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Review

Cold Agglutinin Disease—Keeping a Cool Head in Diagnosis and Management

Timothy Woo[1], Nicole Japzon[1], Ethan Troy-Barnes[1], Ke Xu[1], Samah Alimam[1], Sofhia Akhtar[2], Mari Thomas[1], Jindriska Lindsay[1], Charalampia Kyriakou[1], Shirley D'Sa[1], Jahanzaib Khwaja[1,*]

[1]Department of Haematology, University College London Hospitals NHS Foundation Trust, NW1 2PG London, UK
[2]Health Services Laboratory Blood Transfusion, University College London Hospitals NHS Foundation Trust, W1T 4EU London, UK
*Correspondence: j.khwaja@nhs.net (Jahanzaib Khwaja)
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Abstract

Cold agglutinin disease (CAD) is a rare autoimmune haemolytic anaemia characterised by autoantibodies, known as cold agglutinins (CA), to erythrocyte antigens that react at low temperatures. CA, which are typically of immunoglobulin M (IgM) isotype, often bind to erythrocytes at acral sites of the circulation. This results in red cell agglutination and activation of the complement cascade. These lead to the clinical manifestations of cold-induced circulatory symptoms and chronic haemolysis, which can decompensate during intercurrent febrile illness or surgery. A heightened risk of thrombosis may be seen in active CAD. The underlying pathophysiology of CAD is driven by a small clonal B-cell population in the bone marrow, but the diagnosis requires the exclusion of an overt malignancy, infection, or autoimmune disorder that can be associated with a secondary cold agglutinin syndrome. The workup of CAD is multimodal, incorporating serological (often requiring meticulous temperature-controlled handling), radiographic, and histological investigations. The mainstay of CAD management is cold avoidance, and treatment is indicated in patients with symptomatic disease. In the emergency setting, rescue therapies such as complement inhibitors can be considered alongside warmed blood transfusions. Targeting the pathologic B-cell clone is the primary therapeutic strategy; however, head-to-head randomised controlled trials comparing different approaches are lacking. Given its rarity, CAD can present with diagnostic and therapeutic challenges to clinicians across a range of environments. Involvement of a multidisciplinary team of healthcare professionals incorporating haematologists, histopathologists and the transfusion laboratory is crucial in optimising the care of patients with this condition.

Keywords: cold agglutinin disease; autoimmune haemolytic anaemia; B-cell lymphoproliferation; complement; blood transfusion

1. Introduction

Cold agglutinin disease (CAD) is a distinct autoimmune haemolytic anaemia (AIHA) characterised by autoantibodies against antigens expressed on the surface of erythrocytes that bind at low temperatures [1].

Cold agglutinins (CA) represent immunoglobulins that agglutinate red cells below central body temperature. CAD is a distinct clinicopathological entity formally classified by the World Health Organization (WHO) and defined as an AIHA that arises from monoclonal CA related to an underlying indolent clonal B-cell proliferation in the bone marrow [2,3]. It accounts for approximately 15–30% of cases of AIHA, with an incidence of 0.5 to 1.9 per million persons per year and affects mainly middle-aged to elderly individuals with a female preponderance [4,5,6]. There is substantial geographic variation in the frequency of CAD, with a four-fold increase in prevalence in colder climates [5]. The monoclonal CA is typically of the immunoglobulin M (IgM) kappa isotype with anti-I specificity [7]. Less commonly, these may be immunoglobulin G (IgG) or immunoglobulin A (IgA) [8,9]. Binding of CA to the I antigen on the cell membrane of erythrocytes, occurring predominantly at acral sites of the circulation, results in agglutination and activation of complement via the classical pathway (Fig. 1). Opsonisation of erythrocytes by complement component 3b (C3b) leads to phagocytosis in the reticuloendothelial system with consequent extravascular haemolysis. In severe cases, there may also be a component of intravascular haemolysis. While CA demonstrate highest antigen affinity at a temperature of 0 to 4 ℃, depending on their thermal amplitude—defined as the highest temperature where agglutination occurs—this can reach up to 37 ℃ in some patients [10]. The I antigen is found on leucocytes and platelets as well; however, cytopenias affecting these lineages are rarely observed [11,12,13]. As such, where haemolytic anaemia and immune thrombocytopenia co-present, alternative diagnoses including Evans syndrome should be considered [14].

Primary CAD should be distinguished from cold agglutinin syndrome (CAS) (Table 1). In CAS, haemolysis arises secondarily to malignancy (namely B-cell lymphomas), infection (particularly *Mycoplasma pneumoniae* or Epstein-Barr virus [EBV]) or autoimmune disease [15].

