PROMOTING RECOGNITION, DIAGNOSIS AND TREATMENT OF COLD AGGLUTININ DISEASE

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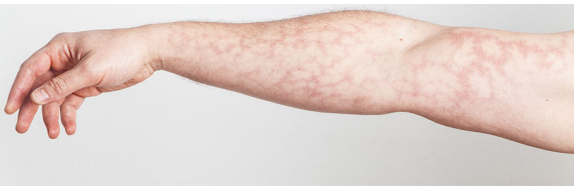

OVERVIEW

Cold Agglutinin Disease is a rare, chronic hemolytic disorder, representing approximately 20 percent of all autoimmune hemolytic anemias. Catherine M. Broome, MD and Alexander Röth, MD provide an understanding of the pathogenesis of this disorder caused by cold-reacting IgM autoantibodies and reveal common clinical features. Because of the high mortality rate among those suffering from CAD, it is important to be aware of the diagnostic criteria, as well as the current and novel treatment options with the goal of increasing hemoglobin levels, avoiding the need for transfusions, and improving circulatory symptoms. Dr. Broome discusses the most recent advancements in treatment based on global collaborative efforts and the importance of participating in the CADENCE Registry. Significant findings from the phase 3 CARDINAL trial are also presented along with an interactive case scenario.

CONTENT AREAS

Pathogenesis | Clinical Features | Diagnostic Markers | Treatment | CADENCE Registry | CARDINAL Trial

LEARNING OBJECTIVES

At the conclusion of this activity, participants should be better able to:

- Describe the pathogenesis of cold agglutinin disease (CAD), the mechanism of disease, its symptoms, and its possible consequences
- Diagnose patients suspected of having CAD
- Appropriately treat and monitor patients with CAD using non-pharmacologic and pharmacologic options
- Describe the mechanism of action, safety, and efficacy of new and emerging treatments for CAD

FACULTY

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TARGET AUDIENCE

This activity is intended for adult hematologists, hematologist-oncologists, hematology-oncology nurse practitioners, emergency physicians, primary care physicians, and other clinicians involved in the management of patients with cold agglutinin disease.

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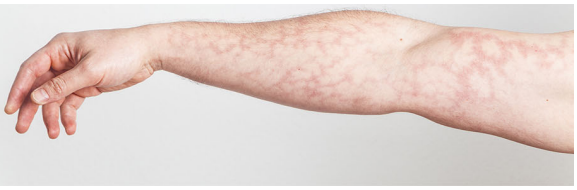

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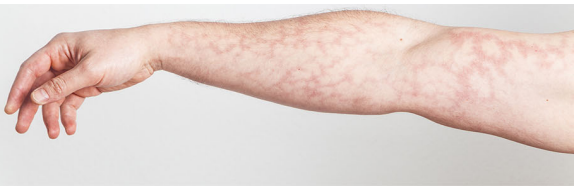

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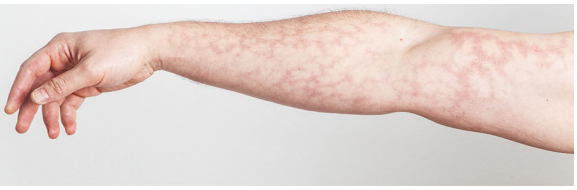

Editor's Note: This is a transcript of a CME presentation. It has been edited and condensed for clarity.

Pathogenesis and Clinical Features of Cold Agglutinin Disease

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Hi, I'm **Dr. Catherine Broome** from MedStar Georgetown University Hospital. We're going to be talking today about recognition, diagnosis, and treatment of cold agglutinin disease.

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We're going to talk about the learning objectives. We're going to describe the pathogenesis of primary cold agglutinin disease, the mechanism of this disease, its symptoms, and some of the possible clinical consequences. We're going to talk about how to diagnose patients who are suspected of having cold agglutinin disease. We're going to discuss treatment and monitoring of patients with cold agglutinin disease, using both nonpharmacologic and pharmacologic options, and we're going to describe the mechanism of action, safety, and efficacy of some of the new and emerging treatments for cold agglutinin disease.

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So, this first module is going to talk about the pathogenesis and some of the clinical features of cold agglutinin disease. Autoimmune hemolytic anemias and cold agglutinin disease, in particular, are rare hematologic disorders. They can present acutely in the emergency room or with more chronic symptoms to primary care physicians or other specialists. They can be challenging at presentation, especially if the clinician is unfamiliar with this rare condition. We all need to be aware of the pathogenesis, signs, symptoms, and potential risks associated with cold agglutinin disease, as well as familiarizing ourselves with some of the treatment options.

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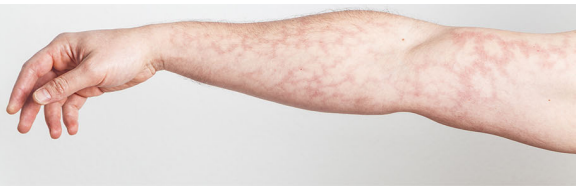
Primary cold agglutinin disease, or idiopathic cold agglutinin disease, is a subtype of autoimmune hemolytic anemia. It's caused by IgM [Immunoglobulin M] autoantibodies, which tend to react with the antigen at cold temperatures. Cold agglutinins bind to RBC [red blood cell] antigens at less than core body temperatures. Secondary cold agglutinin syndrome is also an autoimmune hemolytic anemia mediated by IgM autoantibodies. The difference is that cold agglutinin syndrome is associated with systemic disorders, most commonly infections or malignancy, often associated with mycoplasma pneumonia, Epstein-Barr virus infections, or indolent lymphomas, such as CLL [Chronic Lymphocytic Leukemia] and follicular lymphoma.

