CASE REPORT

Cold agglutinin-induced haemolysis in association with antinuclear antibody-negative SLE

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SUMMARY

Systemic lupus erythematosus (SLE) is a chronic relapsing autoimmune disease associated with several autoantibodies targeted to nuclear and cytoplasmic antigens. Serum antinuclear antibody (ANA) is considered an important diagnostic marker of SLE. However, 2–3% of patients with typical clinical picture of SLE may have persistently negative ANA tests. Autoimmune haemolytic anaemia (AIHA) in SLE is usually mediated by warm IgG anti-erythrocyte antibodies. Our report describes a female patient who presented with clinical manifestations of SLE including photosensitivity, joint pains and AIHA. Further workup revealed high cold IgM agglutinin titres. A comprehensive workup for infectious aetiologies was negative. Autoimmune studies revealed negative ANA, but positive anti-double-stranded DNA and antiphospholipid antibodies. Lymphoproliferative disorder was excluded by imaging studies. Initial treatment with steroids proved of little benefit; however, rituximab resulted in significant clinical improvement. To the best of our knowledge, this is perhaps the first report of ANA-negative SLE presenting with cold AIHA.

BACKGROUND

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disorder with protean clinical manifestations and is associated with significant morbidity and mortality. Although a number of factors contribute to the pathogenesis of the disease, a complete picture of disease aetiology remains elusive. The diagnosis of SLE can be made by using the revised criteria of the American College of Rheumatology (ACR).¹ Antinuclear antibody (ANA) is generally considered an important diagnostic marker in SLE. However, a small number of patients (about 2–3%) with distinctive clinical picture of SLE may remain persistently negative for ANA.² Haematological manifestations in lupus are common and include anaemia, leukopenia and thrombocytopenia. Anaemia is present in 50% of patients with SLE.³ While anaemia of chronic disease is the most common cause of anaemia in SLE, autoimmune haemolytic anaemia (AIHA) is not uncommon (10%) and is included in ACR classification criteria for SLE. AIHA in SLE is typically mediated by warm-IgG type anti-erythrocyte antibody.⁴ The presence of IgM cold antibody leading to AIHA is a rare phenomenon in SLE.

We herewith report a unique case of cold antibody-mediated AIHA with ANA-negative SLE.

CASE PRESENTATION

A 42-year-old woman presented to the Emergency department with progressively worsening fatigue and exertional dyspnoea over a period of 3 weeks. She also reported of mild right upper quadrant abdominal discomfort. A detailed review of systems was additionally remarkable for arthralgias and photosensitivity.

Her medical history included hypertension, hyperlipidaemia and obesity. Her only home medication was depot medroxyprogesterone. There was no history of autoimmune disease in other family members. Her immunisation status was up-to-date. She denied any recent history of travel.

On examination, the patient was pale and icteric. Her blood pressure was 130/85 mm Hg, pulse 96 beats/min, respiratory rate 18/min, temperature 98°F and SpO₂ 98% on room air. Cardiovascular examination revealed a soft systolic ejection murmur at the apex. Her chest was clear and abdomen was soft, non-distended and non-tender with no organomegaly.

INVESTIGATIONS

Her initial laboratory workup revealed normal white cell count (9 600/UL), low haematocrit of 22%, increased bilirubin (total 3.6 mg/dl, indirect 3.1 mg/dl), elevated lactate dehydrogenase (811), reticulocyte count (3%) and a low haptoglobin. Peripheral smear showed spherocytosis. Further workup revealed a positive direct Coomb's test and high-level of cold IgM agglutinin titres. Coagulation studies were normal. ECG showed normal sinus mechanism without any ST-T changes. Urine analysis was unremarkable.

CT scan of abdomen with oral and intravenous contrast, performed for abdominal pain, revealed splenomegaly and multiple non-enhancing splenic lesions likely consistent with small haemangioma (figure 1A). MRI of the abdomen subsequently performed revealed multiple small splenic lesions with enhancement characteristic of haemangioma. The laboratory workup for infections including *Mycoplasma*, *Ehrlichia*, *Babesia*, *Bartonella*, *Legionella*, Lyme, cytomegalovirus, Epstein-Barr virus, herpes simplex viruses, viral hepatitis, tuberculosis and HIV were negative. Autoimmune workup revealed a negative screening ANA (<100 AU/ml), but positive anti-double-stranded DNA (anti-dsDNA=11 IU/ml) and antiphospholipid antibodies (IgM: 26 MPL U/ml). Serum protein

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Figure 1 (A) CT scan of abdomen with oral and intravenous contrast, reveals splenomegaly and small non-enhancing splenic lesions likely consistent with haemangioma. (B) Positron emission tomography scan shows no evidence of active adenopathy or focal spleen abnormality.

electrophoresis with immunoglobulin quantification and cryoglobulins were normal.

DIFFERENTIAL DIAGNOSIS

1. Autoimmune haemolytic anaemia
2. Lymphoproliferative disorder
3. Infection
4. Paroxysmal cold haemoglobinuria
5. Paroxysmal nocturnal haemoglobinuria.

