CASE REPORT

Cold agglutinin-mediated autoimmune haemolytic anaemia associated with diffuse large B cell lymphoma

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SUMMARY

Cold agglutinin-mediated autoimmune haemolytic anaemia is associated with the development of autoantibodies that can agglutinate red blood cells at cold temperatures. While primary cold agglutinin disease is an idiopathic lymphoproliferative disorder, secondary cold agglutinin syndrome (CAS) complicates other diseases such as infections, autoimmune diseases and cancers, mostly low-grade lymphomas. Early recognition, treatment of CAS and treatment of its associated underlying diseases are crucial to a successful outcome. We report a case of CAS in a setting of diffuse large B cell lymphoma, in which the treatment course was complicated by worsened anaemia due to chemotherapy-induced myelosuppression. We reviewed previously reported cases and discussed diagnosis and treatment strategies, including novel complement inhibitors, as potential future therapy.

BACKGROUND

Autoimmune haemolytic anaemia (AIHA) can be classified into three types according to autoantibodies: warm type, cold type and mixed type.^{1–3} Warm-antibody AIHA (WA-AIHA) accounts for approximately 75% of the cases. Warm antibodies show optimal binding at 37°C and are mostly IgG subtype. Red blood cells (RBCs) coated with IgG with or without complement fragments are preferentially removed via Fc-gamma receptor-mediated phagocytosis in the spleen, whereas RBCs coated with activated C3b, are destroyed via complement-receptor mediated phagocytosis in the liver. Both mechanisms lead to extravascular haemolysis.²

For cold-antibody AIHA (CA-AIHA), the antibodies optimally bind to RBCs at 0°C–4°C and are mostly IgM subtype. Most cold agglutinins are specific for antigens of the I/i/Pr systems. Because IgM forms a pentameric or hexameric structure, it has a high efficacy to activate the complement system resulting in C3 cleavage and deposition of C3b on erythrocyte surface. C3b binds to the complement receptors on phagocytes leading to erythrocytes destruction in the liver. C3b is converted to C3d on the surviving erythrocytes leading to a positive direct antiglobulin test (DAT) for C3d, a key diagnostic feature of CA-AIHA.^{1 2 4 5} In some cases, complement activation may progress beyond the C3b formation step, resulting in C5 activation, formation of the membrane attack complex and finally intravascular haemolysis.^{1 2 6 7}

CA-AIHA accounts for 25% of AIHA and can be further subdivided based on the presence of underlying or associated disorder, primary cold agglutinin disease (CAD) and secondary cold agglutinin syndrome (CAS).¹ Primary CAD is a rare idiopathic clonal lymphoproliferative disorder with an incidence of 1:1 000 000.^{1 4 8} In a case series of CAD, more than 90% of patients had detectable serum kappa light chain monoclonal IgM antibodies or clonal bone marrow lymphoid cells, supporting the notion of CAD as a lymphoproliferative disease.⁵ In some cases, lymphoma diagnoses may become apparent long after the original CAD presentation, suggesting occult lymphoma as an aetiology of CAD.⁹ A diagnostic workup of CAD should, therefore, include investigating the underlying diseases, as treating the underlying disease is required for a successful long-term disease control.^{7–10} Primary CAD has well-defined characteristics and should be separated from secondary CAS which is a syndrome associated with an autoimmune condition or infections (*Mycoplasma pneumoniae*, Epstein-Barr virus and HIV) in half of the cases. The remaining cases are commonly associated with underlying clonal B-cell disorders.^{1 4 6 8} CAS in other cancers including sarcoma, metastatic melanoma and chronic myeloproliferative disorder have been reported in case report forms.^{8 10} The incidence of CAS and the casual relationship of CAS to these cancers are difficult to determine. Some cases are possibly misdiagnosed based on the lack of uniform diagnostic criteria and molecular or immunohistochemical technologies to support the diagnosis beyond the pathological appearance. A few cases of sarcoma associated CAS that were reported in the 1980s might have been misclassified, as the described pathology is more fitting with the current WHO classification of splenic marginal zone lymphoma.¹¹

Patients with CAS with diffuse large B cell lymphoma (DLBCL) have been reported,^{9 12–19} however the disease course and response to therapy when CAS is associated with DLBCL are still unknown due to its rarity. We describe an elderly patient with severe CAS and DLBCL, summarise other previously published cases and discussed novel therapies including complement inhibitors.