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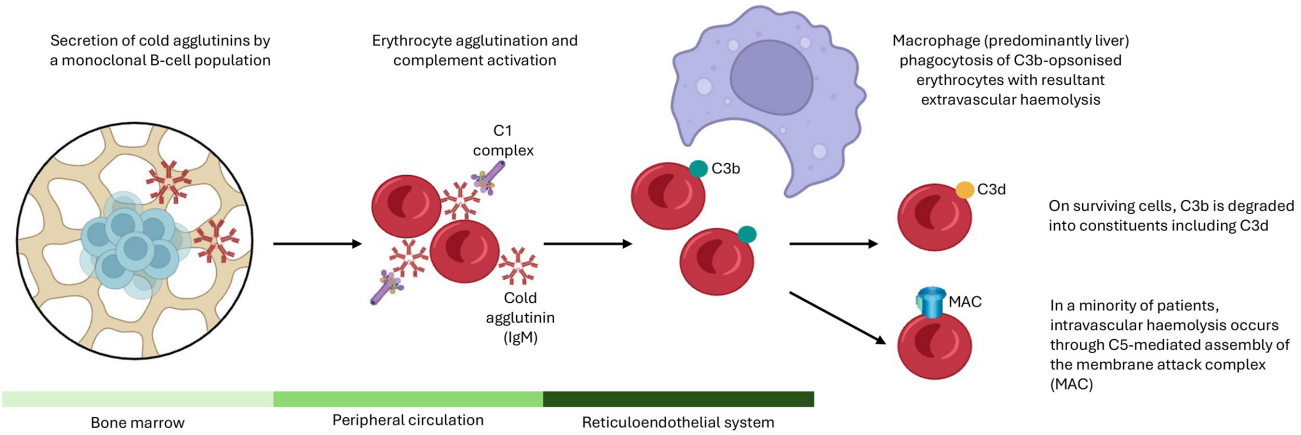

Fig. 1. Pathophysiology of cold agglutinin disease. Illustration created on BioRender (https://app.biorender.com/). C1, complement component 1; C3b, complement component 3b; C3d, complement component 3d; C5, complement component 5; IgM, immunoglobulin M.

Table 1. Key differentiating features of cold agglutinin disease versus cold agglutinin syndrome.

Characteristic	Cold agglutinin disease	Cold agglutinin syndrome
Aetiology	Primary autoimmune disorder	Secondary to infection (e.g., Epstein-Barr virus, *Mycoplasma pneumoniae*), malignancy (e.g., B-cell lymphoma), or autoimmune disease
Clonality of cold agglutinins	Monoclonal (typically IgM kappa)	Polyclonal predominant*
Bone marrow	Small, clonal B-cell lymphoproliferation histologically distinct from other B-cell lymphomas	Bone marrow findings depend on the aetiology; there may be infiltration by a B-cell lymphoma in lymphoma-associated CAS
Treatment	Treatment is directed at the pathogenic B-cell clone or involves inhibition of the complement cascade	Treatment is of the underlying disorder driving CAS

*The CA in CAS may be monoclonal in cases of lymphoma-associated CAS. Therefore, monoclonal CA alone cannot reliably distinguish CAD from all secondary CAS. IgM, immunoglobulin M; CA, cold agglutinins; CAS, cold agglutinin syndrome; CAD, cold agglutinin disease.

CA in CAS are predominantly polyclonal but may also be monoclonal in lymphoma-associated CAS; as such, a monoclonal CA does not definitively differentiate CAD from CAS.

The clinical manifestations of CAD are predominantly those of anaemia and cold-induced ischaemic symptoms, such as acrocyanosis and Raynaud's phenomenon [5,9]. These are often exacerbated during intercurrent febrile illnesses owing to upregulation of complement factors [16]. Gangrene or ulceration may occur in more extreme cases but should prompt evaluation for a concurrent cryoglobulinaemia, which has been reported to co-exist with CAD in over 50% of cases in a retrospective cohort analysis [17]. Concurrent clonal IgM type I or II cryoglobulinaemia may be a result of the same clone and is not considered a secondary CAS.

Patients with CAD have an increased risk of venous thromboembolism (VTE), particularly in those with highly active and brisk haemolysis [18,19,20]. CAD is also associated with a greater risk of arterial events such as ischaemic stroke.

Because of its relative rarity, CAD can present in varied clinical environments, posing diagnostic and therapeutic challenges. Furthermore, recognising the need for close liaison with the local specialist integrated haematological diagnostic service is integral to the management of this disorder. This review aims to outline the clinical presentation, diagnostic evaluation and management of CAD.

2. Diagnostic Workup and Laboratory Investigations

A diagnosis of CAD requires clinical and laboratory criteria to be met (Table 2). Chronic haemolysis is demonstrated by the presence of anaemia alongside reticulocytosis, hyperbilirubinaemia that is predominantly unconjugated, raised lactate dehydrogenase and low haptoglobin. It is important to concomitantly assess for other common contributors to anaemia, including haematinic deficiency and occult blood loss. A peripheral blood film should be examined, which may identify agglutinated erythrocytes when prepared from a sample at room temperature as well as spherocytes (Fig. 2). It is noteworthy that erythrocyte ag-

