Cold Agglutinin Disease: Where Do We Stand, and Where Are We Going?

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Abstract: Primary cold agglutinin disease (CAD) is characterized by a very indolent bone marrow clonal B-cell lymphoproliferative disorder that initiates an autoimmune hemolytic anemia. The clonal B cells produce a monoclonal autoantibody termed cold agglutinin, most often of the immunoglobulin (Ig) Mκ class. After binding to its antigen, the IgM initiates a complement classical pathway–driven erythrocyte destruction, predominantly mediated by opsonization with complement protein C3b and extravascular hemolysis in the liver. We review the molecular biology, histopathology, clinical features, and diagnostic procedures in CAD. Some patients are only slightly anemic and do not require treatment, but moderate or severe anemia frequently occurs, and the disease burden has been underestimated. CAD should not be treated with corticosteroids. Several B-cell–directed treatment options are available, and complement-directed approaches are being rapidly developed. Current and possible future therapies are reviewed.

Introduction

Cold agglutinin disease (CAD), also known as primary or idiopathic CAD, is a clonal B-cell lymphoproliferative disorder of the bone marrow that results in an autoimmune hemolytic anemia (AIHA).[1-3] CAD predominantly affects elderly or middle-aged people, but it may occur in people as young as 30 years.[4,5]

The autoantibodies that initiate the hemolytic process are termed cold agglutinins owing to their ability to agglutinate erythrocytes at an optimum temperature of 3° to 4°C.[6,7] Cold agglutinins also may show activity at 28° to 30°C, and occasionally at temperatures approaching 37°C. This makes them pathogenic, given that such temperatures occur in acral parts of the body at normal ambient temperatures. The biological activity of a cold agglutinin is usually assessed by its titer, expressed as the inverse value of the highest serum dilution at which agglutination of erythrocytes can be detected.[7]

Cold agglutinins that are associated with CAD are monoclonal and most often of the immunoglobulin M (IgM) class with κ light-chain restriction.[4,5,8] After an IgM cold agglutinin binds to its antigen on the erythrocyte surface, complement is activated by the

classical pathway, and complement activation is essential for hemolysis.[9,10]

Most effective therapies have aimed to target the pathogenic B-cell clone.[2,11-13] Currently, however, novel complement-directed therapies are being rapidly developed.[14] In this review, we address the recent achievements in the basic understanding of CAD, as well as established and future therapeutic approaches.

Disease Definitions

AIHA and related concepts have often been addressed in the literature without being defined, and the terms CAD and cold agglutinin syndrome (CAS) have been used interchangeably.[15,16] This may make it difficult to find generally accepted criteria for use in clinical trials, to compare trials or descriptive studies, and to apply the results from studies in clinical practice.

We support a definition of CAD as an AIHA with a monospecific direct antiglobulin test result that is strongly positive for C3d and negative or weakly positive for IgG, along with a cold agglutinin titer of 64 or greater at 4°C. It should be recognized that occasional patients may have a cold agglutinin titer of less than 64. Patients may have a B-cell clonal lymphoproliferative disorder detectable in blood or marrow, but without any clinical or radiological evidence of malignancy.[2,3,15,16]

In contrast, we define CAS as an AIHA with a monospecific direct antiglobulin test result that is strongly positive for C3d and negative or weakly positive for IgG, along with a cold agglutinin titer of 64 or greater at 4°C. Patients have an associated condition, such as a specific infection, an autoimmune disorder, malignancy, or overt evidence of lymphoma (clinical or radiological).[15,16] Only CAD is further addressed here.

Epidemiology

A hospital-based study from Norway found a prevalence of 16 per million inhabitants and an incidence of 1 per million inhabitants per year.[4] The median age of patients at the time of the study was 76 years (range, 51-96 years), and the median age at clinical onset was 67 years (range, 30-92 years). These figures were on the same order of magnitude as those in a recent, registry-based study from Denmark (prevalence, 12.6 per million inhabitants; incidence, 1.2 per million per year).[17] The prevalence and incidence are probably underestimated in both the Norwegian and Danish epidemiology studies. Possible geographical variations remain to be confirmed or excluded, as does the notion that anemia may be less severe in warmer climates. The male-to-female ratio has been estimated at 0.54.[4] Thus, a slight female predominance is noted even when the higher proportion of women in an elderly population is taken into consideration.

