How I Treat Cold Agglutinin Hemolytic Anemia

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Overview

- Cold agglutinin disease is an immune-mediated hemolytic anemia characterized by complement on the red cell surface.
- In half of patients, observation is appropriate because the hemolysis is compensated.
- A polyclonal form and a monoclonal form exist; the former is self-limited, and the latter results in chronic sustained hemolysis.
- Therapy includes reduction in the production of the immunoglobulin M monoclonal protein responsible for mediating hemolysis and, most recently, complement inhibitor therapies, as yet not approved, to eliminate complement-mediated hemolysis.

Patient Presentations

Patient 1

A 67-year-old man with a hemoglobin level of 9.6 g/dL was referred to a local oncologist. The oncologist found an immunoglobulin M (IgM) kappa monoclonal protein, with an IgM level of 376 mg/dL. During monitoring over the next 17 years, the IgM monoclonal protein level rose to 1330 mg/dL. The local oncologist diagnosed Waldenström macroglobulinemia and recommended chemotherapy, and the patient sought a second opinion.

The patient had a hemoglobin level of 10.2 g/dL, a reticulocyte count of 3%, a monoclonal protein peak of 1 g/dL, a haptoglobin level that was not measurable, a total bilirubin level of 1.6 mg/dL, and a direct bilirubin level of 0.3 mg/dL. A direct antiglobulin test was positive (2+), as was testing for anticomplement direct antiglobulin (2+). A cold agglutinin titer was 1:131,072. A bone marrow biopsy showed red cell hyperplasia and 15% to 20% infiltration with lymphoplasmacytic lymphoma, both nodular and interstitial. Observation was recommended, and the hemoglobin level remained stable for 6 months. After 22 months of observation, the patient developed acute viral bronchitis. This resulted in a hemoglobin level of 6.5 g/dL and a reticulocyte count of 6%. The lactate dehydrogenase (LDH) level rose to 339 U/L, and total bilirubin rose to 2.5 mg/dL. The patient was given dexamethasone, which resulted in no improvement in hemoglobin and a blood glucose level of 610 mg/dL. The patient subsequently received 4 weeks of rituximab (Rituxan, Genentech/Biogen). Two months later, the hemoglobin level was 12.2 g/dL, although the haptoglobin remained unmeasurable. The cold agglutinin titer was 1:65,536. Direct antiglobulin testing remained positive (2+). The IgM level fell to 375 mg/dL.

Patient 2

An 11-year-old child presented with fever and cough, a temperature of 39.4°C, and a heart rate of 140 bpm. The patient's hemoglobin level was 8 g/dL. The white cell count was 20,000 mm^3. The alanine aminotransferase level was 86 U/L. The result of a titer for *Mycoplasma pneumoniae* was 1:320. Peripheral blood smears showed agglutination, and a titer was positive for cold agglutinin. The chest x-ray showed bilateral infiltrates. The patient was initiated on erythromycin therapy. Two days later, the patient's hemoglobin level was 3.8 g/dL, haptoglobin was unmeasurable, and the LDH level was 1397 U/L. The patient received warmed red blood cells and intravenous immunoglobulin and was discharged 13 days later with a hemoglobin level of 8.7 g/dL. The hemoglobin level 10 weeks later was 13.3 g/dL, and cold agglutinin was not detectable at 6 weeks.

Commentary

The first patient has classic cold agglutinin disease with a monoclonal IgM that fixes complement to the red cell membrane, resulting in extravascular hemolysis. The anemia initially was incorrectly attributed to

macroglobulinemia. Such patients would not have developed anemia with marrow involvement of less than 20%, however. Hemolysis was compensated until a viral infection occurred. The patient recovered with supportive care to baseline, with sustained low-grade hemolysis.

The second patient has classic post-infectious cold agglutinin disease. Such disease may be severe, but is generally self-limited and can be managed with supportive care, with complete resolution expected.