Cold agglutinin disease is defined as a rare, chronic, hemolytic disorder caused by anti-red blood cell IgM autoantibodies. They're most often



(more than 90% of the time) monoclonal IgM antibodies, and they're associated with a kappa light-chain restriction. These antibodies recognize the [big] I antigen on the surface of red blood cells. Hemolysis in cold agglutinin disease is complement-dependent with mainly extravascular hemolysis, typically occurring in the liver. Cold agglutinin disease represents somewhere between 15% and 25% of all autoimmune hemolytic anemias, and it may be exacerbated by many things, including cold temperatures.

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How common is cold agglutinin disease? It affects one person per million every year. There are over 5000 people living with cold agglutinin disease in the United States. It's generally going to affect middle-aged and elderly individuals. Average age of onset is around 60 years of age. And, just like most autoimmune diseases, cold agglutinin disease is more common in women than it is in men.

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It's a rare subtype of autoimmune hemolytic anemia, and it is characterized by two main clinical features. The IgM-mediated agglutination of erythrocytes and hemolysis, which is mediated by the activation of the classical complement pathway. Cold agglutinins are IgM autoantibodies that bind to the RBC antigens.[1] We distinguish it from warm antibody autoimmune hemolytic anemia, which is mediated by IgG autoantibodies.

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Acrocyanosis, or agglutination, of the red blood cells, which is mediated by the IgM that is bound to their surface, can cause many signs and symptoms in patients. It can cause *livedo reticularis*, as you see in the bottom right-hand corner of the slide. It can cause vaso-occlusion, as you can see in the top right-hand corner of the slide, leading to cyanotic and necrotic changes in patients' tissue. If we look at a test tube, as you can see on the left-hand side, the cells will stick together or appear to clot in a sample of blood that is maintained at room temperature. This is different from clotting, and when we look at it under a microscope, we can actually see these red blood cells stuck to one another in this form of agglutination.

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The second aspect of cold agglutinin disease is hemolysis, and hemolysis in cold agglutinin disease is mediated by activation of the classical complement pathway. IgM autoantibodies bind to the surface of red blood cells. That IgM antigen complex is a very powerful and efficient activator of the classical pathway via binding to C1.[2] Binding to C1 and the creation of the C1 complex is going to allow a cascade of events to occur, which is eventually going to result in deposition of C3b on the surface of those red blood cells. C3b is a very powerful opsonin, which is recognized

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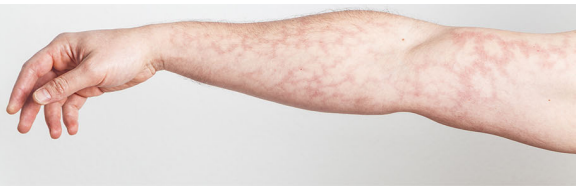

by the monocyte macrophage system, primarily within the liver, and results in extravascular hemolysis related to decreased RBC survival. If the C3b is converted to C3d, the red cell survives. A small amount of complement activation will proceed through the terminal portion to generate C5b, which is the backbone of the membrane attack complex, which will, in some patients, result in intravascular hemolysis related to the cold agglutinin or IgM autoantibody. These patients may have a mixture of both intra- and extravascular hemolysis, although the predominant form of hemolysis in most patients with cold agglutinin disease is extravascular.

Complement Cascade

Let's remind ourselves about the complement cascade. We just talked in generalities about the complement cascade. We talked about the C1 complex and how this IgM is going to bind to the surface of the red blood cell. That IgM antigen complex is then going to activate C1 and create the C1 complex, which is going to lead to this cascading through the complement pathway to generate C3b, which is going to result in that extravascular hemolysis, and then also can proceed through the terminal portion of the cascade to activate all the way through to C5b, which is going to be the backbone upon which the membrane attacks complex forms and generates intravascular hemolysis. I want to also draw your attention to the fact that proceeding through this classical pathway of complement activation is also going to generate both C3a and C5a, which are very powerful inflammatory molecules. And so, in addition to being a disease of agglutination and acrocyanosis, hemolysis and anemia, cold agglutinin disease is also a chronic inflammatory condition as well.

