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Case Series of Cold Agglutinin Syndrome: A Malaysian Teaching Hospital Experience

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Abstract

Cold agglutinin-autoimmune haemolytic anaemia (CA-AIHA) is characterised by the destruction of red blood cells mediated by IgM autoantibodies that function optimally at 4 °C. CA-AIHA can be categorised into cold agglutinin disease (CAD), a primary or idiopathic clinicopathological entity, and cold agglutinin syndrome (CAS), which occurs secondary to an identifiable underlying cause. This case series presents three patients with CAS due to distinct secondary causes: lymphoma, systemic lupus erythematosus (SLE) and Mycobacterium tuberculosis. It highlights the diagnostic and therapeutic challenges unique to each aetiology and underscores the importance of targeted management. These cases highlight the importance of promptly identifying underlying causes in CAS for effective treatment and improved outcomes.

Keywords
Cold agglutinin syndrome, autoimmune haemolytic anaemia, autoimmune disorders, lymphoma, Mycobacterium tuberculosis

Introduction

Cold agglutinin-autoimmune haemolytic anaemia (CA-AIHA) is a rare condition characterised by the destruction of red blood cells, mediated by IgM autoantibodies that function optimally at 4 °C. It is categorised into cold agglutinin disease (CAD) and cold agglutinin syndrome (CAS). CAD pertains to idiopathic cold agglutinins, whereas CAS refers to cases with a discernible underlying cause.[1,2] The diagnostic criteria for CAS require evidence of haemolytic anaemia (elevated reticulocytes, lactate

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dehydrogenase (LDH), indirect bilirubin, low haptoglobin) and a positive direct antiglobulin test (DAT) for complement fragment C3d only. A cold agglutinin titre of ≥1:64 at 4°C, especially if active near 30°C, supports the diagnosis. Key clinical features include acrocyanosis and Raynaud's phenomenon, in addition to excluding secondary causes. Identifying secondary causes of CA-AIHA is crucial as it directs therapeutic decisions.[3] This paper presents three cases of CAS, each associated with a different secondary cause: neoplastic, autoimmune, and infection-related.

Case 1

A 61-year-old diabetic Malay man presented with exercise intolerance, weight loss, digital numbness, and cyanosis due to cold exposure. He exhibited jaundice with splenomegaly but without lymphadenopathy. Laboratory tests indicated macrocytic anaemia, elevated mean cell haemoglobin, and leucocytosis with an absolute lymphocytosis. The peripheral blood film (PBF) revealed numerous atypical lymphocytes and significant red cell agglutination, suggesting CA-AIHA.

A positive DAT of 3+ with monoclonal anti-C3d and a significant cold agglutinin titre of 1:2048 at 4°C, demonstrating anti-I specificity, confirmed the diagnosis of CA-AIHA. An elevated reticulocyte count, along with increased serum total bilirubin and LDH levels, indicated ongoing haemolysis. The bone marrow biopsy revealed atypical lymphoid cells, and immunohistochemistry confirmed the presence of CD5 and cyclin D1-positive B-cell lymphoma, consistent with mantle cell lymphoma (MCL).

Pre-treatment computer tomography (CT) scanning revealed splenomegaly, a suspicious thyroid nodule, and sub-centimetre mediastinal and cervical lymph nodes. The patient was treated with rituximab and bendamustine for MCL.

Case 1 had diagnostic features of CA-AIHA, evidenced by a positive DAT for C3d, a high cold agglutinin titre with anti-I specificity, and laboratory signs of haemolysis, including elevated reticulocytes, LDH, and bilirubin. The bone marrow biopsy revealed MCL, identifying it as the secondary cause of CAS.

Case 2

A 33-year-old Malay woman with systemic lupus erythematosus (SLE) and lupus nephritis class V presented with a one-week history of lethargy and exertional dyspnoea. She had a history of elevated anti-double-stranded DNA antibodies (dsDNA) (>400 IU/ml) and a positive anti-nuclear antibody (ANA) (1:320). Previous treatment with cyclophosphamide was complicated by varicella zoster infection and pulmonary tuberculosis (PTB), which led to the deferral of further cyclophosphamide administration.

