REVIEW

Cold Agglutinin Disease – Diagnostic and Therapeutic Challenges in the Clinical Setting

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ABSTRACT

Cold agglutinin disease (CAD) is a rare form of autoimmune hemolytic anemia (AIHA), in which IgM-specific antibodies cause the agglutination of red blood cells (RBCs) at temperatures < 37°C and activate the classical pathway of complement leading to extravascular hemolysis, C3b-coated RBCs are phagocytosed by the macrophages of the reticuloendothelial system (predominantly in the liver). Up to date there are two clinical pathologic entities recognized as distinct with different therapeutic implications: cold agglutinin disease and cold agglutinin syndrome (CAS). Primary CAD is recognized as clonal B-cell lymphoproliferative disorder of the bone marrow, clinical and imagistic evidence of associated malignancy. CAS arises in the setting of an underlying disorder such as infection, autoimmune disease, or malignancy (non-Hodgkin lymphoma or other malignant process). The diagnosis of CAD is often delayed due to the unpredictable clinical course. In spite of the current therapeutic options which are directed at the pathogenic B cells or the complement system, the low response rates and frequent relapses lead to challenges regarding the management of this disease.

Keywords: *autoimmune hemolytic anemia, cold agglutinin disease, cold agglutinin syndrome, lymphoproliferative disorders, complement inhibitors, therapy*

Introduction

Cold agglutinin disease (CAD) is a complement-mediated immune hemolytic disease, accounting for 15–25% of autoimmune hemolytic anemia (AIHA) and, also, a distinct clonal B-cell lymphoproliferative disorder of the bone marrow. The autoantibodies in CAD are immunoglobulin M (IgM) that are able to agglutinate erythrocytes at an optimum temperature of 3- 4°C but are also able to react at a higher temperature, depending on the thermal amplitude (TA). If the thermal amplitude of the cold agglutinin allows binding to its antigen in vivo, this will result in classical complement pathway-mediated AIHA and agglutination-mediated ischemic symptoms affecting the acral circulation (acrocyanosis, Raynaud phenomenon). (1). CAD is a chronic, systemic condition, with a prevalence OF about 20 cases per 1 million. (2)
CAD, also known as primary CAD was recognized as an indolent B-cell lymphoma in the World Health Organization (WHO) classification revised in 2022 (3, 4). This entity should be distinguished from secondary cold agglutinin syndrome (CAS), an AIHA associated with an underlying condition (5,6,7). The nature of this clonal B-cell lymphoproliferative disorder was investigated and the major findings included the following:

a) No evidence of an extramedullary lymphoma (no lymphadenopathy, splenomegaly or abnormal lymphocytes in the peripheral blood).

b) Bone marrow lymphoid infiltration of nodular B-cell aggregates, with mature plasma cells surrounding the aggregates.

c) Immunophenotyping demonstrated lymphocytes B expressing IgM with a kappa light chain, positive for CD19, CD20, CD22, CD79b, and FMC7; CD23 was negative.

d) Specific features of lymphoplasmacytic lymphoma (LPL), such as para trabecular growth, fibrosis, lymphoplasmacytic cell morphology, or an increased number of mast cells surrounding the lymphoid aggregates, were not seen. In contrast to LPL, the testing for the MYD88 L265P mutation, commonly seen in LPL, is negative in all cases. (8)

Cold agglutinin syndrome (CAS) is associated with an underlying condition such as a viral infection (M. pneumoniae, EBV, SARS-CoV2, HIV, Parvovirus B19, Mycobacterium tuberculosis), autoimmune disorder (systemic lupus erythematosus), B-cell lympho-ma (Chronic lymphocytic leukemia, Waldenström macroglobulinemia, Castleman disease) or other malignancy (ovarian teratoma, thymoma). Infections and autoimmune disorders are more frequent in the younger population diagnosed with CAD, and malignancies should be suspected in older populations. Most cases of CAS associated with a lymphoid malignancy are monoclonal, and the majority associated with a viral infection are polyclonal. (8,9)