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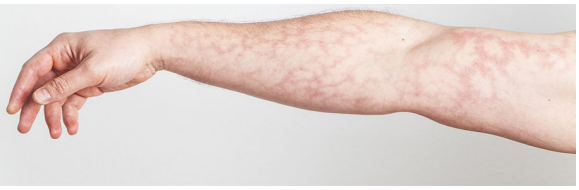
We can use a variety of different medications in order to interact with the classical pathway to try to stop this pathophysiologic reaction, which is occurring related to the activation of the classical pathway by these IgM autoantibodies. We can use C5 inhibitors, which are eculizumab, ravulizuzmab and crovalimab, which can inhibit the generation of the C5b complex, which will decrease and eliminate intravascular hemolysis. But, remember, we talked about the majority of hemolysis in patients with cold agglutinin disease being extravascular. We need to think about interacting with the complement cascade at a point that's higher than C5. We do have available C1 inhibitors, including sutimlimab, ANX005, and C1-esterase inhibitors. We could also potentially interact at the C3 level, with pegcetacoplan, which is a C3 inhibitor. We can impact the continued activation of complement related to Factor B and Factor D with Factor B inhibitors, which is iptacopan, and Factor D inhibitor, which is danicopan.[3]



So, what is happening to these patients? Well, many of them are going to have some fairly nonspecific symptoms, right? They're going to have hemolysis and hemolytic anemia, fatigue, dyspnea, which may be out of proportion to the degree of anemia, as oftentimes is the fatigue. They may have hemoglobinuria and jaundice. All of these symptoms are going to be mainly complement-driven. They can also develop these symptoms that are related to agglutination of those red blood cells, which would include acrocyanosis, Raynaud's phenomena, *livedo reticularis* and, in extreme cases, sometimes even gangrenous tissue.[4–10]

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What is the **clinical phenotype**? From papers that have been published by Dr. Berentsen in *Blood*, as recently as 2020,[11] we see that about 70% of patients are going to have mostly hemolytic anemia with relatively minor circulatory symptoms. About 21% of patients are going to have hemolytic anemia with more severe circulatory symptoms. And about 10% of patients are going to have mostly circulatory symptoms, with fairly well-compensated hemolysis. It's important to think about what is the clinical phenotype of a patient that you are managing or diagnosing with cold agglutinin disease so that we can think about how to best direct our therapy. Remember, we just talked about how the hemolytic component is mediated by complement. The acrocyanotic or circulatory symptom component is mediated by IgM. And our therapies may be different, depending on which pathophysiologic mechanism we're hoping to trigger or target.

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So, consequences of cold agglutinin disease for our patients, right? Fifty percent of patients have been considered to be transfusion-dependent for short or long periods of time. In multiple studies, we now understand that patients with cold agglutinin disease have an increased risk of developing both venous and arterial thromboembolic events, 62% higher than a matched cohort population.[12] They have reduced quality of life related to need for transfusions, doctors' visits, fatigue, shortness of breath, modifying their daily activities. And when we look at some mortality data, which were presented and published by Dr. Hill and Lauren Bylsma, we notice that patients who are diagnosed with cold agglutinin disease vs a non-cold agglutinin disease matched population, have a higher mortality rate.[1,13]

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Supportive Care

What can we do for our patients? Well, certainly we advise them to potentially avoid cold temperatures. This is mostly going to decrease the amount of agglutination of those red blood cells in the peripheral tissues, like the fingers and the toes, or the tip of the nose. This does not generally modulate the amount of hemolysis that's ongoing. We know

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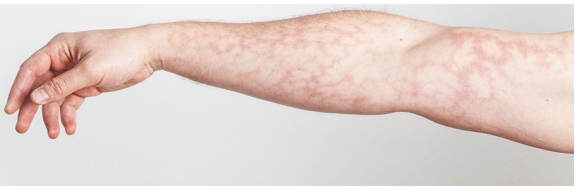

that things that can increase complement activity, such as infections, can trigger hemolytic crises. So, these patients need to be on early and adequate antibiotic therapy. Viral infections also need to be taken quite seriously in these patients because they can trigger these hemolytic crises.

Transfusions, when indicated, need to be kept warm in a blood warmer. Patients with chronic hemolysis need folic acid supplementation, and potentially B12 or iron supplementation if they're deficient. Adequate hydration is critical during hemolytic crises and, in patients who are having an exacerbation of hemolysis, prophylaxis against thrombotic events with low-molecular-weight heparin (LMWH) or other heparin compounds has been recommended.[5,14–19]

CAD Management

These management options are generally unsatisfactory for our patients. There is no approved treatment available. Many times, patients are not offered any therapeutic intervention until their anemia is quite severe.

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We talked a little bit earlier about this being a systemic inflammatory state, and many of these patients have a significantly impacted quality of life, even if the degree of anemia is not severe, related to chronic complement activation and chronic hemolysis. Avoidance of cold environments can be somewhat helpful, but [it] is not appropriate management for these patients in general. If we notice that this is cold agglutinin syndrome and a secondary problem, we can treat the underlying disorder and hope that the hemolysis and the anemia will improve. We should give these patients transfusions when necessary. Sometimes, we have to have emergency therapy for these acute hemolytic crises, and we'll talk about what some of those should look like. Steroids, alkylating agents, and splenectomy, which we often use to treat IgG-mediated autoimmune hemolytic anemia, is not effective in cold agglutinin disease and really should not be part of our management plan.[5,14–19]

Mortality Data Among Patients with CAD

We talked about mortality and here we are going to dive into it a little bit deeper. We looked at a Danish National Patient Registry from 1999 to 2013,[13] and this was really the first attempt to evaluate in a large cohort mortality in a cold agglutinin disease cohort vs a matched comparison cohort. You can see that, in the first year after diagnosis, the cold agglutinin probability was only 83% compared to 96% of the matched comparisons. At 3 years, down to 75% compared to 89% and, at 5 years, down to 60% compared to 82%. The mean survival in the cold agglutinin