CASE PRESENTATION

A 75-year-old woman with underlying hypertension, asthma and hypothyroidism presented with shortness of breath and dyspnea on exertion for 2 weeks with no history of abnormal bleeding. Physical examination revealed a tachycardic patient in

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Figure 1 H&E staining of peripheral blood smear. (A) x50 magnification, (B) x100 magnification. Peripheral blood smear shows anisopoikilocytosis with spherocytes, red blood cell agglutination and nucleated red cells. (C) H&E staining of lymph node biopsy shows large mononuclear cell infiltration, confirmed by immunohistochemical staining to be positive for CD20, CD10, CD45, BCL6, BCL2 and MUM1, compatible with diffuse large B cell lymphoma. (D) Staining for Ki67 shows near 100% expression suggesting for highly proliferative tumour. H&E staining of bone marrow aspirates: (E) x20 magnification, (F) x100 magnification. The bone marrow aspirates reveal hypercellular marrow with both erythroid and myeloid hyperplasia, with left shift. There was no evidence of bone marrow involvement by diffuse large B cell lymphoma.

mild distress with pale and icteric sclerae. There were no bruises, hepatosplenomegaly or lymphadenopathy. Complete blood counts showed an haemoglobin of 3.9 g/dL with an absolute reticulocyte count of $0.3 \times 10^{12}/L$, white cell count of $4.9 \times 10^9/L$ and a platelet count of $174 \times 10^9/L$. A peripheral blood smear showed anisocytosis and poikilocytosis with spherocytes, RBC agglutination, polychromasia and nucleated red cells (figure 1A, B). Blood chemistry showed lactate dehydrogenase (LDH) of 702 U/L, haptoglobin less than 6 mg/dL, total bilirubin 3.4 mg/dL, indirect bilirubin 2.8 mg/dL. DAT was positive for C3d and negative for IgG and IgM. Cold agglutinin titer was 1:1280. Serology for *M. pneumoniae*, Epstein-Barr virus, viral hepatitis and HIV were negative. Serum protein electrophoresis, immunofixation and serum free light chain were unremarkable. Diagnosis of CA-AIHA was made. The patient was transfused with 6 units of packed RBCs over 48 hours and started on prednisone 1 mg/kg per day and 1 g/kg of intravascular immunoglobulin (IVIG) with a modest improvement in haemoglobin to 5 g/dL. Once the diagnosis of CA-AIHA was made, rituximab 375 mg/m² was given. After the first week of hospital care, the haemoglobin remained at 5–5.5 g/dL.

To investigate for the underlying lymphoproliferative disorders, she underwent CT of chest, abdomen and pelvis, which revealed adenopathy at multiple sites including left axillar, left hilum, mediastinum, periaortic region, periceliac region, porta hepatis and right groin. Groin node aspiration and biopsy showed atypical kappa-restricted lymphoid cells which were positive for CD20, CD10, CD45, BCL6, BCL2 and MUM1, compatible with DLBCL with high proliferative activity (Ki67 is near 100%) (figure 1C,D). An immune-chemotherapy regimen, rituximab-cyclophosphamide-doxorubicin-vincristine-prednisone (R-CHOP), was started. Absolute reticulocyte count response significantly declined from $287 \times 10^9/L$ in the first 3 days of chemotherapy to $77 \times 10^9/L$. Concurrently, white cells and platelets declined to the lowest level on day 7 of the cycle at $2.0 \times 10^6/L$ and $98 \times 10^9/L$, respectively. She continued to have a lack of response to transfusion, likely due to ongoing haemolysis, now complicated by chemotherapy-induced myelosuppression. Erythropoietin, iron, B₁₂ and folate, as well as, oxygen supplementation were given. She also continued to receive weekly rituximab to complete four doses and went to receive full dose R-CHOP for two more cycles with no major

complication. A bone marrow biopsy was done 14 days after R-CHOP showed hypercellular marrow with both erythroid and myeloid hyperplasia, with a left shift. There was no evidence of bone marrow involvement by DLBCL (figure 1E,F). Reticulocyte count improved to $80 \times 10^9/L$ by day 18 post R-CHOP and haemoglobin improved to 6.8 g/dL. The repeat cold agglutinin titre was 64 before she was discharged home.

OUTCOME AND FOLLOW-UP

Haemoglobin continued to improve to 11.6 g/dL within the first week of discharge. The patient continued weekly rituximab to complete four doses and went on to receive full dose R-CHOP for two more cycles with no major complications. A repeat CT scan at a community hospital demonstrated improved nodal enlargement after the first three cycles of therapy. She received three more cycles with a plan for repeat staging after the end of six cycles.