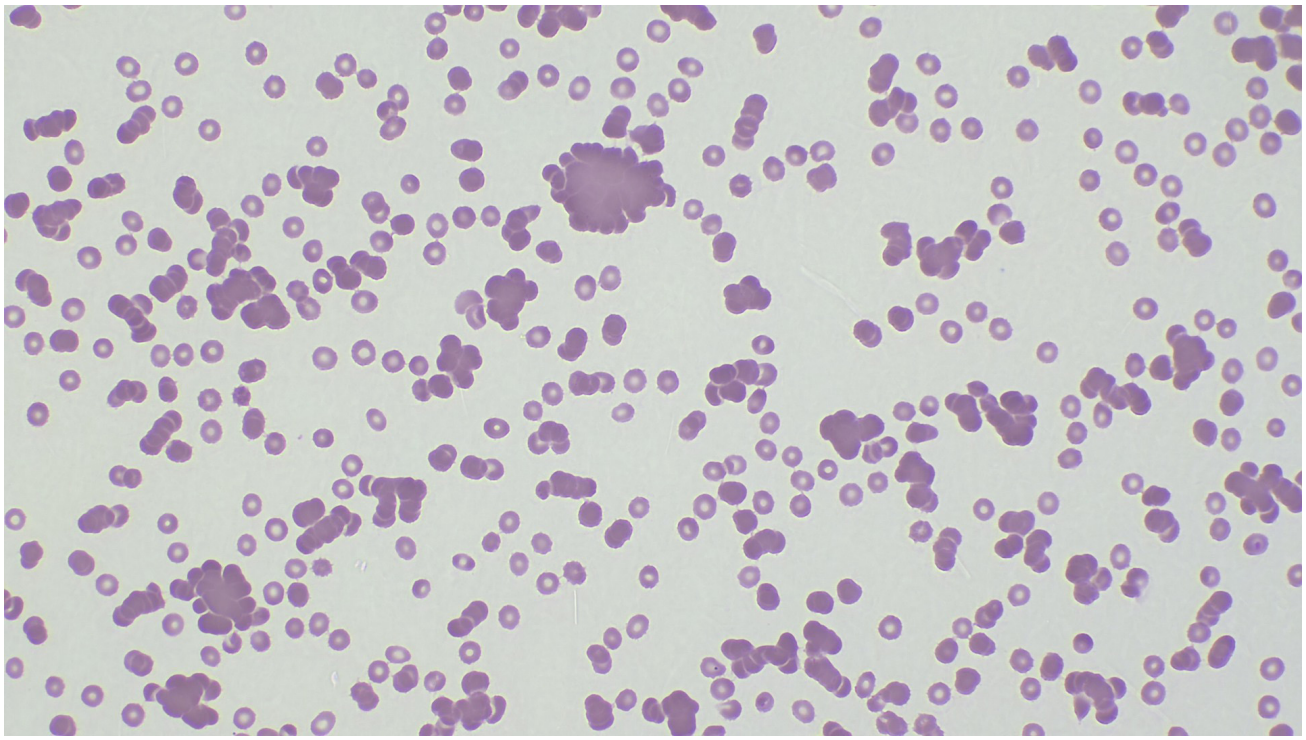

Fig. 2. Peripheral blood film (May-Grünwald-Giemsa stain, magnification ×400) demonstrating red blood cell agglutination.

glutination on a blood film can be an *in vitro* phenomenon. Thus, correlation with clinical and laboratory features is prudent.

The differentials for haemolysis are broad. A direct antiglobulin test (DAT) is an important investigation for identifying immune-mediated haemolysis. In CAD, the monospecific DAT is typically strongly positive for complement component 3d (C3d) and negative for IgG; however, in up to 20% of patients, the DAT is weakly positive for IgG as well [6,21]. A detailed transfusion history should be obtained to exclude the possibility of an alloimmune haemolytic transfusion reaction, which can occur up to 30 days post-transfusion. All patients should undergo manual blood group typing and antibody screening (group and screen [G&S]). However, strongly reacting CA will interfere with this, resulting in false positive results. Prewarming of samples to 37 ℃ in the laboratory is a necessary step to overcome this, although the process requires significantly more time than routine pre-transfusion testing to complete. An extended red cell phenotype and/or genotype may also be performed to facilitate blood component selection. Early liaison with the transfusion laboratory is therefore essential, especially when the blood group or antibody screen cannot be resolved; samples may need to be sent away to the reference red cell immune-haematology (RCI) service for further investigations.

Quantification of the CA titre is mandatory to formally diagnose CAD, and will be prompted by a C3d-positive DAT. This is determined through serial dilutions of serum incubated with reagent red cells at 4 ℃, until agglutination is no longer detectable *in vitro*. A titre of 1 in 64 or greater fulfils the diagnostic threshold, although this alone does not predict disease severity [22]. The thermal amplitude of CA is measured by combining serum with reagent red cells at increasing temperatures to the point where agglutination is not observed, with 28 ℃ or above considered the threshold for clinical significance [23]. Both the CA titre and thermal amplitude should be interpreted in the context of each other, alongside the patient's clinical presentation. Based on international consensus it is preferable that, where possible, blood is collected into 37 ℃-warmed ethylenediaminetetraacetic acid (EDTA) vacutainer tubes and transported in a temperature-controlled container (for example, a flask) until analysis in the laboratory [10,24]. However, this can be logistically challenging. Alternatively, EDTA-anticoagulated specimens may be re-warmed to 37 ℃ before testing, a practice that is supported by guidelines and adopted at many local centres [10]. It is therefore of utmost importance that clinicians communicate clearly with laboratory staff and adhere strictly to sampling requirements in accordance with their local standard operating procedures, as practices may vary, to minimise inappropriate specimens, preanalytical errors and delays in the provision of compatible blood.