Introduction

Cold agglutinin hemolytic anemia is a form of immune-mediated hemolytic anemia. The pathophysiology is an IgM protein, which can be monoclonal (cold agglutinin disease) or polyclonal (usually post-infectious). The IgM protein fixes complement to the red cell surface. C3 is deposited on the surface of the red blood cells. C3 convertase removes C3a, and the red cells are then covered with C3b. The mononuclear phagocyte system recognizes the C3b, leading to binding and removal of a fragment of the red cell, which results in spherocytes in the peripheral blood smear. The C3-coated red cells can be recognized with direct antiglobulin testing for complement. If a direct antiglobulin test is negative for complement in a patient with anemia, the likelihood of finding a cold agglutinin is only 1%. Therefore, if direct antiglobulin testing is negative, cold agglutinin testing is probably not clinically indicated.[1] Hemolysis is usually moderate because the C3b-coated red cells are eventually cleaved, leaving C3d on their surface. C3d does not interact with the mononuclear phagocyte system, and these cells become resistant to hemolysis. This can result in a well-compensated chronic hemolytic state, and these patients may not be transfusion-dependent.

The term "cold agglutinin hemolytic anemia" is a bit of a misnomer because it does not refer to the temperature of the external environment but rather to the behavior of the red cells in vitro in the blood bank laboratory. Red blood cells that are coated with IgM agglutinate in vitro only when antiglobulin antisera is added and the cells are incubated at 37°C, thus the term "warm immunohemolytic anemia." When hemolysis is mediated by an IgM antibody, the size of the protein bridges the space between red blood cells and results in agglutination without the addition of human antiglobulin at a temperature of 3°C, thus the term "cold agglutination."

When an IgM antibody fixes complement to the red cell surface, it may be a polyclonal IgM or a monoclonal IgM. Polyclonal IgM is part of the primary immune response to infection. Polyclonal cold hemolysis tends to occur in the pediatric population. The most commonly described infections are *Mycoplasma pneumoniae* and infectious mononucleosis mediated by the Epstein-Barr virus. However, a whole host of infections can cause hemolysis, ranging from influenza to malaria. Hemolysis has also been reported as an adverse event associated with anti–programmed death ligand 1 inhibitors.[2] Because the IgM level is part of the primary immune response, it declines over weeks and, as a result, the hemolysis tends to be transient and requires only supportive care. The IgM antibody associated with cold agglutinin disease has specificity for the I antigen group ubiquitously found on all red cells. There have been reports of anti-Pr cold agglutinins.[3] The resulting positive direct antiglobulin testing will interfere with red cell cross-matching, making transfusional support through the post-infectious interval challenging. In specialized laboratories, the immunoglobulin can be eluted from the red cell surface, allowing for appropriate cross-matching of blood. However, in life-threatening circumstances, the transfusion of ABO- and Rh-compatible red cells will suffice to prevent acute cardiovascular compromise and, even in the presence of unrecognized alloantibodies, would result in a delayed hemolytic transfusion reaction, which can then be managed when cardiovascular dynamics are improved.

The more common scenario is an elderly patient whose IgM protein is monoclonal. This monoclonal IgM is usually at a low level, typically less than 2 g/dL, and many patients do not fulfill criteria for Waldenström macroglobulinemia (10% bone marrow infiltration with clonal cells). The presence of the serum monoclonal IgM that fixes complement to the red cells results in a chronic immunohemolytic anemia. In a retrospective analysis of 377 patients with an IgM monoclonal protein, 16 (4.2%) had cold agglutinin hemolysis.[4]

It is important to recognize that low-titer cold agglutinins are found during routine screening of blood donors. These cold agglutinins are low titer and have low avidity to the red cell membrane, and do not result in red cell membrane loss. These red cells can be safely transfused.[5]

Clinically relevant titers of cold agglutinins are generally greater than 1:64 and cause typical findings of extravascular hemolysis. Reticulocytosis, elevation of LDH, consumption of haptoglobin, and elevation of the indirect bilirubin are typical. Exacerbations can be precipitated by acute viral infections, as in Patient 1. When this happens, there can be signs of intravascular hemolysis, including elevations of serum-free hemoglobin and hemoglobin in the urine. Patients with cold agglutinin disease have high levels of complement on the red cell membrane and, as a consequence, the standard therapies that are used for warm-mediated hemolysis fail. Corticosteroids and splenectomy consistently fail to

provide benefit for patients with cold agglutinin disease. A suggested diagnostic testing assessment is given in Table 1.