Pathogenesis

Molecular Biology of CAD

The first experiments analyzing cold agglutinins are more than 50 years old.[8,18] Later studies have shown that almost all patients display circulating monoclonal antibodies encoded by the immunoglobulin heavy-chain gene *IGHV4-34*, and the framework region 1 (FR1) of *IGHV4-34* encodes a Gln^{6}-Trp^{7} (QW) and Ala^{23}-Val^{24}-Tyr^{25} (AVY) sequence that determines binding to the I antigen.[19-21] A recent analysis described mutations in the N-glycosylation sequon located within the complementarity-determining region 2 (CDR2) of the *IGHV4-34* gene. This sequon is variably mutated, and patients with inactivating mutations have a significantly reduced hemoglobin level.[22] Because the N-glycosylation site is located within the antigen-binding pocket, inactivating mutations preventing attachment of glycan likely modulate the binding.[23,24] A mutation hotspot, Lys^{90}-Leu^{91}-Ser^{92} (KLS), within framework region 3 (FR3) of *IGH* was also found. Patients who had CAD with more mutations in this hot spot had particularly low hemoglobin levels.[22]

Malecka and coworkers[22] reanalyzed previously published *IGKV* gene sequencing data of CAD, using the international ImMunoGeneTics (IMGT) information system and the Immunoglobulin Basic Local Alignment Search Tool (IgBLAST).[20,22,25-27] Reanalysis of published data shows that the Ig light-chain gene *IGKV3-20* was used in most published CAD cases (12 of 16); we also found that the *IGKV3-20* gene and, to a lesser extent, the similar *IGHV3-15* gene are used in most patients (74%) and might therefore contribute to I-antigen binding. Of interest, the *IGKV3-20* CDR3 region is highly homologous in a subgroup of patients and correlated with younger age at diagnosis, a finding consistent with specific antigen selection in this group of patients. A low level of mutations in *IGKV3-20* was also correlated with a younger age at diagnosis.[22]

Although CAD antibodies are almost exclusively encoded by *IGHV4-34*, in rare instances anti-I cold agglutinins may also be encoded by *IGHV3* family genes.[28] Marks and colleagues used *IGHV3-23* instead of *IGHV4-34* to isolate human antibody with specificity against I antigen.[29] More recently, we reported on 1 of 27 patients with CAD who had cold agglutinins encoded by *IGHV3-23* and *IGKV3-20* genes.[22]

The use of next-generation sequencing, together with flow cytometry–assisted cell sorting of bone marrow, enabled us to identify recurrent mutations of *KMT2D* (11/16, 69%) and *CARD11* (5/16, 31%) in CAD.[30]

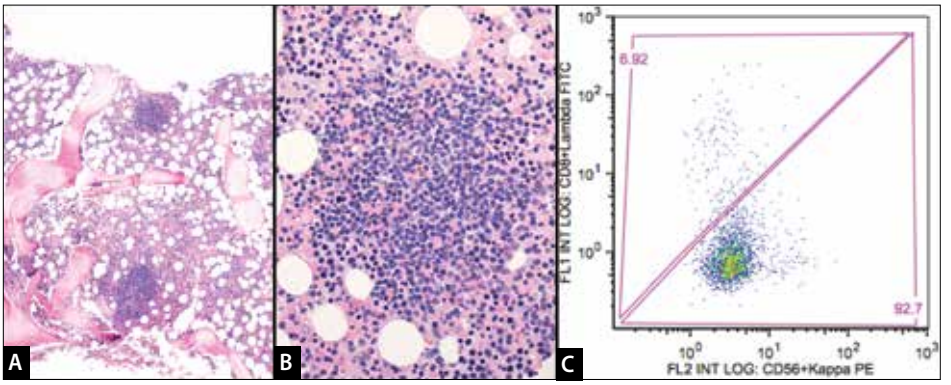

Figure 1. CAD-associated lymphoproliferative disease. Panel A shows the nodular infiltration pattern. Panel B highlights the resemblance to marginal zone B-cell infiltration. Panel C shows the typical flow cytometry finding of a monoclonal κ+ B-cell population (gated on CD19+ B cells).

Seven identified *KMT2D* mutations were high-impact mutations that resulted in a C terminal–truncated protein that lacked the SET domain and was therefore enzymatically inactive. Two missense mutations were located in the C-terminal domain, which may have a negative effect on the activity of the SET domain or result in KMT loss of function.[30,31] All 5 mutations of *CARD11* were located within a 20–base pair fragment of exon 6 coding for the BAR domain of the coiled-coil region of *CARD11*. Mutations localized in the BAR domain and coiled-coil region of *CARD11* were previously demonstrated in diffuse large B-cell lymphoma and shown to induce constitutive activation of the nuclear factor–κB (NF-κB) pathway.[32] It is likely, therefore, that *CARD11* mutations identified in CAD have a similar impact on NF-κB pathway activation. The mutations in *KMT2D* and *CARD11* were concurrent in 4 patients.