Secondary causes must be excluded, namely malignant, infective and autoimmune disorders. The presence of B symptoms—fevers, drenching night sweats, and unintentional weight loss—lymphadenopathy and/or organomegaly would point towards an underlying lymphoma. Cross-sectional imaging, such as computed to-

Table 2. Diagnostic workup of cold agglutinin disease.

Tests		Specimen handling*	Key findings in CAD
Blood			
Haematology	• Full blood count • Reticulocyte count (absolute and percentage) • Blood film	Collection in EDTA tubes	Chronic haemolysis as evidenced by haematology (anaemia, reticulocytosis) and biochemistry (high lactate dehydrogenase, high total and unconjugated bilirubin, low haptoglobin) tests Erythrocyte agglutination, spherocytes and polychromasia on blood film
Biochemistry	• Renal profile • Liver function tests, including total and unconjugated bilirubin • Lactate dehydrogenase • Haptoglobin	Collection in serum separator tubes	
Transfusion	• G&S • DAT • Titre of CA • Thermal amplitude of CA	Collection in EDTA tubes Three samples for G&S are recommended, as these may need to be sent away to the RCI laboratory for further testing; if a patient does not have a historical blood group record, an additional specimen is necessary Samples for CA titration and thermal amplitude analysis should preferably be maintained at 37 ℃ from collection until analysis in the laboratory, with typical turnaround time for results being up to 10 days; where continuous temperature control cannot be maintained, re-warming to 37 ℃ in the laboratory is an alternative approach	Strongly positive monospecific DAT for C3d A CA titre of 1 in 64 or greater
Immunology	• Serum protein electrophoresis and immunofixation • C3 and C4 • ANA and ANCA	Collection in serum separator tubes Specimens for immunoglobulin analyses (including paraprotein quantification and immunofixation) should be strictly maintained at 37 ℃ from collection until separation of serum and clot in the laboratory	Monoclonal protein, typically of an IgM isotype Hypocomplementaemia from consumption Consider screening for autoimmune connective tissue and vasculitic disorders if there are suggestive clinical features
Microbiology and Virology	• *Mycoplasma* serology • Viral serologies for EBV, HIV, HBV, HCV and CMV	Collection in serum separator tubes	No evidence for an infective precipitant
Imaging			
Computed tomography of neck, chest, abdomen and pelvis		N/A	No evidence for a malignant pathology, especially lymphoma
Bone marrow			
Aspirate and trephine biopsy	• Morphology and cytochemistry • Flow cytometry • Cytogenetic and molecular analyses	Aspirate smears Aspirate in EDTA tubes Trephine in formalin	Clonal B-cell lymphoid proliferation on histology or flow cytometry No evidence for a B-cell lymphoma

Abbreviations: EDTA, ethylenediaminetetraacetic acid; G&S, group and screen; DAT, direct antiglobulin test; RCI, red cell immune-haematology; CA, cold agglutinins; ANA, antinuclear antibodies; ANCA, anti-neutrophil cytoplasmic antibodies; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; HBV, hepatitis B virus; HCV, hepatitis C virus; CMV, cytomegalovirus; C3, complement component 3; C4, complement component 4; IgM, immunoglobulin M; N/A, not applicable. *Local practices may vary between hospitals, so liaison with the affiliated laboratory is crucial.

mography (CT), can be a useful adjunct to localise clinically occult lesions. Furthermore, consideration should be given to relevant microbiological investigations, including *Mycoplasma* and viral (including EBV) testing, especially where the clinical assessment is suggestive of an infective syndrome. If the presentation raises suspicion for an associated autoimmune connective tissue disorder or vasculitis, for example with cutaneous and joint symptoms, then testing for antinuclear antibodies (ANA) and anti-neutrophil cytoplasmic antibodies (ANCA) could be indicated. Complement component 3 (C3) and complement component 4 (C4) are often consumed in both CAD and CAS [25].

A bone marrow aspirate and trephine biopsy is required for all patients with suspected CAD. It should be evaluated by morphology, immunophenotype (via flow cytometry), cytogenetic and molecular analyses. In CAD, the bone marrow trephine will typically demonstrate a clonal, kappa light chain-restricted B-cell infiltrate, often organised into nodular aggregates, surrounded by plasma cells, without histological features of other B-cell lymphoma types, for example, the presence of lymphoplasmacytic cells [3,26,27]. Serum protein electrophoresis and immunofixation may identify an IgM paraprotein, which would support the premise of an associated clonal lymphoproliferative disorder. Samples for immunoglobulin analyses should be sent in a temperature-controlled manner at 37 ℃ until laboratory processing to prevent irreversible integration of antibodies within the clot. Confirmation of the B-cell clone occurs in up to 90% of cases, although its absence does not preclude a diagnosis of CAD, as detection may be limited by the diagnostic sensitivity for low-level clones [9]. Indeed, a large study of bone marrow histology in patients with cold AIHA reported that only 11% of CAD cases fulfilled the full WHO morphological criteria for CAD-associated lymphoproliferation [2,27]. The *MYD88* L265P mutation, characteristic of lymphoplasmacytic lymphoma, is usually not detected [3,27,28]. Accordingly, dedicated histopathology input is vital to the diagnosis of CAD.