Table 1. Suggested Tests for Cold Agglutinin Disease

- Complete blood count, with particular attention to the mean corpuscular volume
- Reticulocyte count
- Inspection of peripheral blood film for spherocytes and agglutination
- Total and direct bilirubin
- Direct antiglobulin testing; if testing is positive, assess for surface immunoglobulin and complement
- Serum electrophoresis and immunofixation
- Quantitative immunoglobulins
- Cold agglutinin titer (should be unnecessary if direct antiglobulin C3 is negative)
- Lactate dehydrogenase
- Haptoglobin
- Total hemolytic complement
- C3
- C4 (most likely to be abnormal)

Clinical Manifestations

Although hemolytic anemia is the primary manifestation of cold agglutinin disease, patients also have an increased risk of acrocyanosis and venous thromboembolism. In a retrospective analysis, 31% of patients with cold agglutinin disease had a medical claim for thrombosis, compared with 20% in matched comparisons.[6] In a study in Denmark, the prevalence of cold agglutinin disease was 1.26 per 100,000 persons, and the incidence rate was 0.18 per 100,000 person-years. The median age at diagnosis was 68.5 years. This median age corresponds to the population at risk of developing IgM monoclonal gammopathies. The incidence rate of venous thromboembolism was 52.1 per 1000 person-years, nearly double that of the control population.[7] In an observational study of 29 patients, 7.1 severe anemia events per patient-year were observed during the follow-up period. Transfusions were required in 65% of the cohort, with a mean of 11 transfusions per patient-year.[8] In a review of medical claims records, 31% of patients with cold agglutinin disease had a medical claim for a thromboembolism compared with 20% in the matched group.[6]

Cold agglutinins were first identified more than 100 years ago. The first monoclonal antibody ever identified was a cold agglutinin described by Dacie in 1957.[9] Occasional patients can have IgG or IgA cold agglutinins, but the majority of cold agglutinins are IgM because of their efficiency in fixing complement to the red cell surface. It is estimated that cold agglutinin disease is responsible for 15% of all autoimmune hemolytic anemias. Even in patients who have an antibody that has a low thermal amplitude, red cells can agglutinate in acral parts of the circulation even at mild ambient temperatures, leading to complement fixation and complement-mediated hemolysis. The monoclonal IgM proteins detach from the red cell membrane upon warming in the central circulation. IgM is not found on the surface, but C3b remains bound and triggers clearance of the red cells.

A Norwegian study showed that the incidence of cold agglutinin disease was 16 per million population, with a median age at diagnosis of 67 years. Ninety-one percent had cold-induced circulatory symptoms, 74% had exacerbation of anemia during febrile illnesses, and half had received at least one red cell transfusion. The mean initial hemoglobin at diagnosis was 9.2 g/dL. As mentioned earlier, among 377 patients with an IgM monoclonal protein, 16 (4.2%) had cold agglutinin disease.[4] Table 2 shows the complete blood count of a completely asymptomatic patient whose cold agglutination led to spurious results using a standard Coulter counter to measure blood components. Agglutination resulted in a false elevation of the mean corpuscular volume and an artifactual reduction in the number of counted red cells. The hemoglobin measurement is unaffected because hemoglobin is measured after red cell lysis is performed.[11]