Mechanism of hemolysis

Hemolysis in CAD is entirely dependent on complement activation by the classical pathway. Cold To a lesser extent, C3b proceeds through the terminal activation of complement and generates the membrane attack complex (MAC, C5b-9), resulting in intravascular hemolysis. Typically, complement inhibitors CD55 and CD59 are on the RBC surface, which, unlike inautoantibodies (CA) bind to I/I antigens on the erythrocyte surface during the passage through the peripheral circulation, causing agglutination of RBCs and complement activation by the classical pathway.
The erythrocyte-IgM complex binds the C1q fraction of the complement. C1- esterase activates C4 and C2, leading to the generation of the C3 convertase. This will result in cleavage of C3 into C3a and C3b. Upon warming to 37°C in the central circulation, CA detaches from the cells, allowing agglu

tinated erythrocytes to separate, while C3b remains bound. C3 bopsonized erythrocytes are phagocytosed by macrophages in the reticuloendothelial system, mainly in the liver, resulting in extravascular hemolysis. On the surface of the surviving erythrocytes, C3b is cleaved, leaving high numbers of C3d molecules which can be detected by the direct antiglobulin (Coombs) test. The C3d-coated erythrocytes are protected from hemolysis because of the absence of C3d receptors on macrophages. Paroxysmal nocturnal hemoglobinuria (PNH), is intact in CAD and prevents the classical complement pathway from activating the terminal cascade.

Most patients have secondary low C3 and C4 serum levels by consumption, which leads to a self-limited complement-mediated hemolysis. Conditions associated with increased production of acute phase reactants (infection, trauma, surgery) will exacerbate the hemolytic process in most CAD patients.

Clinical presentation

Hemolysis results in diverse symptoms and a significant chronic burden on the patient's quality of life: profound fatigue, severe anemia, and related symptoms such as shortness of breath or weakness, increased thromboembolic risk, persistent disease manifestations year-round, and increased mortality risk. (10,11) CAD affects mainly elderly or middle-aged people and the median age at presentation is 67 years. Fatigue is the primary symptom affecting CAD patients that may be caused by anemia or by complement activation alone. Cold-induced symptoms include acrocyanosis, livedo reticularis, Raynaud phenomenon, and cutaneous ulceration.

Laboratory findings are consistent with autoimmune, extravascular hemolysis me diated by the classical complement activa tion pathway. The most prominent signs of extravascular hemolysis consist of unconjugated hyperbilirubinemia, reticulocytosis, haptoglobin consumption, a moderate increase of LDH, gallstones, splenomegaly, and iron overload. The blood smear may show RBC agglutination. The cornerstone of the CAD diagnosis is a positive Coombs test for complement C3d and negative for IgG, combined with a titer of IgM cold agglutinins ≥ 64 at 4°C. (12,13)

Therapy

In spite of the considerable progress seen in the last 10 years regarding CAD treatment which targets the two main components of the pathogenesis (B-cell clone or the classical complement pathway-dependent hemolysis), the low response rates and frequent relapses lead to challenges regarding the management of this disease and whenever possible patients with CAD should be included in clinical trials.

Unspecific and supportive therapies

Glucocorticoids and splenectomy are not effective therapies in the majority of patients with CAD because the liver is the main site of erythrocyte phagocytosis in CAD.

For cold-induced symptoms, the main therapy is cold avoidance and symptomatic (gloves, hand warmers) but avoiding cold temperatures is minimally effective, only in very mild CAD. Data suggest that seasonal differences in CAD manifestations are not clinically meaningful.

Many CAD patients require transfusions at some point, but folic acid and vitamin B12 should be given to all patients. Severe or symptomatic anemia requires transfusions. Plasma contains a high amount of complement proteins and so its transfusion should be avoided as prophylaxis for complement-mediated hemolysis. Any bacterial or viral infection should be promptly treated to avoid hemolytic episodes induced by the enhanced production of acute phase reactants (including C3, C4).