CAD lacks the *MYD88* L265P mutation, a very important finding that clearly distinguishes CAD from lymphoplasmacytic lymphoma.[3,30,33,34] Older data suggest that chromosome instability might play a role in CAD because trisomy 3 and 12 were found in a few cases, but this needs to be further investigated.[35-37]

Histopathology and Flow Cytometry Findings

Bone marrow trephine biopsy specimens in patients with CAD typically show a lymphoid infiltration consisting of nodular B-cell aggregates, with involvement varying from 5% to 80% of the intertrabecular surface (Figure 1A).[3] However, some samples show only scattered B cells, making the diagnosis of a lymphoproliferative disease difficult. In these cases, a flow cytometry analysis with the finding of a monoclonal, most often IgMκ-positive, B-cell population is crucial (Figure 1C).[3,34,38]

The infiltration mimics that of marginal zone lymphoma (Figure 1B), and the immunophenotype is not distinct.[3] Mature plasma cells are seen surrounding the lymphoid aggregates and also invariably throughout the marrow between the nodular lymphoid aggregates, but only a few plasma cells are normally seen within the nodular lymphoid aggregates. The plasma cells have the same heavy- and light-chain restriction as the B cells, showing the plasmacytoid differentiation of the B-cell clone. Of note, features associated with lymphoplasmacytic lymphoma, such as paratrabecular growth, fibrosis, lymphoplasmacytoid cell morphology, and an increased number of mast cells surrounding the lymphoid aggregates, are not seen. The patients do not have an extramedullary marginal zone lymphoma, and therefore bone marrow involvement of marginal zone lymphoma can be ruled out.

CAD-associated lymphoproliferative disorder does not have features of hitherto well-characterized types of B-cell non-Hodgkin lymphoma recognized by the World Health Organization classification of tumors of lymphoid tissues, and it should be considered a distinct entity.[2,3,34,39,40]

Autoantibody Characteristics

Identification of the monoclonal Ig in CAD will depend on the sensitivity of the method used and adherence to the optimal handling of samples, as discussed below. In a study of 86 patients, results of protein electrophoresis with immune fixation were available for 84.[4] A monoclonal band had been detected in 79 patients (94%), whereas 5 (6%) had no verified monoclonal Ig. The monoclonal protein was of the IgM class in 71 patients (90% of those with a confirmed Ig), IgG in 3 (3.5%), and IgA in

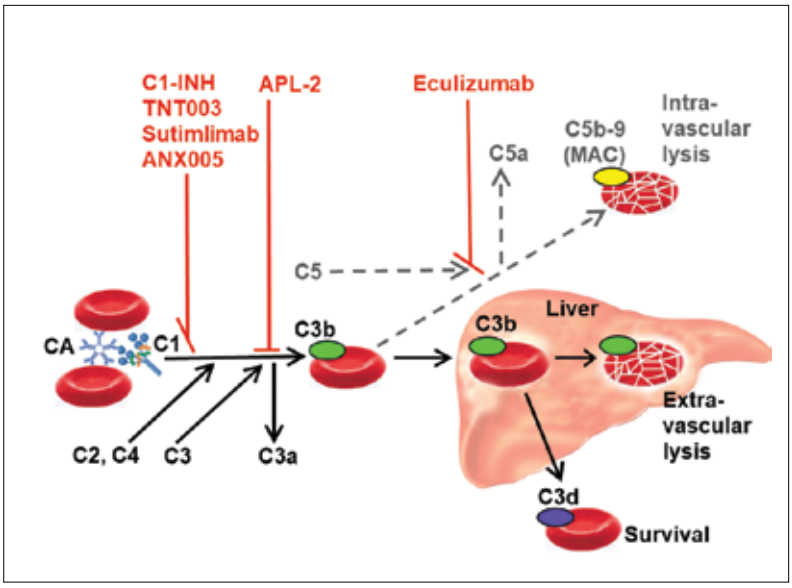

Figure 2. Complement-mediated hemolysis in CAD and possible targets for therapeutic complement inhibition. Only relevant pathways and components are shown. Black lines, major pathways; gray/dotted lines, minor pathways.