DISCUSSION

DLBCL, the most common type of lymphoma, accounts for 30% of all non-Hodgkin's lymphomas (NHLs). Patients present with nodal enlargement, although less common extranodal involvement has also been reported.^{12 16} Anaemia associated with DLBCL is typically secondary to bone marrow involvement or blood loss in the case of gastrointestinal involvement. Immune-mediated haemolysis is a rare cause of anaemia in patients with DLBCL, with over 80% of cases being IgG-mediated WA-AIHA.^{8 12 13} CAS in a setting of DLBCL is rare and the actual incidence is unknown. Hauswirth *et al* reviewed 121 cases of NHL and AIHA or Evan's syndrome previously reported in PubMed and Medline, 10 cases of CAS and DLBCL were noted.¹⁵ Advanced stage with bone marrow involvement occurs in a majority of cases but local extranodal presentation including isolated gastric involvement has been reported.^{12 13 16 17 19} Recurrent chromosomal abnormalities including trisomy 3 and 12 were reported in two cases, one of which also had a translocation (8;22).^{13 14} Trisomy 3 is a frequent aberration in activated B-cell-like subtype, but trisomy 12 is rare.²⁰ The importance of these abnormalities in CAS-associated DLBCL is unknown.

Patients with CAS presented with signs and symptoms of haemolytic anaemia. Laboratory parameters showed an increased reticulocyte count, decreased serum haptoglobin, elevated serum LDH, increased serum indirect bilirubin and positive DAT against complement C3d on the surface of RBCs. Peripheral blood smears reveal spherocytes and RBC agglutination.^{1 3 5 10} The rates of haemolysis and reticulocyte response are key to disease severity and treatment decisions. Non-pharmacological management in CAS such as cold avoidance should be considered in all patients and may be sufficient in mild cases.^{1 10} For elective surgeries or during infection, the application of body-warming blankets and prewarming of intravascular fluids and blood products may minimise the exacerbation of the haemolysis.⁴ In symptomatic cases, despite supportive approaches, drug therapy is indicated. Steroid is generally less effective in CA-AIHA (response rate of 15%–30%) compared with WA-AIHA.¹⁰ Patients may require a higher steroid dose.⁵ While many reports revealed a lack of success of steroid therapy in CA-AIHA, some case reports do indicate that steroids can be effective in some subgroups of patients, particularly those with IgG cold agglutinin.³ Splenectomy is not recommended as a treatment in CAS, as RBC destruction is known to primarily occur in the liver.^{1 3 5} Similarly, IVIG has limited value as noted in our patient who received the IVIG before CAS was

confirmed.³ The B-cell targeting antibody, rituximab is the gold standard to decrease autoantibody production in patients with CAD.^{1–3 5 8} Treating with rituximab 375 mg/m² weekly times 4, yields a 45%–58% complete response, with the median time to response of 1.5 months and the median response duration of 11 months in patients with CAD. The response rate was increased to 76% with rituximab combined with fludarabine, however more toxicities were reported.^{2 4 5} The recent prospective, non-randomised, multicentre trial published in 2017 reported that rituximab–bendamustine therapy was highly efficient, safe and may be considered as a first-line therapy for patients with CAD.²¹

Anaemia at the presentation of CAD can be severe as 50% of patients require a red cell transfusion.^{4 5 8} RBC transfusion only provides a transient improvement of anaemia and should only be considered in severe and acute cases. Transfusions should be done using an in-line blood warmer and started at a slow rate due to increased risk of transfusion reactions. Exacerbations of haemolysis can be triggered by fever, trauma and surgery.^{22–25} Nutritional supplements to support red cell production including folate and vitamin B₁₂, recombinant erythropoietin to drive red cell production and oxygen supplementation to increase oxygen-carrying capacity, are beneficial supportive care.^{1 8}