B-cell-directed therapies

Treatment targeting the pathogenic B-cell clone in the bone marrow is used to reduce the production of monoclonal cold agglu- tinin production. Therapy generally involves Rituximab-containing regimens or Rituximab-monotherapy and should be considered as first-line treatment, depending on individual patient characteristics.
Rituximab mono-therapy has yielded response rates of 50%, only rare complete.

Complement-directed therapies

Therapies that target the classical complement pathway components responsible for extravascular hemolysis in CAD can be used to reduce transfusion requirements and improve anemia and fatigue. Complement-directed therapies do not eliminate the cells producing the cold agglutinins, and they would not be expected to improve cold-induced responses and median response duration of 11-12 months (14). These results can be improved by adding bendamustine or fludarabine, but at a risk of short-term toxicity and with concerns about possible late adverse effects. Rituximab plus Ben-damustine produces high response rates and often complete responses lasting for many years. Rituximab plus fludarabine is more toxic and should be reserved for third-line therapy in selected patients. Toxicities may be greater than with ritux-imab monotherapy, limiting the use of this combination in frail individuals (9). For individuals for whom a rituximab-containing regimen is ineffective can be used bortezomib. Bortezomib has shown a favorable effect, although response rates seem to be relatively low after monotherapy. Ibrutinib can also be considered as a second-line B-cell targeting therapy, although the effect has only been documented in retrospective studies (5). Symptoms related to RBC agglutination, as agglutination is complement independent (16). Furthermore, complement-directed therapies will have to be continued indefinitely, in contrast to cell-directed therapies, which are temporary. Therefore, complement-directed therapies are generally reserved for individuals whose disease does not improve with immuno-suppressive therapies, those who cannot take these therapies, or those who require a more rapidly acting or transient blockade of hemolysis.
Complement component C1 because it has enzymatic activity is a therapeutic target. C1 consists of three subunits (C1r, C1s, and C1q). Sutimlimab, a humanized monoclonal antibody targeting C1s, has the ability to reduce the extravascular hemolysis mediated by C3b. It was approved for CAD in 2022 (16,17). Inhibition of complement component C3 is brings up the possibility to block the entire complement system, including the classical pathway and ensuing C3b opsonization, and the terminal pathway, which is of importance in rare cases with intravascular hemolysis. Pegcetacoplan (anti-C3) is currently under investigation for reducing hemolysis in CAD. (18). Eculizumab targets the more distal complement component C5 and is not expected to lessen hemolysis, which is mostly extravascular and mediated by upstream components of the classical complement pathway, although reports have described a reduction of hemolysis in selected individuals.
For those with cold agglutinin syndrome (CAS) secondary to an overt lymphoma that requires treatment by itself, such as an aggressive B-cell lymphoma, there is no documented therapy apart from appropriate treatment for the specific type of lymphoma. Patients with CAS secondary to infection have a polyclonal cold agglutinin that will resolve spontaneously, typically over two to four weeks following the resolution of the infection. If an underlying autoimmune disorder is identified, treatment should be directed at the underlying

disorder. (1, 5, 16,17,18)
In our clinic, we encountered two cases of cold agglutinin syndrome which were challenging regarding the management and finding the best therapeutic approach.