ANX005, C1q inhibitor; APL-2 (pegcetacoplan), a C3-targeted complement inhibitor; C1, C2, etc, complement proteins; CA, cold agglutinin; C1-INH, plasma-derived C1 esterase inhibitor; MAC, membrane attack complex; TNT003, inhibitor of the serine protease C1s.

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3 (3.5%), whereas 2 patients (2.5%) had biclonal IgM and IgG. The light-chain restriction was κ in 74 patients (94%), λ in 2 (2.5%), and unknown in 3 (3.5%). The 2 occurrences of λ monoclonal light chains were found with the IgG and IgA classes. The further implications of IgG and IgA cold agglutinins are addressed in the next subsection, "Role of Complement in CAD."

Monoclonal cold agglutinins, as found in CAD, are generally more pathogenic than polyclonal cold agglutinins.[41] Cold agglutinins are also characterized by their thermal amplitude—that is, the highest temperature at which the cold agglutinin will react with its antigen.[6,9] The thermal amplitude is assumed to be more important than the titer with respect to pathogenicity.[42] Cold agglutinins with a thermal amplitude below 25° to 28°C will be active only in vitro and are not pathogenic, and individuals who have incidentally detected cold agglutinin without hemolysis or clinical symptoms do not have CAD.[12,43,44]

IgM cold agglutinins in CAD are specific for the erythrocyte surface carbohydrate antigen I (capital *I*) in the vast majority of cases; rare specificities include those for i (lowercase *i*) and the protein antigen Pr.[4,7,8,45,46] A study has found the median cold agglutinin titer in IgM-mediated disease to be 2048 (upper range, 819,200).[4] In clinical practice and some studies, high cold agglutinin titers will tend to be underestimated because the analysis is time-consuming, and many laboratories do not continue the titration until exact determination of the highest dilution that triggers agglutination.

Role of Complement in CAD

At least in typical (IgM-mediated) CAD, hemolysis is complement-driven (Figure 2).[9,47,48] Cooling of blood during circulation through the head and extremities causes IgM cold agglutinin to bind to its antigen at the erythrocyte surface.[7,42] IgM is a potent activator of the classical complement pathway, resulting in fixation of the C1qrs complex with subsequent binding and activation of C4 and C2.[9,49] The resulting C4b and C2a combine to form C3 convertase, which cleaves the next complement protein, C3, into C3a, an anaphylotoxin, and C3b, which remains bound to the cell surface. In general, this reaction may also result from activation of the alternative or lectin pathways, which is assumed not to take place in CAD. The cleavage of C3, therefore, is a point of convergence for all 3 initial activation pathways and essential for further complement activation.[48]

From this step, hemolysis can occur by 2 different mechanisms. The C3 convertase complex can bind to its reaction product, C3b, thus generating membrane-bound C5 convertase, which initiates the terminal complement cascade.[48,49] In this series of reactions, C5 convertase splits C5, resulting in the generation of C5a, a powerful anaphylotoxin, and C5b, which remains surface-bound. C5b then in turn binds C6, C7, C8, and C9, thus forming the C5b-9 complex, also known as the membrane attack complex (MAC). The MAC is able to lyse the cell, and intravascular hemolysis ensues. Concomitantly or alternatively, however, C3b-opsonized erythrocytes can undergo phagocytosis by the mononuclear phagocyte system, mainly in the liver, a process known as extravascular hemolysis.[10,50] On the surviving erythrocytes, C3b is cleaved into fragments, including C3d, which can be detected by the direct antiglobulin test. C3d probably also protects the erythrocytes against further phagocytosis.[50]

Old as well as recent experiments indicate that in most patients with stable CAD, C3 opsonization and phagocytosis comprise the predominant mechanism of hemolysis.[10,50] However, hemoglobinuria has been reported in 15% of cases of CAD,[5] hemosiderinuria occurs relatively frequently,[51] and a beneficial effect of blocking the terminal complement pathway is seen at least in severely hemolytic patients and during acute exacerbations.[52-54] These observations show that terminal pathway activation with intravascular hemolysis does occur in some patients and situations.