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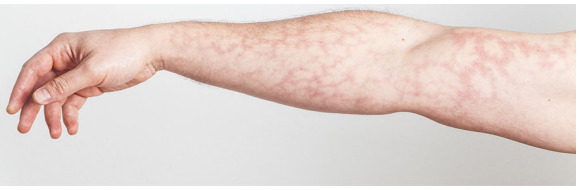

cohort was 8.5 years and had not been reached yet in the matched comparisons.[13]

An additional study, large cohort of patients in the United States, study period 2007–2018, 651 cold agglutinin patients and 3000-plus matched non-cold agglutinin controls were identified.[1] When we looked at events that could lead to mortality, we found that 35% of cold agglutinin patients vs only 20% of non-cold agglutinin patients had experienced one or more thromboembolic events. Mostly female patients were included in both of these cohorts and mostly Caucasian patients, as well. I think the keys here are that mortality does seem to be increased in cold agglutinin disease patients. What exactly is driving that morality, we still have to try to better understand.

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When we look at patients who experienced no thromboembolic events vs those who had one or more, we saw that mortality was affected by having had thromboembolic events.

Diagnosing Cold Agglutinin Disease

So, how do we diagnose cold agglutinin disease? It's really critical to recognize signs and symptoms and accurately diagnose these patients so that appropriate management may be offered to them. Therapeutic management in autoimmune hemolytic anemias relate specifically to antibody type, and we talked about therapies that are appropriate for IgG-mediated autoimmune hemolytic anemias vs those that are appropriate for IgM, and they are not the same. As always, we're going to take a good history and perform a good physical examination. We're going to look at a complete blood count, evaluating hemoglobin levels. We're going to look at a blood smear because we are all good hematologists and we're going to look for evidence of autoimmune hemolytic anemia, which would be spherocytes on the peripheral smear. We're going to think about ordering a direct antiglobulin or "Coombs" test. And remember that the direct antiglobulin is going to detect either immunoglobulin, which would be IgG, complement in the form of C3, or both, on the surface of the red blood cell. When there is evidence of hemolysis, so anemia, an elevation in LDH [lactate dehydrogenase] and bilirubin and a decrease in haptoglobin, we perform the direct antiglobulin test to demonstrate that this is an autoimmune process and to further identify and characterize that autoantibody.[20]

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When we look at the clinical and lab characteristics of patients with cold agglutinin disease as published by Dr. Barcellini in 2020,[21] we see that the

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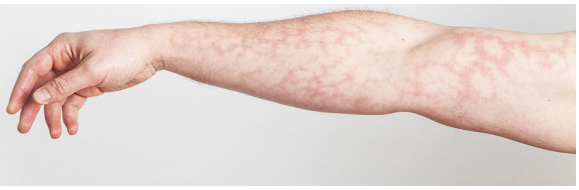

median age at diagnosis was 70, although it's a wide range, 28–94. The median hemoglobin is quite low at 8.2, but again a wide range, 4–13.5. The LDH was 1.4 times the upper limit of normal for a median, but can actually be normal or markedly elevated. Reticulocyte index is another indicator of hemolysis, and the reticulocyte index in most patients with cold agglutinin disease will be elevated. There are, however, a percentage of patients who may have an inadequate reticulocytosis related to marrow function abnormalities.

We have several diagnostic criteria for cold agglutinin disease, including chronic hemolysis; a polyspecific DAT positive. Next step, a monospecific DAT that is strongly positive for C3; a cold agglutinin titer that's greater than or equal to 64. And then we do advise bone marrow evaluation in patients with cold agglutinin disease at diagnosis to look for a monoclonal population of lymphocytes.[2,21]

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Hemoglobin can indicate severity of anemia, and about 36% of patients with cold agglutinin disease are going to have mild anemia characterized by hemoglobin of greater than 10. About 37% are going to be moderate, and about 27% are going to be severe.[11] We are learning, however, that the degree of anemia does not necessarily tell the whole story as to the clinical severity of these patients' disease, and so it's wise to look at markers of hemolysis in conjunction with degree of anemia, to understand the severity of this disorder.

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There may be many reasons for having abnormalities in the markers that we discussed. LDH can be elevated for a variety of reasons. Haptoglobin can be low for a variety of reasons. Reticulocytes may be low or high for a variety of reasons. Bilirubin may also be abnormal for a variety of reasons. So, we have to think about what is potentially going on: acquired causes of abnormalities with regards to hemolysis. Direct antiglobulin test is going to tell us whether this is an autoimmune hemolytic anemia or whether this is a non-immune hemolyticanemia, which may be paroxysmal nocturnal hemaglobinuria or microangiopathic hemolysis, mechanical valve hemolysis, etc.[20]

