had a mild hepatomegaly with a smooth surface measuring 3cm below the subcostal margin, but no splenomegaly. The rest of the examination was normal. She was hemodynamically stable.

Upon investigation, she was found to have a normochromic normocytic anemia with mild thrombocytopenia and absolute lymphopenia. Her inflammatory markers were elevated, including ESR and CRP. Blood picture evidence of polychromasia with spherocytes and reticulocytosis, supported by elevated LDH and indirect hyperbilirubinemia, confirmed ongoing extravascular hemolysis. Her abdominal ultrasound was normal except for a mild hepatomegaly. The direct Coombs test was negative, and further

testing with the gel card method revealed warm IgG-typespecific auto-antibodies. With a background history of polyarthritis and alopecia, she was tested for ANA immunofluorescence with a titer, which revealed a 1:160 titer, and her anti-DS-DNA antibody levels were positive. She had hypocomplementemia, which confirmed the diagnosis of systemic lupus erythematosus with autoimmune hemolytic anemia. Her renal functions were normal, and her urine full report did not show any active sediments. Her chest X-ray, ECG, and 2D echocardiography were normal. Her coagulation profile was normal, with a normal APTT. Therefore, antiphospholipid antibody screening was not carried out. (Table1).

Since the patient completed the entry criteria for SLE with a positive ANA titer and a diagnosis of auto-immune hemolytic anemia being made following antibody positivity with the Gel card method, she was started on intravenous methylprednisolone pulses for 3 days, followed by a high-dose oral prednisolone 1 mg/kg dosage. She responded very well to steroid therapy within 2 days of treatment, and she had improvements in her hyperbilirubinemia and anemia. Follow-up LDH levels showed a declining titer.

She was discharged on prednisolone at a dose of 60 mg/daily, which was gradually tapered off while monitoring her inflammatory markers and for any evidence of active hemolysis. She is on treatment for bone protection and is awaiting a dexa-scan for the assessment of her bone health. She developed steroid-induced diabetes, but no opportunistic infections were detected during the course of treatment. During the period of follow-up, she did not experience any relapses of autoimmune hemolysis or any other manifestations of the disease.

FBC	WBC 4.5×10^3 Hb 7.5mg/dl (MCV 105) PLT 125×10^3
CRP	110mg/dl (<5)
ESR	96mm/1st hour
Blood picture	Polychromasia with reticulocytosis and spherocytes, no schistocytes visible. Supportive of an ongoing extravascular hemolysis. Mild thrombocytopenia and lymphopenia present
LDH	596 IU/L (105-333)
Reticulocyte count	5.6%
Total Bilirubin	5.8mg/dl (0.1-1.2)
Direct Bilirubin	2.8mg/dl (<0.3)
Indirect Bilirubin	3mg/dl
AST	180 U/L (8-33)
ALT	43 U/L (4-36)
USS Abdomen	Mild hepatomegaly of 16.5 cm with no focal lesions or fatty change. No splenomegaly or para-aortic lymphadenopathy. Normal biliary system and kidneys
Coombs test	Negative
Gel-card method	Warm IgG type specific auto antibodies positive
ANA	1: 160
Anti DS-DNA	Positive

Serum Complement levels	C3 25mg/dl (75-175) C4 14mg/dl (15-45)
Serum Creatinine	0.7mg/dl (0.7-1.3)
Urine full report	Pus cells 1-2 /hpf red cells 1-2/hpf Protein Nil Casts Nil
Serum Complement levels	Normal
ECG	Sinus rhythm
2D-Echocardiogram	Good diastolic and systolic function.
Hand X-ray	Normal study. No joint space narrowing or sclerosis.
PT	12 Sec. (10-13)
APTT	34 Sec. (30-40)

Table 1: Investigations

4. Discussion

Systemic lupus erythematosus (SLE) is an auto-immune-related chronic inflammatory disorder that can involve any organ in the body. The etiology of SLE is unknown, and the deposition of immune complexes in target organs, which trigger complements and other mediators of inflammation, is considered to be the underlying pathophysiological mechanism. SLE can mimic many other disorders since it can involve multiple body systems; therefore, suspicion and serology are highly important in the diagnosis.

Hematological manifestations of SLE include leucopenia, lymphopenia, thrombocytopenia, autoimmune hemolytic anemia (AIHA), and autoimmune myelofibrosis. Thrombocytopenia can be secondary to underlying immune thrombocytopenic purpura or thrombotic thrombocytopenic purpura (TTP) [1].