In a retrospective analysis performed at the Mayo Clinic, the median age at diagnosis was 72 years and the most common symptom was acrocyanosis, which occurred in 44% of patients.[12] Cold-triggered symptoms were seen in 39% of patients, 40% received transfusions during observation, and 82% received some form of medication therapy for management. Because virtually all patients have a monoclonal IgM protein, it is common to find a clonal population of B lymphocytes in the bone marrow responsible for the synthesis of the monoclonal protein. In approximately half of patients, the morphology was lymphoplasmacytic lymphoma.[12] In another study, an MYD88 mutation considered diagnostic of lymphoplasmacytic lymphoma was detected in only 25% of patients, suggesting that cold agglutinin disease is a distinct lymphoproliferative disorder, with clonal B cells.[13] Even in patients who do not have detectable lymphoma in the bone marrow, it is possible to demonstrate gene rearrangements of the immunoglobulin heavy and light chain. Nucleotide sequence of IgH V34, which would predict reactivity against the I antigen, has been detected

Table 2. Complete Blood Count of a Patient With Artifactual Changes Induced by Cold Agglutinin

Hematology	
CBC Aggregate	
Hemoglobin	13.6
Hematocrit	! 6.1
Erythrocytes	! 0.53
MCV	! 115.1
RBC distribution width	See comments[a]
Leukocytes	8.0
Platelet count	249
% Reticulocytes	1.39
Absolute reticulocytes	! 17.9

CBC, complete blood count; MCV, mean corpuscular volume; RBC, red blood cell.

[a] Agglutination of red blood cells led to miscounting and underestimating the number of red blood cells, which is used to calculate the hematocrit. The hemoglobin level is measured on a lysate of red cells and is therefore accurate. The hemoglobin is normal, whereas the red cell count and hematocrit are below levels compatible with life. Agglutination of red cells results in the artifactually high mean corpuscular volume.

even in patients without morphologic evidence of bone marrow involvement.[14] One could presume that if sufficiently sensitive techniques were employed, all patients would have a clonal population of B lymphocytes in the bone marrow. Some of these cases would rise to the level of malignant lymphoma, whereas others would fall below that threshold.

Attempts have been made to define a normal range for cold agglutinin titers.[15] When adjusted for age and sex, titers of 1:4 or lower were almost always innocuous incidental findings. Patients with titers of 1:64 or higher were at significant risk of having clinically significant disease.

Special precautions are required for patients with cold agglutinins who are undergoing surgical procedures that require extracorporeal circulation.[16] If the cells are allowed to reach room temperature, agglutination can occur, which can occlude a membrane oxygenator and result in clinically significant anemia intraoperatively. If a cold agglutinin is found during preoperative cross-matching, warm induction and warming of blood during the cross-clamp period can reduce the risk of surgical complications.[17] High-temperature plasma exchange has also been used in a patient with a cold agglutinin with anti-I specificity. The room was warmed to 29°C; warm blankets, heating pads, and 2 blood warmers were used; and heating packs were applied to all exposed tubing, resulting in safe surgery.[18]

Therapy

Approximately half of patients with monoclonal IgM-mediated cold agglutination will have a chronic stable anemia that does not require active therapy beyond folate supplementation (as is required for all patients with chronic hemolysis). However, therapy is appropriate for the significant fraction of patients who have symptomatic anemia or require regular red blood cell transfusions, with the risk of allo-antibody sensitization and iron overload. Initial therapies have all been directed toward reducing the production of the IgM monoclonal protein responsible for complement fixation on the red cell membrane. It bears repeating that neither corticosteroids nor splenectomy should be used to treat cold agglutinin anemia. The majority of therapies used in these patients have been derived from the experience in treating Waldenström macroglobulinemia. Single-agent rituximab was the first intervention reported to be effective. Approximately half of patients respond to rituximab monotherapy. However, the responses are not durable; the median duration of response is less than 1 year.[19] Multiple case series and case reports of single-agent rituximab for the treatment of cold agglutinin disease have been published.[20] It is common to see increases in the hemoglobin level of 2 to 3 g/dL, along with reductions in IgM of greater than 50%, but durable responses are reported infrequently.