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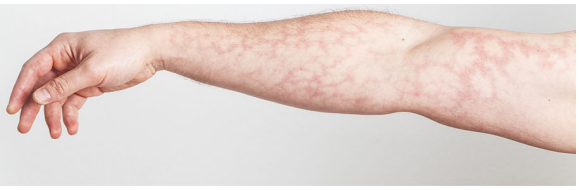
Biomarkers of hemolysis. Hemoglobin reduced in patients with cold agglutinin disease. Reticulocytes are generally going to be increased in hemolysis, and they're an indicator of marrow compensation. The healthier the marrow, the more able a patient is to compensate. An inappropriately low reticulocyte count may represent a medical emergency. It may indicate a patient's temporary or more permanent inability to compensate and should be monitored carefully. LDH is a marker of hemolysis, mostly intravascular, and we said that these



patients are going to have 2 different forms of hemolysis, both intra- and extravascular. Bilirubin levels are going to also be elevated. It's a good marker for both intra- and extravascular hemolysis, and then haptoglobin is going to be decreased. This is a scavenger of free hemoglobin and may be reduced related to hemolysis. And then ferritin may be increased because it's a marker of inflammation, and we talked about this being sort of a chronic inflammatory condition.[2,20,21]

Current Treatment Approaches for Patients with Cold Agglutinin Disease

We're going to address some of our treatment goals in patients with cold agglutinin disease. First and foremost, we would like to improve the anemia and increase hemoglobin levels. We want to avoid transfusions for a whole variety of reasons, including iron overload and potential alloimmunization. We'd also, if possible, like to improve the circulatory symptoms that can be caused by cold temperatures.

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We talked a little bit earlier about some of this supportive care for cold agglutinin disease patients, none of which is really satisfactory in effectively managing this disorder. Nonpharmacologic treatments for mild forms of cold agglutinin disease include thermal protection and cold avoidance. Some patients find that avoiding cold food and beverages is helpful. Transfusions. Steroids should definitely not be used to treat cold agglutinin disease.

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From a pharmaceutical recommendation, we're not going to use steroids which are ineffective in cold agglutinin disease. There's low efficacy in monotherapy treatments, and we should all be highly, highly considering our cold agglutinin disease patients for clinical trials for some of these newer, emerging therapeutic options so we can continue to identify the best therapeutic intervention for patients with cold agglutinin disease.

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First-line treatment strategies that have been utilized, and we do have some published information,[11] include the use of rituximab monotherapy, which would be standard dose rituximab 375 mg/m^2 once a week for 4 doses. We tend to think about this in patients that have multiple comorbidities or who are particularly frail. The response rate is only about 50%, partial responses almost exclusively, and the median duration of response is only about 12 months.

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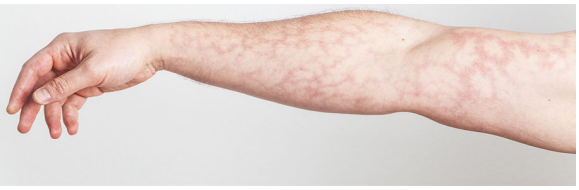
Other therapies have combined rituximab with bendamustine, rituximab 375 mg/m^2 weekly x 4, and bendamustine 90 mg/m^2 on day 1 and day 2 in a 28-day cycle.[22,23] Generally, these are going to be relatively fit



patients who can withstand the potential side effects of neutropenia. It generally has been administered for 4 cycles, and in the clinical trial, there was a 78% response rate with a 53% complete response, and the response duration was greater than 88 months.[22] The problem with this type of combined modality therapy is that the bendamustine can cause neutropenia, which can lead to an increased incidence of adverse events, including neutropenic fevers and other infections.

When we looked at combination therapies, it did certainly increase the response rate over monotherapy. Limitations? More than 25% of patients are still not responding, and some patients continue to hemolyze even though, with the rituximab and the bendamustine, we have significantly reduced the population of lymphocytes that we believe is producing the IgM monoclonal antibody.[11] Again, be aware of the serious adverse events including neutropenia and infections in this older population of patients, which can be serious.

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Other second-line treatment strategies include fludarabine and rituximab. Again, for fit patients, 40 mg/m^2 fludarabine days 1–5. Response rate was about 76%, and there were some sustained remissions, but again, adverse events, including prolonged cytopenias, were seen in this clinical trial.[2,4,21] There is one clinical trial that has been reported and directed against lymphocytes, as well, which is using a drug, bortezomib.[23,24] It has been approved for the treatment of multiple myeloma and mantle cell lymphomas, one cycle, and has been effective in about a third of the patients.

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Common Adverse Events

With all of these combination therapeutic interventions, neutropenia is one of the more serious adverse events. This can result in infection. When we use cytotoxic agents, like fludarabine and bendamustine, we may have some long-term toxicities, such as long-term cytopenias. And remember, there are infusion reactions that may be associated with rituximab that could potentially be problematic for patients who have underlying respiratory or cardiac comorbidities.