3. Case Study

A 62-year-old male, in remission from previously treated Hodgkin's lymphoma since 2016, presented to the Emergency Department with a three-day history of jaundice, fatigue, dyspnoea, abdominal distension, and profound livedo reticularis. On admission, his haemoglobin was 18 g/L, platelet and white cell counts were 293 × 10^9/L and 17.9 × 10^9/L, respectively, bilirubin was 256 µmol/L, and lactate dehydrogenase was 847 U/L. There was marked erythrocyte agglutination on the blood film. A CT abdomen and pelvis scan revealed hepatosplenomegaly without significant lymphadenopathy. Subsequent red cell immunohaematology investigations showed a pan-reactive antibody panel and a strongly positive (3+) DAT for C3d at 37 ℃. Immunofixation identified an IgG paraprotein that was too small to quantify. Serology for *Mycoplasma* was negative, as was an autoimmune disease screen that included ANA and ANCA. His EBV IgG was positive and IgM negative, but the viral load was undetectable by polymerase chain reaction. His bone marrow demonstrated a small, phenotypically abnormal (cluster of differentiation [CD]5^-, 10^-, 19^+, 20^+, 23^-, kappa-restricted) B-cell population that accounted for 0.5% of total cellularity, implying an underlying clonal disorder. No other abnormal lymphoid or plasma cell infiltrate was identified to indicate an associated B-cell lymphoma or plasma cell dyscrasia, respectively. A positron emission tomography scan was also performed, which showed no radiological evidence of relapse of his Hodgkin's lymphoma or another *de novo* lymphoma. The presence of strongly reacting cold antibodies impeded the laboratory measurement of a CA titre. Nonetheless, the clinical presentation in addition to evidence of immune haemolysis with monospecific positivity for C3d on DAT and demonstration of a B-cell clonal lymphoproliferation were collectively consistent with a diagnosis of primary CAD with a rare circulating IgG monoclonal protein.

Recognising the risk of decompensation with life-threatening organ injury from significant immune-mediated haemolytic anaemia, despite the absence of formal serological confirmation to diagnose CAD, emergency treatment was instigated. He was treated initially with 1 g/kg of intravenous immunoglobulin (IVIG) and prednisolone 1 mg/kg, alongside frequent blood transfusions whilst investigations were being arranged. The response was limited, and he commenced treatment with the anti-cluster of differentiation 20 (CD20) monoclonal antibody rituximab at 375 mg/m^2 in combination. He remained refractory to these therapeutic approaches with ongoing severe haemolysis. He was treated with eculizumab 900 mg, an inhibitor of the complement component 5 (C5). He received two doses of weekly rituximab and eculizumab, respectively. An associated cryoglobulinaemia was detected, and plasma exchange was initiated for three days. Haemolysis persisted, and thus daratumumab—an anti-cluster of differentiation 38 (CD38) monoclonal antibody—was initiated.

Daratumumab at 1800 mg was administered weekly for a total of six doses. Despite a mid-treatment delay enforced by a concurrent infection, his treatment course successfully resulted in a reduction in his transfusion requirements. His haemoglobin improved and stabilised to between 90 and 110 g/L.

His disease course was complicated by a prolonged intensive care unit admission for multi-organ support, compounded by sepsis, pneumonitis, acute renal failure and liver injury—highlighting the severity of his illness. He also developed an ischaemic stroke with right-sided middle cerebral artery infarct. This was managed initially with aspirin and then cautiously switched to therapeutic enoxaparin after multidisciplinary discussion between the Stroke and Haematology teams to mitigate his thrombotic risk.