Clinical examination revealed mild pallor without lymphadenopathy or organomegaly. Laboratory tests showed severe anaemia, and the PBF (Figure 1) indicated marked red blood cell agglutination with prominent polychromatic cells. A cold agglutinin titre of 1:512 at 4°C with anti-I specificity and a positive DAT for C3d confirmed the diagnosis. Infectious screening was negative, and there were no signs of malignancy.

The patient was advised to avoid cold environments and continued taking prednisone and hydroxychloroquine for SLE while completing tuberculosis treatment. An immunosuppressant was to be started after tuberculosis treatment.

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Case 2 had diagnostic features of CA-AIHA, evidenced by a high cold agglutinin titre, anti-I specificity, positive DAT for C3d and severe anaemia with peripheral blood features of haemolysis. The elevated anti-dsDNA and ANA, with no evidence of infection or malignancy, confirmed CAS secondary to SLE.

Case 3

A 67-year-old Malay man with uncontrolled diabetes presented with a four-day history of fever, productive cough, and worsening dyspnoea. Upon examination, the patient was tachypnoeic and febrile. A chest X-ray revealed a right upper zone opacity and a blunted right costophrenic angle. Laboratory tests indicated severe anaemia and leucocytosis. PBF revealed marked red cell agglutination and polychromasia (Figure 1).

A cold agglutinin titre of 1:2048 at 4°C, with anti-I specificity and a positive DAT (C3d 4+), confirmed CA-AIHA. Sputum tested positive for acid-fast bacilli, and *Mycobacterium tuberculosis* was cultured. He was assessed with CAS secondary to pulmonary tuberculosis (PTB) and treated with ethambutol, isoniazid, rifampicin, and pyrazinamide (EHRZ).

After the initial discharge, the patient missed follow-up appointments and returned four months later, presenting with fever, weight loss, jaundice, and splenomegaly. Persistent anaemia (Hb 6.9 g/dL) and elevated liver enzymes were observed. A repeat PBF still displayed CA-AIHA features.

Despite treatment with intravenous immunoglobulin (IVIG), prednisone, plasmapheresis, and rituximab, the haemolysis persisted, and the patient developed methicillin-resistant *Staphylococcus aureus* (MRSA) bacteraemia. Further immunosuppressive therapy was withheld while additional investigations were pending. Unfortunately, the patient passed away shortly after.

Case 3 had CAS secondary to pulmonary *Mycobacterium tuberculosis*, evidenced by a high cold agglutinin titre (1:2048 at 4°C), anti-I specificity, and a strongly positive DAT (C3d 4+).

Discussion

CAS primarily occurs in older patients, peaking in the seventh decade, with no widely noted preference for either sex. Cold agglutinins are mainly IgM, and their pentameric structure allows them to agglutinate red blood cells at low temperatures of 4°C.[1,2]

Pathophysiology

CAS may be associated with underlying infections, autoimmune disorders, and both haematological and non-haematological malignancies. Recent infections commonly associated with the Epstein-Barr virus (EBV), *Mycoplasma pneumoniae*, hepatitis B virus (HBV), and hepatitis C virus (HCV), among others, can trigger the production of clinically significant cold agglutinins. Other infectious causes implicated include HIV, varicella-zoster virus, cytomegalovirus, parvovirus B19, rubella, and, to a lesser extent, Chlamydia psittaci.[4–7] Although *Mycobacterium tuberculosis* is not typically recognised as a common cause of CAS, it is increasingly being identified, especially in developing countries with high prevalence of PTB. Several case reports have been published in recent years highlighting this trend.[8–10]

Autoimmune conditions associated with CAS include, but are not limited to, SLE, rheumatoid arthritis, and Sjögren's syndrome. Immune dysregulation is the hallmark of developing CAS in this setting.[11]