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When we think about how to prioritize and how to go through a therapeutic algorithm, we're going to first try our best to differentiate between primary cold agglutinin disease and secondary cold agglutinin syndrome. For cold agglutinin syndrome, we want to definitely try to treat the underlying problem, either infection or malignancy, if possible. For primary or idiopathic cold agglutinin disease, we can always institute all of those supportive care measures we discussed. If our patients are asymptomatic, we can watch them very carefully and wait on any

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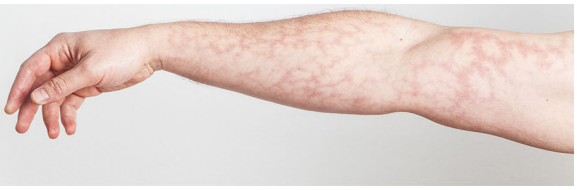

therapeutic trial or other therapeutic modality introduction. If they're symptomatic, they're anemic, they're requiring transfusions, they're having significant circulatory symptoms. We talked about clinical trials, and that would certainly be one of our top suggestions. In emergency situations, acute hemolytic crisis with severe intravascular hemolysis, eculizumab, the C5 inhibitor, has been helpful, and plasmapheresis to try to remove those IgM autoantibodies can also sometimes be helpful.[19]

If the patient's relatively stable, and we're thinking about using either rituximab as monotherapy or a rituximab in combination with other therapeutic interventions, we can think about bendamustine for patients who are fit. If they have no response or relapse, again clinical trials, or we can go to rituximab plus fludarabine, or bortezomib monotherapy.[19]

Emerging Treatments for Cold Agglutinin Disease

So, what are some of the emerging therapies that might be available for cold agglutinin disease in the relatively near future? We know that there are many unmet medical needs. There's a high frequency of persistent hemolysis and anemia that immunochemotherapy is unsuccessful in at least 25% of patients, either treatment failure or toxicity, which is unacceptable. There may be a very small B-cell clone which is responsible for the production of this IgM autoantibody. It may have a very low proliferative activity, and it's difficult to target this clone efficiently. We need rapid-acting therapy, especially for acute, severe hemolytic exacerbations that can be related to the up-regulation of complement activity, infections, surgery, traumas, cardiac surgery, etc.[5,14–18]

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There have been some global collaborative efforts to try to look at evidence-based treatment. In 2017, there was the first international consensus meeting. Dr. Berentsen published a Norway vs Northern Italy study, which is the largest study of patients with verified cold agglutinin disease.[11] And then a variety of population studies have been looked at to try to better understand some of the issues that are associated with cold agglutinin disease that may have been previously unrecognized in this relatively rare disorder.[13,25]

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From that first international consensus meeting which convened in Vienna in 2017,[19] we addressed some issues, which included that there are no licensed treatments for autoimmune hemolytic anemias currently. There are new treatment approaches that are underway, which are trying to target underlying mechanisms of hemolysis. Goals were to improve

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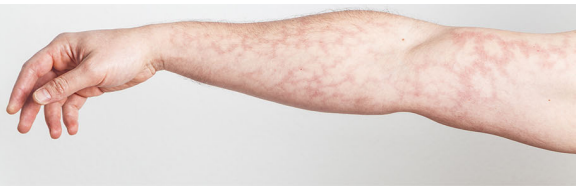

management, provide a basis for clinical trial planning, and establish a need for an international autoimmune hemolytic disease network.

In this Norway vs Italy study, long-term treatment outcomes were looked at, including long-term outcomes from rituximab-bendamustine therapy.[13] Again, that 53% CR rate and response duration of 88 months, 77% estimated 5-year sustained remission. The Danish National Patient Registry, we talked about. Cold agglutinin patients with increased mortality, median survival 8.5 years.

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Status of Potential Targets

What are these potential targets? Well, they are based on better understanding of pathogenesis. There are a few therapies available for symptomatic cold agglutinin patients. No FDA-approved treatments, but several emerging agents are in ongoing clinical trials.

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As of June 2021, we had eculizumab, which was a DECADE trial, phase 2, completed in 2017.[26] It's an inhibitor of C5, monoclonal antibody. The endpoints were the difference in LDH from baseline to the last day of therapy, and it was effective in reducing transfusion requirements. Bortezomib, which is a proteasome inhibitor, is in a phase 2 trial which was completed in 2017.[23] Achieve transfusion independence or a significant rise in hemoglobin were the primary endpoints, but a third of patients—it was a short course—effective in one-third of patients failing previous treatments.

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Sutimlimab, which is a C1s inhibitor, the CARDINAL trial, last update was March 2021.[3] A phase 3 trial. Hemolysis halted, increased hemoglobin, reduced bilirubin, and reduced fatigue. A C1s inhibitor, sutimlimab, in the CADENZA trial, and these are patients that were transfusion-independent at the beginning of the trial.[27,28] We're hoping to see some top-line results, maybe as soon as ASH [American Society of Hematology].

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Pegcetacoplan, which we talked about, is a C3 inhibitor. It's an ongoing phase 2 trial which is going to assess the safety of multiple doses.[29] Interim data showed an increase in hemoglobin and a decrease in bilirubin. And then we have BIVV020, which is a C1s inhibitor, a phase 1 trial is active and recruiting. Multiple IV doses and then a phase 1b is going to assess a single dose of IV therapy.[30,31]

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When we look at the eculizumab data,[26] we see significant improvements in LDH, and we talked about LDH being a marker of intravascular hemolysis. So, by blocking C5, not allowing the generation of C5b, which is the backbone of that membrane attack complex—which is going to be

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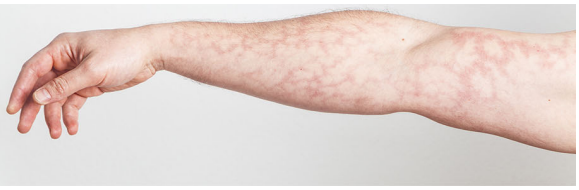

what is the driver of intravascular hemolysis—we're able to significantly reduce the LDH and stop the intravascular component. You can see here (slide 49), when you look at hemoglobinuria in a patient with an acute cold agglutinin exacerbation, you can see, 24 hours after the first dose of eculizumab stopping the intravascular hemolysis, the hemoglobinuria was markedly improved.