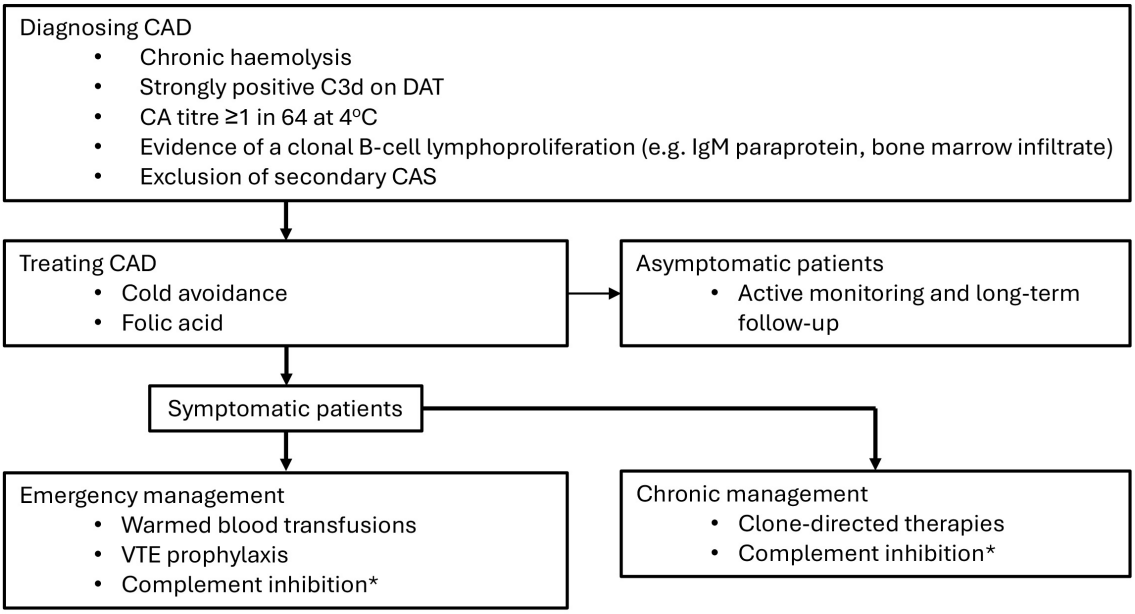

Fig. 3. Diagnosis and management overview of cold agglutinin disease. Empirical emergency treatment should be considered in clinically unstable patients with brisk haemolysis from suspected CAD, even in the absence of complete serological confirmation. Illustration created in Microsoft PowerPoint (version 16.114.3, Microsoft, Redmond, Washington, USA). Abbreviations: CAD, cold agglutinin disease; DAT, direct antiglobulin test; CA, cold agglutinins; CAS, cold agglutinin syndrome; VTE, venous thromboembolism. *Unlike clone-directed therapy, complement inhibition suppresses haemolysis but does not address cold ischaemic symptoms and requires continuous treatment.

Unfortunately, he soon after experienced a haemorrhagic transformation, which was managed conservatively. He was thereafter transferred to a dedicated neurorehabilitation unit for post-stroke recovery.

He now remains off-treatment, transfusion-independent, and under active surveillance as an outpatient with an undetectable paraprotein more than three years since his diagnosis.

Key Learning Points From the Case Study

- Strongly reacting antibodies may interfere with laboratory diagnostic tests, including the titration of cold agglutinins.
- Clinically unstable patients with suspected cold agglutinin disease may commence empirical therapy without full serological confirmation, after due consideration of risks versus benefits.
- Cold agglutinin disease confers a risk of both arterial and venous thromboses. Decisions to anticoagulate require careful balancing of thrombotic and bleeding factors.

4. Management

4.1 General Management

The management of CAD comprises specific pharmacotherapy and general supportive measures (Fig. 3).

Supportive care is crucial for all patients, which most importantly emphasises cold exposure avoidance [24]. This includes utilising a warming device for intravenous infusions, such as blood transfusions, alongside other day-to-day adaptations for thermal insulation, particularly of one's extremities. Cold avoidance prevents the binding of CA to erythrocytes and agglutination, serving to protect against not only ischaemic symptoms but also haemolysis. Patients should also be counselled to be aware that their condition could decompensate during periods of intercurrent illness or surgery, especially in the setting of cardiopulmonary bypass. Peri-operative optimisation is crucial as thermoregulation can be impaired by general anaesthesia and operating rooms are typically cold environments [29,30,31]. Infections should be treated promptly to mitigate the risk of acute phase reaction-driven exacerbation of haemolysis, mediated by upregulation of complement proteins. Long-term folic acid 5 mg once daily supplementation is advisable as these patients have chronic haemolysis [10].

Thromboprophylaxis is recommended for hospitalised patients with acute haemolysis, and there should be a low threshold to screen for deep vein thrombosis or pulmonary embolism if symptoms are suggestive [24]. Patients who develop VTE should be considered for long-term anticoagulation given the chronicity of CAD and their susceptibility to future exacerbations of haemolysis, although no guidance currently exists. This should therefore be reviewed on a case-by-case basis with a haematologist with expertise in haemostasis, factoring in each individual's thrombotic and bleeding risks.

Table 3. Comparison of selected clinical studies of key therapeutic agents for cold agglutinin disease.