Hemolytic anemias can be immune or non-immune, primary or secondary. Secondary causes such as lymphoproliferative syndromes, autoimmune diseases, infections, immunodeficiencies, post-transplantation-associated diseases, and drugs, including novel anti-cancer therapies, need to be kept in mind as these may provide specific management options for the particular underlying disorder.

The diagnosis of hemolytic anemias should be done with a step-wise approach. The presence of anemia combined with reticulocytosis, indirect hyperbilirubinemia, high LDH, and low haptoglobin levels would suggest the possibility of hemolytic anemia. The nature of hemolysis can be understood by the blood picture, where the presence of polychromasia with spherocytes may suggest extravascular hemolysis (most likely autoimmune hemolysis), whereas the presence of schistocytes may suggest intravascular microangiopathic hemolysis.

Autoimmune hemolytic anemias can be sub-categorized as warm IgG-mediated disease, cold IgM-mediated disease, mixed-type disease, cold agglutinin disease, and paroxysmal cold hemoglobinuria. AIHA is reported to have an estimated incidence of 1–3 cases per 100,000 people per year. It is more common after the age of 40. Chronic or relapsing cases are more frequent, particularly if they are associated with autoimmune or

lymphoproliferative disorders [2].

The pathophysiology of autoimmune hemolysis includes red cell destruction via antibody-dependent cellular cytotoxicity (ADCC). When hemolysis is extravascular, hemolysis occurs in the spleen and liver via macrophage-mediated ADCC and C3b-mediated ADCC, respectively. IgM autoantibodies activate the complement pathway, which leads to the formation of the membrane attack complex (MAC-C5-C9), which later causes activation of “perforins” and other cytotoxic factors leading to intravascular hemolysis. It has been calculated that extravascular hemolysis causes the destruction of approximately 420 mL of RBCs in 24 hours for a 70 kg patient, whereas IgM-mediated intravascular hemolysis causes the destruction of about 200 mL of RBCs per hour since it presents with greater clinical severity [3].

The American College of Rheumatologists (ACR) criteria recognize autoimmune hemolytic anemia with reticulocytosis as one of the criteria in the classification of SLE.

Antibodies found in SLE are mainly warm-type IgG. Many antibody types are considered to be associated with autoimmune hemolytic anemia in SLE. Commonly, anti-cardiolipin antibodies (IgG and IgM subtypes) and under-expression of CD55 and CD59 have been detected in patients with SLE-associated AIHA [4]. Even though these associations are described in the literature, no conclusions have been drawn regarding the antigen specificity of anti-erythrocyte antibodies or their therapeutic implications in patient management.

Usually the direct antiglobulin test (DAT) is positive in autoimmune hemolytic anemia, but it can become negative in ~5–10% of patients with auto-immune hemolytic anemia when the antibody titer is too low in their blood to be detected by conventional DAT. In addition, DAT can become falsely positive in healthy individuals after therapies like intravenous immunoglobulin therapy, anti-thymocyte globulin, and daratumumab, following recent transfusions (due to alloantibody formation), and in paraproteinemic conditions with high serum globulins.

In addition to low titer antibody levels, DAT can be negative in the

background of autoimmune hemolysis when the affinity for anti-IgG is low or when the autoantibodies are IgA or IgM in nature. Rarely, the presence of an IgM monomer without a complement fragment may affect the detectability of the antibody by a commercially available antiglobulin reagent [5]. Since there are all these possibilities, the interpretation of a negative DAT should always accompany the clinical and other laboratory findings of the patient. If clinical suspicion is high and non-immune causes are excluded, the addition of more sensitive methods than the standard DAT protocol can be used in the management.

The gel card method, polybrene test, microcolumn, solid phase, washings with cold or low-ionic salt solutions, and immune-radiometric assays for red cell-bound IgG can be used to diagnose AIHA in patients with very low antibody levels that are difficult to detect by conventional DAT [6].

Exclusion of other causes of hemolysis and confirmation of antibody-mediated hemolysis by a sensitive method in the presence of a response to steroid therapy may confirm the diagnosis of DAT-negative autoimmune hemolytic anemia. DAT-negative AIHA has a better prognosis than DAT-positive AIHA [7].

A case-control study carried out in a Japanese population showed that the degree of reticulocytosis and mean corpuscular volume were higher and the hemoglobin levels were lower in DAT-positive AIHA patients when compared to DAT-negative AIHA patients. There was no significant difference in 1-year survival among the two groups. Patients with DAT-AIHA required lower doses of steroid therapy for maintenance when compared to the DAT-positive hemolytic anemia group [7].