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Haematological and non-haematological malignancies may cause CAS. In cases of lymphoma, cold agglutinins are produced by clonal B-cells. Malignant B-cells generate cold agglutinins with a specific idiotype localised in the V4-V3 encoded portion of the variable region. In contrast to the non-pathogenic state, cold agglutinins arise from variable segments distinct from the V4-V3 portion.[12–14]

Cold agglutinins often show specificity for the I blood group antigen on adult RBCs, especially in cases involving *Mycoplasma pneumonia*.[15] Patients with CA-AIHA and high-titre anti-I antibodies tend to experience more severe haemolysis and a rapidly progressive disease course.[16,17] These cold agglutinins, with high thermal amplitude, further complicate ABO phenotyping when transfusions are needed and often require cold autoadsorption of serum to identify underlying alloantibodies.[18] Therefore, a high index of suspicion, prompt diagnosis, and early intervention are crucial in suspected anti-I-mediated CA-AIHA.[16]

Clinical Presentation and Diagnostic Approach

Patients may present with pallor and jaundice, depending on the severity of haemolysis. Acrocyanosis remains an integral clinical feature independent of the underlying aetiology. The nose, earlobes, fingers, and toes are particularly susceptible due to lower temperatures in the peripheral circulation. Other circulatory symptoms may include livedo reticularis and Raynaud's phenomenon. The spleen may be palpable when haemolysis takes a protracted and severe course.[1,4]

CA-AIHA warrants a thorough investigation, including but not limited to a clinical examination, full blood count with a reticulocyte count, DAT and cold agglutinin titres. Tissue biopsies, bacterial and viral infection screening, as well as serum electrophoresis with immunofixation, may be performed as needed to exclude secondary causes.[2,3] In an ideal setting, such laboratory investigations could be included in the standard screening tests for CAS. However, in resource-constrained environments, specialised tests should be conducted exclusively within an appropriate clinical context to ensure the cost-effectiveness of each test.
As shown in Table 1, tailored laboratory investigations confirmed the diagnosis of CAS for each corresponding secondary cause. Abnormal RBC indices and significant red cell agglutination are often the initial laboratory findings that trigger further examination for cold agglutinins. The agglutination typically resolves or diminishes when blood specimens are incubated at 37 °C. Red cell agglutination results in artefactual red cell indices, with a low RBC count and HB levels, while showing high mean cell haemoglobin concentration (MCHC), MCH, and MCV.[19] These fictitious indices were observed in all three cases. Reticulocytosis reflects the increased erythropoietic bone marrow's response to haemolysis. Additional biochemical indicators of underlying haemolysis include elevated serum LDH, increased total bilirubin, and decreased haptoglobin levels.[3]

The diagnosis of CA-AIHA requires a positive polyspecific and monospecific DAT, demonstrating strong positivity for complement fragment C3d, and a cold agglutinin titre of ≥1:64 at 4°C with thermal amplitude active near 30°C.[3,18] Clinically significant cold agglutinins of 1:2048, 1:512, and 1:2048 at 4 °C were noted in cases 1, 2, and 3, respectively. At room temperature, case 3 exhibited the highest titre (1:512), followed by case 1 (1:32) and case 2 (1:4). Regardless of the secondary cause, all three cases displayed specificity to anti-I, which is expected given the age of our patients. Anti-I specificity is confirmed when the antibody agglutinates O+ adult blood at titres that are fourfold or greater than those of O+ cord blood at 4 °C.[4]

Infections are a common cause of CAS. Therefore, it is prudent to perform infective screenings on all patients with clinically significant cold agglutinin titres. All cases were screened and found non-reactive for hepatitis B and C viruses, EBV, syphilis, and human immunodeficiency virus (HIV). In light of his respiratory

symptoms, case 3 was further screened for *Mycoplasma pneumoniae and Mycobacterium tuberculosis* and tested positive for *Mycobacterium tuberculosis*.

In case 1, numerous atypical lymphocytes on the PBF, with associated thrombocytopenia and anaemia, prompted the bone marrow biopsy. The constellation of bone marrow morphology findings and the immunophenotype of the atypical lymphoid cells pinned down the diagnosis of MCL as the secondary cause of CAS. In case 3, the indication for the bone marrow biopsy was persistent severe haemolysis despite pharmacological interventions. The bone marrow was found to be reactive, with no lymphoproliferative disorder. Bone marrow assessment was not indicated for case 2.