Drug(s)	Study design	Sample size	Primary response criteria	Overall response rate (%)	Reference
Rituximab	Prospective, non-randomised	27	Achievement of a complete (normalisation of haemoglobin, absence of haemolysis, resolution of circulatory symptoms, and disappearance of the paraprotein and bone marrow B-cell clone) or partial (normalisation of or increase in haemoglobin by 10 to 20 g/L, reduction in the paraprotein by at least 50%, symptom improvement, and transfusion independence) response [15]	54	[33]
Rituximab	Prospective, non-randomised	20		45	[34]
Rituximab + bendamustine	Prospective, non-randomised	45		71	[32]
Rituximab + bendamustine	Retrospective[#]	45		78	[5]
Sutimlimab	Phase three randomised, placebo-controlled	22	Haemoglobin increase by 15 g/L from baseline*	73	[35]
Sutimlimab	Prospective, non-randomised	24	Composite of haemoglobin normalisation to 120 g/L or an increase by 20 g/L from baseline*	54	[36]

[#]This was a longer-term, follow-up re-evaluation of the patient cohort enrolled on the prospective Nordic 2017 trial [32]. *Without recourse to blood transfusions or medications prohibited by the trial protocol. Adapted from Berentsen (2021) [24].

Patient and clinician education is an important adjunct in supporting the management of CAD through improving understanding of the condition, thereby empowering patient engagement. In recognition of this, accessible information leaflets were developed and are included in **Supplementary Materials 1,2**, aimed at patients and healthcare professionals, respectively.

4.2 Specialist Management

Indications for treatment include symptomatic anaemia, significant circulatory symptoms, transfusion-dependence, or thrombosis [24]. Individuals with fatigue in the absence of anaemia, or mild anaemia who are asymptomatic in general, do not require treatment.

Targeting the pathological B-cell clone driving CAD is the primary therapeutic strategy. Rituximab, an anti-CD20 monoclonal antibody, is long established and considered first-line. Prospective non-randomised studies of rituximab monotherapy dosed at 375 mg/m^2 weekly demonstrated an overall response rate of approximately 50%, with a median response duration and increase in haemoglobin amongst responders of up to 11 months and 40 g/L respectively (Table 3, Ref. [5,15,24,32,33,34,35,36]). Rituximab and bendamustine for four cycles yielded higher response rates of 71 to 78% and more durable median responses of over 32 months [5,32].

Other targeted clone-directed therapies have been utilised for CAD, although evidence in their favour predominantly arises from small-scale, often retrospective clinical studies and case reports. Bortezomib, a proteasome inhibitor, and the anti-CD38 monoclonal antibody daratumumab both constitute the backbone of multiple myeloma treatment but have shown efficacy in the management of CAD [37,38,39,40,41,42,43,44]. The effectiveness of daratumumab likely arises because of its activity against long-lived plasma cells that express CD38, which have been selected out by prior B-cell depleting therapy [38]. Bruton's tyrosine kinase inhibitors, such as ibrutinib, are commonly prescribed in lymphoproliferative disorders including Waldenstrom's macroglobulinaemia and chronic lymphocytic leukaemia [45]. In CAD, they have similarly demonstrated an improvement in haemolytic anaemia and acrocyanosis [46]. There continue to be active trials investigating their use in this disorder. In the United Kingdom, the use of these clone-directed therapies is currently off-label.

Inhibition of the complement system is another therapeutic strategy [35,36,47,48]. However, as complement-directed therapies do not eliminate the CA-producing lymphoid clone, they have no effect on ischaemic symptoms which are mediated by CA in a complement-independent fashion. Moreover, patients treated with complement inhibitors must receive appropriate vaccination against encapsulated bacteria (including meningococcus, pneumococcus, and *Haemophilus influenzae b*) owing to the risk of infective complications.

Sutimlimab is the best studied complement-targeting drug in CAD, although despite being approved for this indication it is not universally funded [35,36]. It is a monoclonal antibody against the complement component 1s, inhibiting activation of the classical pathway. Owing to the chronic nature of CAD, discontinuation of sutimlimab—and indeed complement inhibitors in general—risks recrudescence of haemolysis, necessitating indefinite maintenance treatment [49,50].

The anti-C5 monoclonal antibody eculizumab targets the terminal complement pathway and is likely more beneficial in patients in whom intravascular haemolysis predominates [48]. It is unlicensed in CAD—being more

widely used in other complement-mediated disorders such as paroxysmal nocturnal haemoglobinuria—but may be an option in the event of catastrophic haemolysis.

4.3 Emergency Management

In the acute setting, patients may present with decompensated haemolysis that can be accompanied by life-threatening anaemia and multi-organ dysfunction. Transfusions may be necessary in severe or symptomatic anaemia, and these should be warmed and ABO, Rh-, and K-matched red blood cells that are negative for any atypical erythrocyte antigens that the patient has alloantibodies against. However, in an emergency where cross-match compatible products are not readily available, patients should be given group O, RhD-negative and K-negative blood. Specialist advice may be needed if atypical erythrocyte alloantibodies are detected, particularly in patients who have been extensively transfused. Precipitants for decompensation should be identified and treated in a timely manner.

Where rapid disease stabilisation is required, or where there is co-existing cryoglobulinaemia, plasmapheresis can be considered for direct removal of circulating antibodies, although the evidence base is mostly anecdotal and responses are transient given persistence of the CA-producing clone [10,17,51]. Complement inhibition may halt brisk haemolysis. Trialling a course of corticosteroids (for example, prednisolone 1 mg/kg/day), with prompt tapering, is an option despite variable efficacy with response rates of 14 to 69% [24]. As a result, expert opinion dissuades their routine use, and this is instead established for the treatment of warm AIHA [24]. IVIG, similarly more frequently prescribed in warm AIHA, has also been used as rescue therapy in CAD; however, there is likewise a paucity of data supporting its benefit with a French observational study reporting only two responders amongst six patients [52]. Plasmapheresis, corticosteroids, and IVIG therefore have a limited role in the first-line treatment of CAD and may be reserved for emergency rescue in selected cases.