TREATMENT

The patient was treated for SLE-related intravascular haemolysis. Packed red blood cell (RBC) transfusions were given and intravenous steroids were initiated. However, treatment with steroids proved ineffective and the patient required further blood transfusions for symptomatic anaemia. Steroids were then stopped and rituximab was initiated resulting in significant clinical improvement. Her haematocrit improved from 22% to 34%. The patient was subsequently discharged from the hospital, and positron emission tomography (PET) was scheduled as outpatient to rule out remote possibility of indolent lymphoproliferative disorder.

OUTCOME AND FOLLOW-UP

The patient was continued on rituximab as outpatient and did not require any further blood transfusions. Her follow-up haematocrit levels remained stable (ranging 32–34%).

Outpatient PET scan did not show any evidence of active adenopathy or focal spleen abnormality (figure 1B). Over 1-month follow-up course, she showed remarkable improvement in her symptomatology and other clinical parameters including normalisation of her haemoglobin levels.

DISCUSSION

About 2–3% of patients with SLE may have truly negative ANA; therefore, for the diagnosis of SLE, positive test for a specific antibody may be more important than a negative ANA.⁵ Historically, one of the proposed mechanisms for ANA-negative SLE was the use of low-sensitive mouse liver substrate in indirect immunofluorescence assay.⁶ However, nowadays exclusive use of human epithelial (Hep-2) substrate has increased the sensitivity of ANA assays. As a result, incidence of true ANA-negative SLE has markedly decreased. Other reported mechanism of ANA-negative finding include inability to detect immune complex-bound ANA in serum by indirect immunofluorescence and loss of ANA through kidney in a patient with profuse proteinuria.^{7–8} Some field experts

also provide a reasonable argument that negativity or positivity of ANA is also dependent on laboratory reporting techniques. Depending on the laboratory's reporting habits, a test that is positive at 1:10 or even 1:40 may be called either negative or weakly positive. Most laboratories count 1:80 and higher as clearly positive. Our hospital laboratory reports ANA as positive at or above >100 AU/ml. The point is that ANA negative does not always precisely mean completely negative.⁹

Using the Hep-2 substrate for ANA, the prevalence of patients with a positive anti-DNA assay despite a negative ANA test has been reported to be about 0–0.8%.¹⁰ Therefore, unless there is a reasonable suspicion that the ANA may be falsely negative, testing for anti-DNA antibody is generally not advocated in ANA-negative patients.¹⁰ Thus, in an appropriate setting where clinical suspicion for the disease remains high despite a negative screening ANA, it may be reasonable to proceed with additional specific antibody testing including anti-dsDNA, anti-Sm, anti-Ro/SSA, anti-La/SSB and anti-RNP.

Our patient meets 4 of the 11 revised ACR criteria for SLE, viz. arthritis, photosensitivity, haemolytic anaemia and positive anti-dsDNA and antiphospholipid antibodies. However, clinicians should always keep in mind that the 1997 revised ACR criteria for SLE were established for the differentiation of SLE from other autoimmune diseases and not for the direct diagnosis of SLE.¹¹

Though extremely rare, cold antibody AIHA in SLE has been reported.^{12–13} Binding of antibodies to RBCs activates the classical pathway of the complement system leading to the formation of membrane attack complex and intravascular haemolysis. On the other hand, if classic pathway is ineffective, RBCs are opsonised with complement proteins (particularly C3b and C4b) which enhances phagocytosis in liver and spleen, presenting as extravascular haemolysis. In our patient, haptoglobin levels were low, suggesting intravascular haemolysis.

Rituximab has been shown to be very efficacious in the past for AIHA with SLE refractory to conventional therapy.¹⁴ Rituximab is also known to maintain long-term remission rate in patients with primary cold agglutinin AIHA.¹⁵ Peñalver *et al*¹⁶ showed rituximab to be extremely effective and safe therapeutic alternative in patients with intractable and severe AIHA. We used rituximab as our second-line agent mainly based on its safety profile. In conclusion, our case represents a unique combination of cold antibody AIHA-negative and ANA-negative SLE and re-emphasises the importance of maintaining and evaluating a broad differential.

Learning points

- ▶ Autoimmune haemolytic anaemia occurs in about 10% patients of systemic lupus erythematosus (SLE) and is almost always mediated by warm autoimmune antibodies. Only very few cases of cold autoimmune antibody-mediated haemolytic anaemia in SLE have been reported in the literature so far.
- ▶ Antinuclear antibody (ANA) is positive in most patients of SLE; however, it can be absent in about 2–3% of the patients. Thus, if the clinical suspicion for the disease remains high, it is advised to pursue tests for a specific antibody.
- ▶ Rituximab has been shown to offer a promising therapeutic effect in cold autoimmune haemolytic anaemia with SLE.
- ▶ To the best of our knowledge, this is perhaps the first report in the literature of ANA-negative SLE presenting with cold antibody-mediated haemolytic anaemia.

Competing interests None.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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