Exacerbation of hemolytic anemia during febrile infections and other conditions with acute phase reaction, originally described by Ulvestad and colleagues as "paradoxical" hemolysis, has been found to occur in at least 70% of these patients.[4,53,55] Constant complement consumption during steady-state disease results in low serum levels of C3 and, in particular, C4, which appears to be rate-limiting for classical pathway–dependent hemolysis. Acute phase reaction has been shown to enhance the production of these components; the levels are replete, complement activity increases, and exacerbation of hemolysis ensues.[56] Therefore, patients with CAD should probably not receive transfusion with complement-rich blood products (eg, plasma), although this has not been systematically studied.

CAD with non-IgM cold agglutinin has been described in a small percentage of patients, in particular those with IgG as the involved class.[4,46,57-60] The IgG autoantibodies are generally found with lower cold agglutinin titers than are IgM autoantibodies,[4] and IgG is also a weaker complement activator than IgM. It is mainly the IgG3 subclass and, to a lesser extent, IgG1 that are able to act as classical pathway triggers, whereas IgG2 is a very weak activator and IgG4 does not activate complement.[61,62] Furthermore, in contrast to those with IgM cold agglutinins, some patients with IgG-mediated CAD have been reported to respond to corticosteroids or splenectomy.[59] These observations may indicate a different mechanism of hemolysis in IgG-mediated disease.

In most descriptions of IgA cold agglutinins, the involved subjects did not have hemolysis.[46,63] A case observation of a patient with clinical CAD presenting with monoclonal IgAλ revealed 2 unrelated bone marrow B-cell clones: one of the IgAλ phenotype in accordance with the paraprotein, and one of the IgMκ phenotype as in typical CAD, but with no corresponding monoclonal Ig detectable on electrophoresis.[57] Cell-bound IgA does not activate complement.[58,64] The existence of IgA-mediated CAD can be questioned, therefore, or a non–complement-mediated hemolysis must be present. In patients who have CAD presenting with an apparent IgA phenotype, 2 independent clones should be suspected, and the monoclonal serum IgA may not be identical to the cold agglutinin.[57]

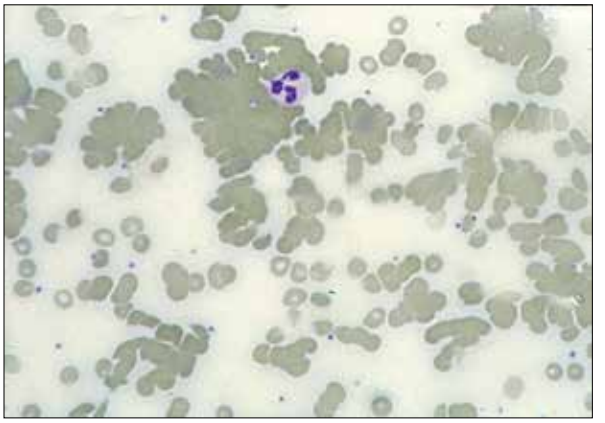

Figure 3. Peripheral blood smear from a patient with cold agglutinin disease. Agglutination of erythrocytes dominates the picture.

Diagnosis and Clinical Features

Hemolysis is assessed by the biochemical markers lactate dehydrogenase, bilirubin, indirect bilirubin if required, and haptoglobin.[11,65] Although the reticulocyte count is not necessarily elevated in all patients with AIHA, determination of an elevated absolute reticulocyte count can also be confirmative of increased erythrocyte turnover. An autoimmune pathogenesis is confirmed by a positive direct antiglobulin test result.

The specific criteria for a diagnosis of CAD have been listed in the section "Disease Definitions."[11,16] Monospecific direct antiglobulin testing and cold agglutinin titration are essential for a reliable diagnosis. Examination of a peripheral blood smear will usually be informative and show erythrocyte agglutinates, anisocytosis, and polychromasia (Figure 3).[66] Mean corpuscular volume is often spuriously high and red cell counts tend to be falsely low, so that calculated hematocrit values are unreliable.[67]

All patients with suspected CAD should have a bone marrow examination, including trephine biopsy and flow cytometric assessment for a B-cell clone.[2,3] Ig class quantification, protein electrophoresis, and immune fixation are mandatory.