5. Current Challenges and Future Perspectives

Achieving a diagnosis of CAD requires demonstration of the CA titre, supported by measurement of its thermal amplitude. These tests are prone to pre- and intra-analytic errors, and their turnaround time can reach up to 10 days as input from specialist RCI centres is often needed. Delays in securing formal diagnostic verification could risk destabilising patients with active and uncontrolled haemolysis. This emphasises a need to refine laboratory workflows, and a recognition that clinicians may be expected to commence treatment empirically in the face of incomplete data, particularly amongst unwell patients.

There have been advances in defining the histopathological CAD entity. Exclusion of a secondary CAS is imperative. Recent findings demonstrated "CAD-like" molecular profiles despite morphological features resembling lymphoma in the bone marrow that would otherwise be compatible with CAS under the current WHO criteria [53]. This underpins the importance of incorporating wider genetic analyses in diagnostic criteria, although validation on larger cohorts is required. Moreover, there is a need to optimise the sensitivity of diagnostic assays to low-level B-cell clones in the bone marrow.

Despite recent advances in therapeutic options for CAD, head-to-head randomised controlled trials comparing different approaches are lacking. Furthermore, complement inhibitors remain hindered by their need for continuous treatment and a lack of efficacy in addressing circulatory symptoms. There is also a need to better understand the risk of VTE beyond acute haemolytic episodes, and the role of thromboprophylaxis in chronic CAD. These collective limitations are in no small part a reflection that CAD is a rare disorder and can be phenotypically highly variable between patients. Thus, current management guidance is reliant on the recommendations of experts. Centralising prospective patient data in an international registry may improve cross-collaborative efforts to enhance patient stratification and individualisation of optimal treatment regimens.

6. Conclusion

CAD represents a complex disease that frequently poses investigational and management difficulties to clinicians. Mishandling of specimens for serological testing is a common pitfall, requiring fastidious adherence to local laboratory practices. However, confirmation of the diagnosis can still be fraught with multiple challenges, and in certain scenarios the decision to initiate treatment may be made based on composite diagnostic reasoning. Therefore, where there is a high index of suspicion clinically, correlated morphologically and by haemolytic biomarkers, empirical CAD-directed therapy should not be delayed pending verification of the diagnosis, especially in unstable patients, although complete diagnostic workup should still be pursued for clinically stable individuals. Supportive care is important for all individuals with CAD. Where there is symptomatic disease, specific pharmacotherapy targeting the B-cell clone and/or inhibiting the complement pathway should be considered, although management approaches are predominantly derived from expert consensus rather than randomised trials.

Key Points

• Cold agglutinin disease is a rare autoimmune haemolytic anaemia, driven by cold agglutinins from an underlying clonal B-cell lymphoproliferation in the bone marrow.

• Cold agglutinins are typically IgM that react with erythrocytes optimally at a temperature of 0 to 4 ℃, resulting in complement-mediated haemolysis.

• Patients with CAD present with haemolytic anaemia and cold-induced ischaemic symptoms often affecting the extremities, which can be exacerbated by intercurrent febrile illnesses or surgery.

• Diagnostic confirmation is achieved by a strongly positive monospecific DAT for C3d, CA titre of 1 in 64 or greater, and exclusion of secondary causes; demonstration of a paraprotein and a bone marrow B-cell clone are supportive of the diagnosis.

• Close liaison with laboratory staff and adherence to local protocols are vital to ensure diagnostic samples are handled appropriately, minimising preanalytical errors.

• Cold avoidance is the mainstay of management, and where treatment is indicated in symptomatic patients, this is typically through B-cell-targeting therapies in the first line. Corticosteroids and IVIG have limited efficacy in CAD and are reserved only for emergency rescue.

Availability of Data and Materials

Patient-level images and related data cannot be shared publicly to protect confidentiality; anonymised supporting materials are available from the corresponding author on reasonable request.

Author Contributions

TW: Writing—original draft, Formal analysis; NJ: Resources, Formal analysis; ETB: Formal analysis; KX: Resources, Formal analysis; SAli: Formal analysis; SAkh: Formal analysis; MT: Formal analysis; JL: Formal analysis; CK: Formal analysis; SD: Conceptualisation, Formal analysis, Supervision; JK: Conceptualisation, Formal analysis, Supervision. All authors contributed to the important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Fig. 2 is an original laboratory image with no identifiable patient information. Informed consent was waived because the risk of identifying any individual is minimal. Written informed consent was obtained from the patient for the publication of the case reported in the "Case Study" section. This study conforms to the ethical principles outlined in the Declaration of Helsinki.

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Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/BJHM57786.

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