Management of CAS

The cornerstone of CAS management lies in addressing the underlying cause. Infection-associated CAS should be treated with appropriate organism-specific antiviral or antibiotic therapy.[2] Although antibiotic therapy is effective in managing *Mycoplasma pneumoniae*, it is important to note that the onset of CAS frequently occurs after antimicrobial treatment has been initiated or even completed. In such cases, CAS is often self-limiting and may require only supportive care [2,20] *Mycobacterium tuberculosis* requires standard anti-tuberculosis therapy (rifampicin, isoniazid, pyrazinamide, and ethambutol). In most cases, this regimen resolves the associated haemolysis, but immunosuppressive agents like prednisone or rituximab may be necessary in severe cases. It is important to consider the drug interactions between rifampicin and these agents, reducing the efficacy of prednisone and rituximab.[8]

Immunosuppressive therapies, such as prednisone and rituximab, are frequently used for autoimmune conditions. However, the response rate is lower than that observed in warm AIHA.[4,21] Malignancy-associated CAS necessitates protocol-guided management of the underlying malignancy. Various chemoimmunotherapy regimens are available for lymphoproliferative neoplasms.[22] Case 1 involves an elderly patient with MCL who was treated with rituximab and bendamustine.

Plasmapheresis, IVIG, and splenectomy are ineffective treatments for CA-AIHA and should not be used as standalone therapies.[2,23–25] Patients with CAS secondary to lymphoproliferative disorders limited to the spleen may inadvertently benefit from splenectomy.

If transfusion support is necessary, blood grouping, antibody screening, and donor unit compatibility testing should be performed at 37 °C with IgG-specific anti-human globulin. Additionally, in-line blood warmers should be utilised to minimise the binding of cold agglutinins to the transfused cells.[1,18] Haematinics, such as folate and iron supplements, can be administered to reduce transfusion demands.[1] Thermal protection from cold environments prevents the exacerbation of haemolytic episodes.[21]

Prognostic Factors and Long-Term Outcomes

The prognosis of CAS depends on the clinical course of the underlying disease and resolves when that condition resolves. CAS resulting from self-limiting conditions or those that respond to therapy generally has a better prognosis than cases associated with protracted disease courses, such as HIV or uncontrolled autoimmune diseases. The remission status of lymphoma influences the clinical manifestation of CAS. In *Mycobacterium tuberculosis* infection, anaemia is often caused by chronic inflammation, nutritional deficiencies, malabsorption, and bone marrow suppression. When CAS is also present, these factors are compounded by increased haemolysis, further exacerbating anaemia.[10] Prolonged exposure to cold environments may worsen haemolysis and lead to ischemic complications. Additionally, chronic severe anaemia may result in multi-organ failure.

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Future Directions and Research Needs

Establishing standardised treatment protocols remains elusive due to the low prevalence of CAS. Therapeutic approaches rely on expert experiences and case reports; therefore, multicentre retrospective studies may be valuable. Complement inhibitors such as sutimlimab and eculizumab show good efficacy and safety profiles for CAD; however, limited data are available on their use in CAS. The frequency of anti-I specificity in CAS and its impact on the disease course and management warrant future research on targeted therapies for severe anti-I-mediated haemolysis.[2,3]

Table 1: Baseline Characteristics, Haematological Parameters, and Immunohematology Results