Samples for cold agglutinin titration, Ig analyses, and if needed thermal amplitude determination must be handled as described in the Table.[66,68] Failure to comply with these requirements will result in poor sensitivity and falsely low values. Even in ethylenediamine tetraacetic acid (EDTA) blood, hemoglobin levels and cell counts may be difficult to obtain because of erythrocyte agglutination in the tube. This problem can usually be prevented by prewarming to 37° to 38°C for up to 2 hours; if this does not work, a 1-minute preheating at 41°C can be tried.[69]

Up to 90% of the patients with CAD in a Norwegian study had cold-induced symptoms owing to agglutination

Table. Cold Agglutinin Disease: Handling of Samples

Analysis	Material	Sampling	Handling of Sample
Hemoglobin, blood cell counts	Blood	EDTA vacutainer	Prewarm at 37°-38°C before analysis if problems with agglutination.
Cold agglutinin titer, thermal amplitude, immunoglobulin quantification, electrophoresis, immune fixation	Serum or plasma	Blood is drawn into prewarmed vacutainers (for serum: no gel or additive). Place in warming cabinet or water bath at 37°-38°C.	Keep at 37°-38°C until serum/plasma has been removed from the clot/cells, after which the sample can be handled at room temperature.
Flow cytometry	Bone marrow aspirate (sensitivity too low if performed in peripheral blood)	Add EDTA or heparin.	Prewarming before analysis will often be sufficient. If not, wash cells at 37°-38°C.[a]

EDTA, ethylenediamine tetraacetic acid.

[a] For more-detailed information, see Ulvestad E et al. *Eur J Haematol*. 1999;63(4):259-266.[7]

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in the cooler parts of the circulation: acrocyanosis and occasionally disabling Raynaud-like symptoms.[4,5] The percentages would possibly be lower in warmer climates. Such symptoms do not correlate with the severity of hemolytic anemia.[4,70]

Anemia in CAD is often mild to moderate, but it may be severe. The median hemoglobin level among patients with CAD living in cool climates has been estimated to be 8.9 g/dL, with median levels in the lower and upper tertiles of 8.0 g/dL and 10.4 g/dL, respectively. Several reports show that these patients occasionally have a hemoglobin level as low as 4 g/dL.[4,6,70] On the other hand, fully compensated hemolysis is not unusual.[4,70] Data on transfusion requirements are highly variable, probably because of patient selection and differences in transfusion criteria. Studies of larger, unselected cohorts have indicated that 40% to 50% of patients with CAD have received transfusions.[4,5]

Many hemolytic anemias are associated with an increased risk for venous and arterial thromboembolic events.[71] In CAD, such a risk has been documented in severely anemic patients,[52] but it has been difficult to show for CAD patients in general, partly because of methodology issues with the studies.[17,72] Further descriptive studies may better answer this question.

Current and Emerging Therapies

Nonpharmacological Management and Unspecific Therapies

The term "cold" relates primarily to the biological properties of the cold agglutinins, not the clinical picture, and the notion of a beneficial effect of cold avoidance is based on clinical experience and anecdotal reports.[6,73] Still, it seems reasonable to maintain recommendations regarding protection against exposure to cold, in particular of the acral parts of the body, and avoidance of cold infusions.[11,12]

Any bacterial infection should be treated promptly.[55,56] Transfusions can be safely administered if the patient and the extremity chosen for infusion are kept warm and an in-line blood warmer is used.[11,12] Sometimes, it is necessary to perform the screening for irregular antibodies and crossmatching (if needed) at 37°C, but the compatibility problems seen in warm antibody AIHA are not encountered in CAD.

No prospective trials on corticosteroid therapy have been published, but most retrospective data indicate low response rates (probably <20%) and the frequent need for unacceptably high maintenance doses among the few responders.[4,5,74] Therefore, corticosteroids should not be used to treat CAD.[2,11,12,65,74] No evidence is available for treatment with azathioprine, cyclosporine, or other unspecific immunosuppressive therapies.

Folic acid supplementation has traditionally been recommended[11,12] on the basis of theoretical considerations and old studies of nonimmune hemolytic anemias.[75] In our experience, however, many patients with CAD have normal folate levels even in the absence of supplementation. A systematic study might clarify this issue.

Erythropoietin (EPO) at a relatively high dose has been shown in 2 retrospective studies (one with 12 patients, and the other with 29 patients) to improve hemoglobin levels in patients with AIHA.[76,77] Most participants with a diagnosis of CAD responded, and increasing hemoglobin levels were seen even among those with some endogenous EPO response.[77] EPO may be considered as a temporary adjunct in selected patients until a more specific treatment has taken effect.

Until more data have been collected regarding the risk for thromboembolic events, we would not generally recommend antithrombotic prophylaxis in CAD. Such measures should be considered, however, in severely anemic patients, in those with acute exacerbations, and if additional risk factors are present.