First Case study

A 52-year-old woman, with a history of asymptomatic hairy cell leukemia (HCL) and asymptomatic cold agglutinin disease since April 2021, with no indication for therapy presented for evaluation in 2022 after falling with hematuria and scleral icterus. The laboratory findings showed an acute intravascular hemolysis episode: mild anemia (Hb=9g/dl), reticulocytosis, highly increased LDH (1362U/L), hemoglobinuria, unconjugated hyperbilirubinemia (TBIL(ser)= 4.01 mg/d, DBIL(ser)=0.6 mg/dl). Folic acid supplementation was administered. Serum protein electrophoresis with immunofixation and serum immunoglobulins showed a monoclonalprotein IgMλ (IgM= 1980 mg/dL, SFLC lambda=3.29 g/L). A lymphoplasmacytic lymphoma IgMλ associated with hairy cell leukemia was suspected, hence we decided to initially treat the patient with purine analogs (Cladribine), followed by a bone marrow examination. Administration of corticosteroids was avoided as a consequence of a high risk of infection. One month later on reevaluation the patient presented without anemia and signs of hemolysis, but with persistent elevated IgM monoclonal component (1830mg/dL), and the myelogram analysis and immunophenotyping of the bone marrow cells demonstrated the absence of hairy cell infiltrate and the presence of a lymphoplasmacytic cell infiltrate. CT chest, abdomen, and pelvis examination revealed only hepatomegaly, without lymphadenopathy or splenomegaly. We decided to initiate the Rituximab/Ibrutinib protocol for the treatment of the lymphoplasmocytic lymphoma/Waldenstrom macroglobulinemia. After the first four weekly administration of Rituximab (375mg/m2) and daily Irtinib(420mg/day), the patient had a significantly improved clinical state with the disappearance of the hemolytic anemia. CAD is seen in less than 5% of HCL cases. An association with second malignancy has been reported in HCL patients.

Second Case study

A 59 year old woman with a history of epilepsy, asthma and osteoporosis, was diagnosed in January 2021 with primary myelofibrosis with JAK2V617F mutation and treated with Ruxolitinib (15mg x2/day) until April 2022 when disease progression was suspected based on the severe anemia, thrombocytopenia and anemia related symptoms. The patient presented to a private clinic in Vienna where the repeated HP and IHC examination of the bone marrow biopsy showed an accentuated fibrotic state with a mature monocytic infiltrate CD14+ of 50% and the NGS analysis showed the presence of ASXL1 and SRSF2 mutations, both associated with a poor prognosis in primary myelofibrosis. It was decided to initiate the pomalidomide/ dexamethasone protocol. After 3 months of treatment, she was admitted in our clinic with a fair medical state, with profound fatigue, pallor, scleral icterus, hepatomegaly and grade 5 splenomegaly, signs of portal hipertension. The CBC showed a severe leukoerythroblastic anemia (Hb=5.3g/dl) moderate thrombocytopenia (PLT=84x10^3/µL), monocytosis (MONO=36.38 x10^3/µL), elevated hemolysis markers (LDH=539U/L, TB=2.30mg/dL, dB=0.5mg/dL), a positive polyspecific and C3d DAT. A diagnosis of autoimmune hemolytic anemia with cold agglutinins was established. The patient continued the treatment with the Pom/Dex protocol associated with Cyclophosphamide for the AIHA and red cell transfusion.
The prolonged anemic syndrome and severe anemia imposed the cessation of oral chemotherapy and off-label administration

of a four-weekly dose of Rituximab (375mg/m2) was initiated. Despite the continuous red cell transfusions there was no significant improvement of Hb level, but with the disappearance of the hemolytic markers and persistent severe fatigue. The persistence of peripheral monocytosis imposed the differential diagnosis with chronic myelomonocytic leukemia (CMML) and, as a consequence, we repeated the examination of the bone marrow biopsy and immunophenotyping of the peripheral blood. The HP and IHC of the bone marrow biopsy revealed primary myelofibrosis with important monocytosis (>50% monocytes), without dysplasia, polymorphic megakaryocytes. The peripheral blood immunophenotyping showed 16% immature monocytes CD14+CD16+. The patient did not meet the revised 2022 WHO diagnostic criteria for CMML, and so a definite diagnosis of PMF with monocytosis was established. The concurrent presence of JAK2 V617F, monocytosis and bone marrow fibrosis can be observed in both chronic myelomonocytic leukemia (CMML) and primary myelofibrosis (PMF). We consider Fedratinib as a therapeutic option, which will be initiated when it becomes available for use in our country and depending on the hematological parameters.

Conclusions

The diagnosis of CAD is often delayed due to the unpredictable clinical course. Considering the fact that CAD is not such a benign condition, but may even be life threatening, a rapid and accurate diagnosis is important to a correct treatment and management of CAD. In spite of the current therapeutic options which are directed at the pathogenic B cells or the complement system, the low response rates and frequent relapses lead to challenges regarding the management of this disease.

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