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Sutimlimab is a drug that targets C1s and inhibits classical complement pathway activation. If you remember earlier, we talked about how that IgM antigen complex is going to interact with C1 and generate that C1 complex. If we bind C1s with this monoclonal antibody, the C1 complex cannot be generated, and therefore activation through the classical pathway is completely halted.[3] There is no generation of C3 and there is no generation of C5.

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CARDINAL was an open-label, multicenter, phase 3 study of sutimlimab in patients with primary cold agglutinin disease who had had a recent history of blood transfusions.[3] Baseline hemoglobin less than 10, more than one transfusion within 6 months of enrollment, and no treatment with rituximab or other therapies within 6 months. Primary endpoint was a composite responder analysis, including an increase in hemoglobin of greater than 2g from dL or normalization and the absence of transfusion and no use of protocol-prohibited CAD medications. A very important secondary endpoint in these patients was a pretty rigorous quality of life assessment, measuring fatigue by FACIT fatigue score.

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Twenty-four patients, mean age 71, mean transfusions in the prior 6 months 3.2, but it ranged from 1–19. Most patients had received more than one prior targeted therapy within the last 5 years, and the mean baseline hemoglobin was 8.6g. We can see all of those characteristics there (slide 53).

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So, mean hemoglobin greater than 11 maintained from week 3 through the end of the study. The mean bilirubin normalized by week 3; 71% of patients did not get any transfusions from weeks 5–26. There was a clinically meaningful reduction in fatigue observed by week 1 and maintained throughout the study. There was no placebo arm, so 92% of patients had at least one adverse event, 29% had one serious adverse event not related to the investigational agent. There were no thromboembolic events. And, as we inhibit complement, we recognize its role in helping us deal with encapsulated bacterial infections. There were no meningococcal infections although everyone who participated in the trial was vaccinated.

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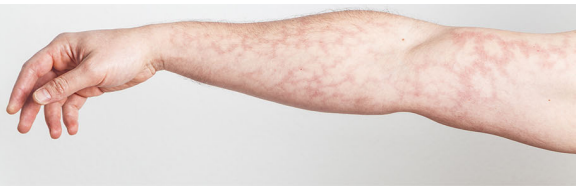

So, a summary of CARDINAL includes that it's a first-in-class selective inhibitor of the classical complement pathway. Sutimlimab demonstrated a rapid and sustained efficacy in cold agglutinin disease. The drug prevented hemolysis, increased hemoglobin and improved quality of life. It has the potential to change treatment practices for patients with cold agglutinin disease.[3,32]

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CADENZA is a phase 3 trial of sutimlimab in cold agglutinin disease in patients without a recent history of blood transfusion. The primary endpoint is the number of patients responding to treatment with a greater than 1.5 point increase in hemoglobin level above baseline, and no blood transfusions between week 5 and 26. Very important secondary outcomes are going to include haptoglobin measures, reticulocyte counts and a variety of assessments of quality of life including the FACIT fatigue score.

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A single case study of daratumumab,[33] which is a drug that targets CD38-positive cells—and remember that CD38 is expressed on plasma cells and lymphoplasmocytes. It shows some immunomodulatory influence on cytokines, like interleukins, interferons and TGF. This patient had a long history of multi-treated cold agglutinin disease, and the patient did not experience a complete response, but did have a hemoglobin increase 3g/dL resulting in transfusion independence and improvement in circulatory symptoms. It's a potential additional therapeutic option for patients with refractory disease.

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CADENCE Registry

There is a cold agglutinin registry, which is called CADENCE. It's a global registry that's going to try to help provide perspective longitudinal data with regards to patients with cold agglutinin disease, help us with a better understanding of demographics, clinical presentation and characteristics, comorbidities and disease burdens, patterns and use of cold agglutinin disease treatments, long-term clinical outcomes, health-related quality of life and different geographic locations. You can all register at this potential website: https://coldagglutininnews.com/2021/01/07/cadence-registry

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Of interest with regards to cold agglutinin patients is a case report in the *American Journal of Hematology* regarding a hemolytic crisis secondary to COVID vaccination in a woman with cold agglutinin disease.[34] Of note, patients on anti-complement agent affecting B-cell function may not be as prone to these exacerbations, and we have to have more than symptomatic management for these cold agglutinin disease patients.