Indications for Specific Therapy

Patients with mild anemia and tolerable circulatory symptoms should be followed without treatment.[11,12,65] However, series of unselected patients show that despite the restrictive older recommendations, 70% to 80% have received therapy.[4,5] Together with the median and lower-tertile hemoglobin levels referenced above, this finding indicates that the disease burden has traditionally been underestimated and previous recommendations have been too restrictive. Today, a considerable proportion of the patients should receive treatment because efficacious therapeutic approaches with sufficiently low toxicity are available. No evidence-based cutoff exists for the initiation of therapy, but most of the recent literature recommends treatment for patients with clinical symptoms of anemia, hemoglobin levels below approximately 10 g/dL, a transfusion requirement, or disabling cold-induced circulatory symptoms.[11,12,65]

Therapies Directed at Clonal B Cells. Monotherapy with rituximab (Rituxan, Genentech/Biogen) is the oldest documented approach in patients with CAD.[11,78,79] In 2 prospective nonrandomized trials using similar criteria, 4 weekly infusions of rituximab at 375 mg/m^2 resulted in overall response rates of 45% to 54%, with a median response duration of approximately 1 year.[80,81] Complete responses were rare, as defined by criteria that included the disappearance of any sign of a clonal B-cell disorder. Responders achieved a median increase in hemoglobin levels of 4.0 g/dL, and the median time to response was 1.5 months.[80] Of 10 patients who relapsed, 6 responded to re-treatment.

In a nonrandomized prospective trial, 45 patients received rituximab at 375 mg/m^2 on day 1 and bendamustine at 90 mg/m^2 on days 1 and 2 of 28-day intervals for 4 cycles.[70] Of the 45 patients, 32 (71%) responded; 18 (40%) achieved a complete response and 14 (31%) achieved a partial response. Among 14 patients previously treated with rituximab or fludarabine/rituximab, 7 (50%) responded to bendamustine plus rituximab. Hemoglobin levels increased by a median of 4.4 g/dL among those who achieved a complete response and by 3.9 g/dL in the partial response group. Median time to response was 1.9 months (upper value, 12 months), with an even longer time to an optimal response in some patients. Grade 4 neutropenia occurred in 9 patients (20%), but only 5 (11%) experienced infection with or without neutropenia. After 32 months, fewer than 10% had relapsed.

The combination of rituximab plus fludarabine yielded an overall response rate similar to that achieved with rituximab/bendamustine, but with a lower complete response rate (21%) and more toxicity. The observed response duration was 6 years.[82] In a prospective trial of patients receiving bortezomib (Velcade, Millennium/Takeda Oncology) monotherapy administered as a single cycle, the overall response rate was 31% (6/19), with 3 complete responses and 3 partial responses.[83] A strong theoretical rationale exists for studying the effect of Bruton tyrosine kinase (BTK) inhibitors and other novel B cell–targeted therapies, but no such studies have been published.

Rituximab/bendamustine should be considered as first-line treatment in severely affected patients without relevant comorbidities or contraindications to chemoimmunotherapy. For other patients, rituximab monotherapy should be the first choice. Bortezomib-based therapy (which might possibly be improved by using combinations and/or an extended duration) or rituximab/fludarabine should be considered second-line alternatives.

Complement-Directed Therapies. Following the success in treating paroxysmal nocturnal hemoglobinuria with the anticomplement C5 monoclonal antibody eculizumab (Soliris, Alexion),[84] several novel complement inhibitors are being rapidly developed for therapeutic use in complement-mediated hemolytic anemias.[85]

Case reports have found a beneficial effect of eculizumab in some patients with CAD.[53,54,86] A subsequent nonrandomized prospective trial confirmed a reduction in transfusion requirements and intravascular hemolysis as assessed by lactate dehydrogenase levels, but the increase in hemoglobin levels was marginal, and no significant improvement in quality of life was noted.[52] On the basis of the limited role of terminal pathway activation in CAD (Figure 2), these findings were as expected.

In theory, attacking the complement system upstream in the classical pathway or at the C3 level would be more likely to succeed. Treatment with high doses of plasma-derived C1 esterase inhibitor (C1-INH), which is approved for hereditary angioedema, has been successful as rescue therapy in single cases of complement-mediated warm antibody AIHA and severe CAS.[87,88] However, because patients with CAD are not deficient in C1-INH, and the in vivo half-life of C1-INH is relatively short, frequently repeated high doses will probably be required. Therefore, this approach does not appear attractive for long-term therapy.