First-line treatments for AIHA are steroids, and usually a dramatic response is seen with the administration of steroids. If the diagnosis is not clear, this criterion may be helpful as a supportive criterion. Splenectomy, rituximab, and intravenous immunoglobulin therapy are the second-line treatment options available for patients who poorly respond to the initial steroid therapy. Rituximab reduces the degree of inflammation and the number of B lymphocytes that are involved in the mediation of antibody-mediated cytotoxicity. Also, plasmapheresis can be utilized as a therapeutic option as it removes immune mediators from the system. Recombinant erythropoietin therapy can be used to increase compensatory erythropoiesis [3]. Supportive blood transfusions are said to be avoided as much as possible since blood transfusions may lead to hyperhemolysis in these patients. In cases of blood transfusion, it is important to check for the presence of allo-antibodies to avoid the generation of new allo-antibodies since these patients may require further transfusions in the future, which will prevent the transfusion-mediated hemolytic process. Small-volume, slow transfusions are recommended to avoid transfusion reactions. In cases of cold AIHAs, blood should be pre-warmed before transfusion [8].

The presence of anemia with indirect hyperbilirubinemia and spherocytes with polychromatic red cells in the blood picture supported the possibility of hemolytic anemia in our patient.

Since her background history suggests a possible autoimmune disorder, even though her Coombs test was negative, she was further evaluated with the gel card method, which detected warm-type IgG-specific auto-antibodies. Therefore, her ANA positivity, constitutional symptoms, small joint arthritis, and autoimmune hemolytic anemia completed the diagnostic score for SLE according to the EULAR criteria for the diagnosis of SLE [9].

5. Conclusion

When AIHA presents with negative DAT and anemia, once non-immune causes of hemolytic anemia are excluded, if clinical and other laboratory findings support the possibility of autoimmune hemolytic anemia, clinicians should not be misled by the negative Coombs test. More sensitive methods for detecting antibody levels are available, and they need to be carried out in this setting. A restricted transfusion policy with steroids followed by rituximab in refractory cases may show a dramatic response in cases of AIH, which may further support the diagnosis.

Contribution

Dr Tilan Aponso and Dr.W.M.D.A.S. Wanninayake did the literature review and writing of the initial manuscript was done by Dr.W.M.D.A.S. Wanninayake. Dr. Manohari Seneviratne and Dr.Dhanapala Dissanayake finalized the manuscript and gave expert opinion. All the authors read and approved the final manuscript.

Consent for Publication

Informed written consent for publication of details was taken from the patient. Consent form can be made available to the editor on request.

Competing Interests

Authors declare that they have no competing interests.

Availability of Data and Materials

The data is available from the corresponding author on reasonable request.

References

1. Fayyaz, A., Igoe, A., Kurien, B. T., Danda, D., James, J. A., Stafford, H. A., & Scofield, R. H. (2015). Haematological manifestations of lupus. *Lupus science & medicine*, 2(1), e000078.
2. Barcellini, W., Zaninoni, A., Giannotta, J. A., & Fattizzo, B. (2020). New insights in autoimmune hemolytic anemia: from pathogenesis to therapy. *Journal of clinical medicine*, 9(12), 3859.
3. Barcellini, W., & Fattizzo, B. (2022). Diagnosis and Management of Autoimmune Hemolytic Anemias. *Journal of Clinical Medicine*, 11(20), 6029.
4. Ruiz-Argüelles, A., & Llorente, L. (2007). The role of complement regulatory proteins (CD55 and CD59) in the pathogenesis of autoimmune hemocytopenias. *Autoimmunity reviews*, 6(3), 155-161.
5. Rodberg, K. (2022). DAT-Negative Autoimmune Hemolytic

-
- Anemia. *Hematology/Oncology Clinics of North America*, 36(2), 307-313.
6. Behzadifard, M., Arianezhad, A., Bandehzadeh, A., & Gholampour, M. (2022). Severe hemolysis with negative direct antiglobulin test: A case report. *Annals of Medicine and Surgery*, 81.
 7. Kamesaki, T., Toyotsuji, T., & Kajii, E. (2013). Characterization of direct antiglobulin test-negative autoimmune hemolytic anemia: a study of 154 cases. *American journal of hematology*, 88(2), 93-96.
 8. Ueda, Y. (2018). Transfusion of patients with autoimmune hemolytic anemia. [*Rinsho Ketsueki*] *The Japanese Journal of Clinical Hematology*, 59(10), 2354-2361.
 9. Fava, A., & Petri, M. (2019). Systemic lupus erythematosus: diagnosis and clinical management. *Journal of autoimmunity*, 96, 1-13.

Copyright: © 2024 W.M.D.A.S.Wanninayake, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.