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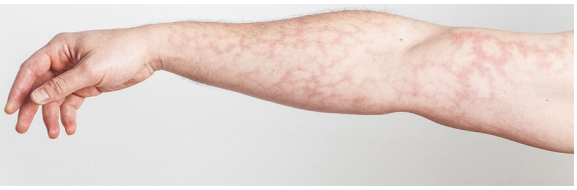

Interactive Case Scenario

So, we're going to talk about a case report and an actual case patient. In December of 2010, a 62-year-old lady began feeling fatigue and shortness of breath. She saw her primary care physician but was offered no diagnosis or explanation for her symptoms. A few months later, due to continued complaints of fatigue and shortness of breath, she was referred to see a cardiologist, but no cardiac pathology was detected. Subsequent to that, she was seen by a pulmonologist, with no pathology detected. Finally, after about 18 months, in January of 2012, she was noted to be anemic, and her evaluation revealed cold agglutinin disease. And remember we talked about that evaluation including measurements of hemoglobin, reticulocyte count, LDH, haptoglobin, bilirubin and, most importantly, a DAT, which is going to be positive for C3, and then also measurement of cold agglutinin titers. The patient was treated beginning in February of that same year with rituximab and steroids. Remember, we discussed that although this is an autoimmune hemolytic anemia, we should not use steroids in these patients, because it does not respond the same way as an IgG-mediated autoimmune hemolytic anemia.

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By May, she had a partial response, and from May to June 2013 (so almost one whole year), she spent weaning off of the steroids. As soon as she was weaned off the steroids, she began experiencing worsening fatigue, shortness of breath, and recurrent hemolysis. She was again placed on high-dose steroids—not a good idea—and another course of rituximab. Six months later, she was tapered off steroids.

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In October of 2014, she had recurrent hemolysis, shortness of breath, and fatigue again. Placed on steroids, no benefit. Again, treated with rituximab. Relatively stable for almost 2 years, but then again had fatigue and shortness of breath. In January 2017, a fourth round of rituximab with no benefit. Remember when we went through the algorithm. We talked about rituximab as a first-line monotherapy, but then if patients are relapsing or having recurrent disease, we want to think about moving on, maybe to a combination therapy with rituximab and bendamustine, rituximab and fludarabine, and certainly, we definitely want to try to avoid steroids in these patients.

In April 2017, the patient presented to Georgetown, where I work, with anemia, shortness of breath. She also had some necrotic lesions on her ear and on her thumb. She was found to have a pulmonary embolus, and because she had these necrotic areas, which we know are mainly mediated by the amount of IgM that's causing the agglutination or the

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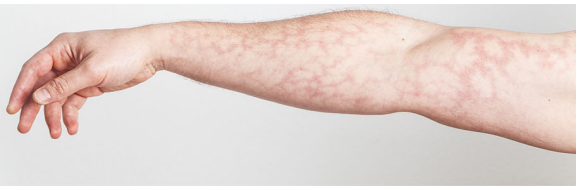

red blood cells to stick together, [we] started her on plasma exchange. She had two times a week plasma exchange, plus cyclophosphamide, for about a year, which managed to hold her relatively stable; no more lesions or other circulatory symptoms, but she was still anemic, with a hemoglobin around 9 and having persistent fatigue.

So, one question to think about is in a patient who has an underlying cold agglutinin disease and experienced a thrombotic event in the form of a pulmonary embolus, what should the duration of the anticoagulation be for this patient? Three months; 6 months; until hemolysis has resolved; or indefinite?

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I think the correct answer is indefinite. We know from multiple trials and evaluation of cohorts of patients with cold agglutinin disease that these patients remain at risk for both venous and arterial thrombotic events, even when the hemolysis appears inactive. This is believed to be related to chronic complement activation, which damages endothelial cells and sets up the framework for the development of thromboses.

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What are the takeaways from all of this? Diagnostic markers for cold agglutinin disease include anemia, an increased reticulocyte count, an elevated LDH, an elevated bilirubin and a reduced haptoglobin. We have to remember to order all of these various tests so we can assess whether anemia is related to hemolysis. There are many novel therapeutic approaches that are on the cusp of approval for the management of cold agglutinin disease. As clinicians, we should always discuss potential clinical trials with patients who fail first-line and/or second-line therapy. Discuss the registry and register your patients, if at all possible. And try to stay abreast of all of the investigational therapeutic options that are in the pipeline for these patients with this incredibly rare, but potentially life-altering and debilitating disease.

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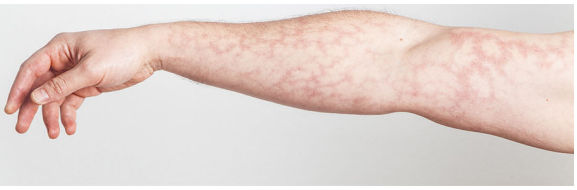
Thank you so much for your attention.

PROMOTING RECOGNITION, DIAGNOSIS AND TREATMENT OF COLD AGGLUTININ DISEASE



ABBREVIATIONS

AIHA, autoimmune hemolytic anemia
CAD, Cold Agglutinin Disease
DAT, direct antiglobulin test
IgG, Immunoglobulin G
IgM, Immunoglobulin M
LDH, lactate dehydrogenase
LMWH, Low-molecular-weight heparin
RBC, red blood cells

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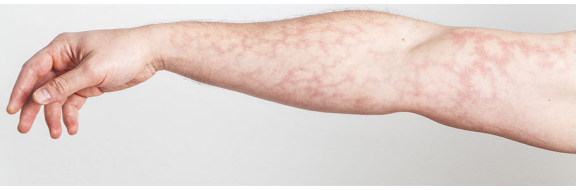

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