While B-cell targeting treatments decrease further production of monoclonal IgM, they do not clear circulating IgM nor inhibit haemolysis of Ag-Ab complex bound red cells. Plasmapheresis can be utilised to remove the circulating IgM autoantibodies in severe cases to transiently attenuate haemolysis until a more specific therapy to decrease autoantibody achieves the therapeutic effects.^{1 3 4} However, such a strategy requires a central venous catheter, which is difficult to place in an unstable patient, and is technically challenging as despite warming of exposed tubing and intravenous fluid, RBC agglutination may still occur as blood passages through the apheresis machine causing clots in the tubing and microthrombi when blood passes back to the patient. In the past few years, novel pathway inhibitors have become available, and some are already approved for other complement-mediated haemolysis such as the C5 inhibitor eculizumab which is approved for use in paroxysmal nocturnal haemoglobinuria.^{2 5 26} A case of successful use of eculizumab in CAD was reported by Röth and colleagues.²⁷ Since the driver complement that mediates haemolysis in CAD is C3b, complement inhibitors that block directly or upstream of C3 would have the best efficacy. C3 is an intersecting point of classical and alternative complement activation pathways. The disadvantage of C3 targeting is infection risk, particularly as eculizumab has already been associated with meningococcal infection.^{2 26} A mouse monoclonal antibody TNT003 and a humanised monoclonal antibody TNT009 target the C1s subunit of C1. They have promising efficacy in vitro and in animal models and are currently undergoing early phase clinical trials.^{26 28 29} In a study of 40 CAD patient samples, TNT003 prevented cold agglutinin-mediated deposition of complement opsonins, and completely inhibited in vitro haemolysis. The production of anaphylatoxins C4a, C3a and C5a were also inhibited.²⁸ In theory, TNT003 and TNT 009 should block only the classical pathway and inhibit C3b-mediated extravascular haemolysis without interfering the alternative and lectin complement pathway, therefore not interfere with the generation of anaphylatoxins C3a and C5a in response to infection.²⁸ These inhibitors should provide for rapid control of haemolysis, while the underlying disease is determined. To date, complement inhibitors have only been explored in CAD.² Whether this treatment could rapidly stabilise haemolysis and allow patients to tolerate chemotherapy

better in cases of CAS associated lymphoma, warrants future research. Treatment of the underlying diseases remains the mainstay of CAS therapy.

The clinical course of CAS associated with lymphoma is less clear due to the heterogeneity of the underlying diseases. Treatment regimens for lymphoma commonly contain rituximab, in combination with some chemotherapy including alkylators (bendamustine or cyclophosphamide) or purine analogue (fludarabine); R-CHOP.^{12 19} Myelosuppression from these agents is a common side effect and its extent is dose-dependent.⁸ Myelosuppression resulting in a reduced reticulocyte response and a decline in haemoglobin is a major concern, particularly in cases of the elderly with low cardiopulmonary reserve. In our case, R-CHOP for DLBCL is likely the cause of the delayed haemoglobin response as the reticulocyte counts dropped to a very low level at mid cycle of chemotherapy. This hypothesis was supported by the bone marrow hyperplasia and a rapid rebound of reticulocytes production prior to the subsequent cycle of therapy. Dose attenuation of chemotherapy may strike a good compromise between controlling CAS and DLBCL, although there is no published data to support this practice. In addition, there is no data comparing sequential therapy, that is, weekly rituximab for CAS until haemoglobin is stable followed by treatment of the underlying lymphoma, to concurrent therapy.

Learning points

- Cold agglutinin syndrome (CAS) is a rare presentation of diffuse large B cell lymphoma.
- Treatment of the underlying disease will continue to be the mainstay of therapy in patients with CAS.
- Immuno-chemotherapy, the standard treatment for lymphoma, may cause myelosuppression, slow down reticulocyte response and transiently worsen anaemia.
- New therapy includes complement inhibitors which may provide a rapid control of haemolysis is under investigation in clinical trials.