Sutimlimab (TNT009, BIVV009) is a humanized monoclonal antibody against C1s.[89] In vitro experiments with TNT003, the murine precursor of sutimlimab, found

complete inhibition of C3 deposition and phagocytosis of erythrocytes when patient serum was used as a source of cold agglutinin and normal human serum as a source of complement.[10] A clinical phase 1b trial in 10 patients with CAD showed that weekly intravenous administration of sutimlimab increased hemoglobin levels by a median of 1.6 g/dL within the first week and by 3.9 g/dL within 6 weeks.[90] Bilirubin levels mostly normalized within 24 hours, and all 6 previously transfusion-dependent patients became transfusion-free. Patients relapsed 3 to 4 weeks after discontinuation, but re-exposure to sutimlimab restored the remission. Drug-related adverse events were not seen. Participants were vaccinated against *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*, but they did not receive prophylactic antibiotics.[90] Sutimlimab is currently being studied in phase 2 and 3 trials in CAD (NCT03347396 and NCT03347422).

Other C1 inhibitors are ANX005, which is a humanized monoclonal antibody against C1q,[91] and peptide inhibitor of complement C1 (PIC1), which is a group of smaller molecules that block the activation of C1 serine proteases and subsequent classical pathway activation.[92] Although no clinical data have been published on PIC1, a safety trial of ANX005 in healthy volunteers has just finalized accrual (NCT03010046).

The compstatin analogue pegcetacoplan (APL-2) is a pegylated cyclic peptide designed for subcutaneous administration. Pegcetacoplan blocks C3 activation and thereby prevents the formation of C3b.[93,94] This agent therefore has the potential to block the entire complement system. A safety study in healthy volunteers has not shown a risk for infection thus far, but experience is limited and the participants were vaccinated.[94] High rates of efficacy and safety were recently found in a phase 1b trial in paroxysmal nocturnal hemoglobinuria.[95] A phase 2 trial in AIHA has reported promising response data, at least in the CAD subgroup.[96] Further studies of pegcetacoplan in CAD are warranted.

The Future

Existing or Emerging Therapies

As described, several options exist or are being developed for patients with CAD who need treatment. Still, a minority of patients fail to respond, experience adverse effects, or need a treatment associated with a shorter time to response.

Although the high efficacy rate, long response duration, and acceptable toxicity of the rituximab/bendamustine regimen make this an attractive option, unmet needs remain, given that relapsed or refractory disease must be expected in up to 30% of patients.[70] Response rates achieved with bortezomib monotherapy are low[83]; however, the possibility of longer treatment duration or bortezomib-based combinations should be explored. Furthermore, the effect of BTK or B-cell lymphoma/leukemia 2 (BCL2) inhibitors should be studied.[97,98] Although a particularly strong mechanistic rationale exists for BTK inhibition in *MYD88* L265P–positive lymphoproliferative disorders,[97] such therapies have also proved efficacious in several B-cell lymphoproliferative disorders that are negative for this mutation.[98]

The complement-directed approaches, particularly those targeting C1 or C3, should be further investigated.[14] The potential risk for infectious or autoimmune complications will have to be carefully addressed, although preliminary data indicate a favorable safety profile.[90,94,99] A disadvantage of these options will be the need for indefinite continuation of treatment, in contrast to the B-cell–directed therapies, which are temporary and often provide long-lasting remissions. Moreover, the peripheral circulatory symptoms are not complement-mediated and will not be relieved. In profoundly anemic patients and in those with severe, acute exacerbations, however, it is to be hoped that rapidly acting complement modulation can provide a bridge to the more definitive and causal—but slower-acting—B-cell–directed therapies.

New Theoretical Prospects

Histone deacetylase inhibitors have been used in lymphoma and myeloma and might have a therapeutic potential in CAD with *KMT2D* mutations.[30,100] Therapies aimed to counteract the effect of *CARD11* gain-of-function mutations are currently being evaluated in diffuse large B-cell lymphoma.[101] Furthermore, the finding that the cold agglutinin light chains are encoded mainly by *IGKV3-20* or the homologous *IGKV3-15* and *IGKV3-11* genes in a smaller number of patients might suggest anti–light-chain vaccination as therapy for CAD.[22] Off-the-shelf vaccines with *IGKV3-20*–encoded proteins, known to be immunogenic, are being considered as treatment in other lymphoproliferative diseases.[102] On the basis of the high efficacy rate of chemoimmunotherapy and promising activity of anticomplement therapies, however, it may turn out to be difficult to explore these potential approaches in CAD.

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