Characteristic	Case 1	Case 2	Case 3
Age/Sex	61/Male	33/Female	67/Male
Ethnicity	Malay	Malay	Malay
WBC (x10^9/L)	59.99	5.34	16.9
RBC (x10^12/L)	0.76	1.53	5.1
HB (g/dL)	3.9	7.3	6.9
MCV (fL)	111.8	86.3	106
MCH (pg)	51.3	38.6	32.6
PLT (x10^9/L)	104	401	324
Reticulocytes (%)	7.7	5.3	10
Peripheral Blood Film	Marked red cell agglutination, atypical lymphocytes (75%)	Marked red cell agglutination, polychromasia	Marked red cell agglutination, polychromasia, neutrophilia
Cold Agglutinin Titre and Specificity	-1:2048 at 4°C -1:32 at RT - Anti-I Specificity	-1:512 at 4°C -1:4 at RT -Anti-I Specificity	-1:2048 at 4°C, -1:512 at RT -Anti-I Specificity
LDH (U/L)	406	237	106
Total Bilirubin (umol/L)	27	6	86
Infective Screen	Non-Reactive	Non-Reactive	*Mycobacterium tuberculosis* was cultured
Bone Marrow Findings	Diffuse infiltration by abnormal lymphoid Cells	Not Done	Erythroid hyperplasia secondary to haemolysis
Secondary Cause	Mantle Cell Lymphoma	Systemic Lupus Erythematosus	Pulmonary Tuberculosis

*Results obtained at diagnosis. RT: Room temperature.

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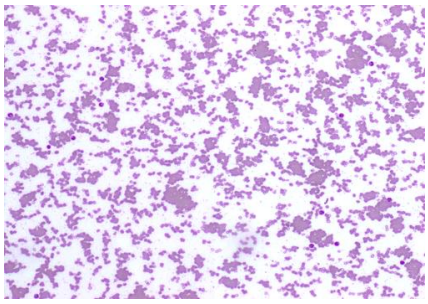
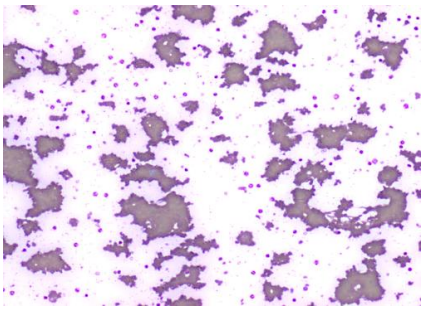
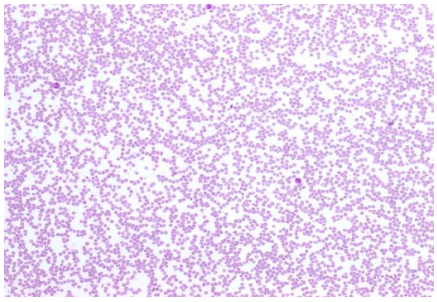
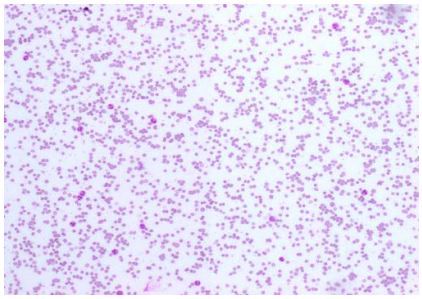
	Case 2	Case 3
Pre-warming Blood Film		
Post-warming Blood Film		

Figure 1: Illustrates the diagnostic peripheral blood films (Wright's stain x10) for cases 2 and 3. Pre-warmed films exhibit significant red cell agglutination. Post-warmed films, incubated in a warm bath at 37°C for 30 minutes, demonstrate resolution of the red blood cell agglutination.
*Peripheral blood films for case 1 could not be located.

Conclusion

We provide an overview of a case series of three patients with CAS who were diagnosed and treated at a teaching hospital in Malaysia. Our cases highlight autoimmune disorders and neoplastic causes as well-defined causes of CAS. Notably, we identify *Mycobacterium tuberculosis* as an emerging infectious cause of CAS. We suggest maintaining a high index of suspicion for *Mycobacterium tuberculosis* in patients with CA-AIHA, especially in developing countries where the prevalence of *Mycobacterium tuberculosis* remains high.

CA-AIHA cases are rare in warmer tropical regions like Malaysia, and CAS cases are further underreported worldwide. To enhance clinical research opportunities for patients with CAS, we recommend establishing patient registries similar to those developed for patients with bleeding disorders.

Conflict of Interest

The authors have nothing to declare.

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