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REFERENCES

- 1 Berentsen S, Tjønnfjord GE. Diagnosis and treatment of cold agglutinin mediated autoimmune hemolytic anemia. *Blood Rev* 2012;26:107–15.
- 2 Berentsen S. Role of complement in autoimmune hemolytic anemia. *Transfusion Medicine and Hemotherapy* 2015;42:303–10.
- 3 Petz LD. Cold antibody autoimmune hemolytic anemias. *Blood Rev* 2008;22:1–15.
- 4 Berentsen S. How I manage cold agglutinin disease. *Br J Haematol* 2011;153:309–17.
- 5 Swiecicki PL, Hegerova LT, Gertz MA, et al. *Br J Haematol* 2013;122:1114–21.
- 6 Randen U, Trøen G, Tierens A, et al. Primary cold agglutinin-associated lymphoproliferative disease: a B-cell lymphoma of the bone marrow distinct from lymphoplasmacytic lymphoma. *Haematologica* 2014;99:497–504.
- 7 Genty I, Michel M, Hermine O, et al. [Characteristics of autoimmune hemolytic anemia in adults: retrospective analysis of 83 cases]. *Rev Med Interne* 2002;23:901–9.
- 8 Valent P, Lechner K. Diagnosis and treatment of autoimmune haemolytic anaemias in adults: a clinical review. *Wien Klin Wochenschr* 2008;120(5-6):136–51.
- 9 Sandhaus LM, Raska K, Wu HV. Diffuse large-cell lymphoma with monoclonal IgM kappa and cold agglutinin. *Am J Clin Pathol* 1986;86:120–3.
- 10 McNicholl FP. Clinical syndromes associated with cold agglutinins. *Transfus Sci* 2000;22(1-2):125–33.
- 11 Diebold J, Reynes M, Tricot G, et al. [Splenic lympho-plasmocytic sarcomas discovered by exploratory laparotomy in 2 cases of cold agglutinin disease]. *Sem Hop* 1978;54(43-44):1325–30.
- 12 Eskazan AE, Akmurad H, Ongoren S, et al. Primary gastrointestinal diffuse large B cell lymphoma presenting with cold agglutinin disease. *Case Rep Gastroenterol* 2011;5:262–6.
- 13 Chng WJ, Chen J, Lim S, et al. Translocation (8;22) in cold agglutinin disease associated with B-cell lymphoma. *Cancer Genet Cytogenet* 2004;152:66–9.
- 14 Michaux L, Dierlamm J, Wlodarska I, et al. Trisomy 3 is a consistent chromosome change in malignant lymphoproliferative disorders preceded by cold agglutinin disease. *Br J Haematol* 1995;91:421–4.
- 15 Hauswirth AW, Skrabbs C, Schützinger C, et al. Evans' syndromes, and pure red cell aplasia in non-Hodgkin lymphomas. *Leuk Lymphoma* 2007;48:1139–49.
- 16 Airaghi L, Greco I, Carrabba M, et al. Unusual presentation of large B cell lymphoma: a case report and review of literature. *Clin Lab Haematol* 2006;28:338–42.
- 17 Níainle F, Hamnvik OP, Gulmann C, et al. Diffuse large B-cell lymphoma with isolated bone marrow involvement presenting with secondary cold agglutinin disease. *Int J Lab Hematol* 2008;30:444–5.
- 18 Sallah S, Sigounas G, Vos P, et al. Autoimmune hemolytic anemia in patients with non-Hodgkin's lymphoma: characteristics and significance. *Ann Oncol* 2000;11:1571–7.
- 19 Kosugi S, Watanabe M, Hoshikawa M. Primary bone marrow lymphoma presenting with cold-type autoimmune hemolytic anemia. *Indian J Hematol Blood Transfus* 2014;30:271–4.
- 20 Lenz G, Wright GW, Emre NC, et al. Molecular subtypes of diffuse large B-cell lymphoma arise by distinct genetic pathways. *Proc Natl Acad Sci U S A* 2008;105:13520–5.
- 21 Berentsen S, Randen U, Oksman M, et al. Bendamustine plus rituximab for chronic cold agglutinin disease. *Blood* 2017;130:537–41.
- 22 Mandigers CM, Keuning JJ, Booi AC. A patient with fatal cold haemagglutinins. *Neth J Med* 1996;49:209–11.
- 23 Rousey SR, Smith RE. A fatal case of low titer anti-PR cold agglutinin disease. *Am J Hematol* 1990;35:286–7.
- 24 Seldon M, Isbister JP, Raik E, et al. A fatal case of cold autoimmune hemolytic anemia. *Am J Clin Pathol* 1980;73:716–7.
- 25 Berentsen S, Ulvestad E, Langholm R, et al. Primary chronic cold agglutinin disease: a population based clinical study of 86 patients. *Haematologica* 2006;91:460–6.
- 26 Wouters D, Zeerleder S. Complement inhibitors to treat IgM-mediated autoimmune hemolysis. *Haematologica* 2015;100:1388–95.
- 27 Röth A, Hüttmann A, Rother RP, Hu A, Du U, et al. Long-term efficacy of the complement inhibitor eculizumab in cold agglutinin disease. *Blood* 2009;113:3885–7.
- 28 Shi J, Rose EL, Singh A, et al. TNT003, an inhibitor of the serine protease C1s, prevents complement activation induced by cold agglutinins. *Blood* 2014;123:4015–22.
- 29 Bartko J, Derhaschnig U, Gilbert J, et al. Safety and efficacy of the C1S complement inhibitor tnt009 in a first-in-human trial. *Clin Ther* 2017;39:e6–7.

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