Bortezomib (Velcade, Millennium/Takeda Oncology), also used in Waldenström macroglobulinemia, has been shown to be beneficial in anemic patients with relapsed cold agglutinin disease.[21] However, the objective response rate is less than 50%, and the follow-ups are relatively short.[22] The combination of fludarabine and rituximab has been reported to result in a 75% response rate, with complete remissions in 20%. However, fludarabine therapy can be quite myelosuppressive as well as immunosuppressive in this population, and its use needs to be carefully weighed against the risks.[23] In one case series, 76% of patients responded, with 21% achieving a complete response and 55% achieving a partial response. The response duration was more than 66 months. Fludarabine is a consideration for long-term management of severely affected patients.[24]

Rituximab and bendamustine (Treanda, Bendeka; Teva) have also been shown to be highly effective for chronic cold agglutinin disease. Of the 32 patients (71%) who responded in a single-arm phase 2 trial, 40% had a complete response and 31% had a partial response. Grade 3 or 4 neutropenia was seen in one-third of patients, but only 11% developed an infection. Rituximab/bendamustine is highly efficient and safe and could be considered as first-line therapy for cold agglutinin disease.[25,26]

A second strategy for the management of cold agglutinin hemolytic anemia does not focus on the production of the IgM but instead on the prevention of fixation of C3 to the red cell membrane. Eculizumab (Soliris, Alexion), an inhibitor of C5 that is used for the treatment of paroxysmal nocturnal hemoglobinuria, has been reported to benefit patients with cold agglutinin disease. However, eculizumab works downstream from C3 and, from a theoretical standpoint, would primarily benefit those patients whose hemolysis is intravascular and in which complement cascade activation through C9 is occurring, resulting in red cell lysis. This scenario applies to a minority of patients.[27] In a case series of eculizumab for cold agglutinin disease, the LDH level—which was the primary endpoint—fell significantly. Unfortunately, the increase in hemoglobin, the primary clinical measure of benefit, rose from 9.35 to only 10.15 g/dL. This increase would be expected to produce minimal clinical benefit, although 8 of the 13 patients in this trial acquired transfusion independence.[28] An inhibitor of C1q demonstrated that agglutination could be blocked in vitro, and a monoclonal antibody that binds with high affinity to C1q was shown to block the classical complement activation and hemolysis in an in vitro system.[29] An antibody that inhibits C1s has also been developed and is currently in active testing.[30] This agent, which is administered subcutaneously by an infusion pump on a daily basis, blocks hemolysis. In a study of 6 patients, all experienced a hemoglobin increase of greater than 3.5 g/dL, with a mean increase of 4.3 g/dL. Haptoglobin levels became normalized in 4 of 6 patients within 1 week.[31] Cessation of the complement inhibitor resulted in rapid recurrence of hemolysis which, after re-exposure, once again was controlled.[32] Further exploration of complement inhibitors in this disorder is important.

Conclusion

Cold agglutinin hemolytic disorder is a complement-mediated hemolysis with positive results on a direct antiglobulin test. In the overwhelming majority of patients, an IgM protein is responsible for complement fixation. The polyclonal form of cold agglutinin disease is a post-infectious hemolysis that is associated with a primary immune response. It can be quite severe, but is self-limited and generally requires supportive care only. The monoclonal form of cold agglutinin disease is usually associated with a lymphoproliferative disorder, which can be lymphoplasmacytic lymphoma or another type of low-grade lymphoproliferative disorder. Treatments can be divided into those that prevent IgM production and those that prevent activation of the complement cascade.

Disclosure

Dr Gertz reports personal fees from Ionis/Akcea, Alnylam, Prothena, Celgene, Annexon, Appellis, Amgen, Medscape, Physicians Education Resource, Research to Practice, and Janssen; grants and personal fees from Spectrum; and speaker fees from Teva, Johnson & Johnson; Medscape, and DAVA Oncology. He has served on the data safety monitoring board of AbbVie and the advisory board of Pharmacyclics and Proclara. He has developed educational programs for i3 Health, has received royalties from Springer Publishing. He has received grant funding from the Amyloidosis Foundation, the International Waldenstrom's Macroglobulinemia Foundation, and the NCI Specialized Programs of Research Excellence MM SPORE 5P50 CA186781-04.

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