REVIEW ARTICLE

Dan L. Longo, M.D., *Editor*

Autoimmune Hemolytic Anemias

Sigbjørn Berentsen, M.D., Ph.D., and Wilma Barcellini, M.D.

Autoimmune hemolytic anemia (AIHA) is defined as increased destruction of red cells through autoimmune mechanisms, usually mediated by autoantibodies against erythrocyte surface antigens.[1-3] During the past decade, important new findings have emerged regarding the cause, pathogenesis, diagnosis, and treatment of this group of disorders. Historically, clinical practice was based on theoretical considerations, case reports, and expert opinion, but more recently, several prospective and other systematic clinical studies have been conducted. The first international consensus on diagnosis and therapy was published in 2020,[1] and British guidelines have also been reported.[4,5] Challenges remain, however, including a wider application of current knowledge, an improved understanding of pathogenesis, development of new therapies, and management of refractory cases. AIHA is a common term for several diseases that differ from one another with respect to cause, pathogenesis, and clinical presentation, and the individual disorders should be addressed according to these differences (Table 1).[1,6,7]

From the Department of Research and Innovation, Haugesund Hospital, Helse Fonna Hospital Trust, Haugesund, Norway (S.B.); and the Hematology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan (W.B.). Address reprint requests to Dr. Berentsen at the Department of Research and Innovation, Haugesund Hospital, Helse Fonna Hospital Trust, P.O. Box 2170, 5504 Haugesund, Norway, or at sigbjorn.berentsen@haugnett.no.

N Engl J Med 2021;385:1407-19.
DOI: 10.1056/NEJMra2033982

CME at NEJM.org

WARM AIHA

In warm AIHA, antibodies have the highest affinity to the antigen at 37°C. The autoantibodies are polyclonal (i.e., produced by nonclonal B lymphocytes and plasma cells).[1,8] They are usually of the IgG class, but IgM warm antibodies may be involved, and in rare cases, IgA warm antibodies may be involved.[3,8-10]

The pathogenesis of warm AIHA is complex (Fig. 1). The mononuclear phagocytic system in the spleen plays a major role in the breakdown of opsonized erythrocytes (extravascular hemolysis).[11,12] The complement system, activated by the classical pathway, is involved in approximately half the cases.[6,13] Most of the complement-mediated erythrocyte destruction occurs through phagocytosis of complement fragment 3b (C3b)–coated cells (extravascular hemolysis). To a lesser extent, cleavage of C5 and activation of the terminal complement cascade may occur, resulting in formation of the membrane attack complex and intravascular hemolysis. The pathogenesis of this disorder also involves the T-lymphocyte system.[14-16] The signal substance cytotoxic T-lymphocyte antigen 4 (CTLA-4) activates regulatory T cells, which are critical for immune tolerance.[17] Polymorphism of the *CTLA-4* gene can confer a predisposition to immunologic disorders, including autoimmune cytopenias.[15] The programmed cell death 1 (PD-1) signal pathway is another checkpoint for immune tolerance, and pharmacologic inhibition of PD-1 carries an increased risk of autoimmune disease.[18]

In nearly 50% of cases of warm AIHA, no underlying or associated disorder can be identified, and the hemolytic disease is classified as primary.[1,6] Slightly more than 50% of the cases are secondary to immunologic or lymphoproliferative disorders (e.g., chronic lymphocytic leukemia, systemic lupus erythematosus, or common variable immunodeficiency).[5,6,14,19] Occasionally, a lymphoproliferative

Table 1. Autoimmune Hemolytic Anemias.

Variable	Warm-Antibody Type	Cold Agglutinin Disease	Secondary Cold Agglutinin Syndrome	Paroxysmal Cold Hemoglobinuria	Mixed Type
Incidence and age at onset	5–10 cases/1 million persons/yr; occurs at any age but frequently in the elderly	0.45–1.9 cases/1 million persons/yr; occurs mainly in the elderly	Rare, any age	Rare in children, ultrarare in adults	Rare, depending on definition
Cause	Unknown in <50% of cases; secondary in ≥50% of cases*	Low-grade lymphoproliferative bone marrow disorder	Secondary*	Postviral (in children); tertiary syphilis, hematologic cancers (in adults)	Unclear
Pathogenesis					
Autoantibody	Warm-reactive, panreactive, polyclonal	Cold agglutinin, anti-I (in rare cases, anti-Pr or anti-IH), monoclonal	Cold agglutinin, anti-I or anti-i, polyclonal or monoclonal	Nonagglutinating, biphasic anti-P, polyclonal	Both warm- and cold-reactive antibodies
Immunoglobulin class	IgG (in rare cases, IgM or IgA)	IgM (in rare cases, IgG)	IgM or IgG	IgG (in rare cases, IgM)	IgG plus IgM
Complement activation†	Frequently none; classical pathway (++), terminal pathway (+)	Classical pathway (+++), terminal pathway (+)	Classical pathway (+++), terminal pathway (+)	Classical pathway (+++), terminal pathway (+++)	Present, details not established
Predominant type of hemolysis	Extravascular (mainly in the spleen)	Extravascular (mainly in the liver); intravascular (in acute exacerbations)	Extravascular (mainly in the liver); intravascular (in acute exacerbations)	Intravascular	Not established
Typical findings					
Direct antiglobulin test	IgG positive C3d negative or positive In rare cases, IgA or IgM positive	C3d positive In rare cases, IgG or IgM positive IgA negative	C3d positive IgG positive or negative In rare cases, IgM positive IgA negative	C3d positive In rare cases, IgG or IgM positive IgA negative	IgG and C3d positive In rare cases, IgM positive IgA negative
Cold agglutinin	Absent	High titer	High titer	Absent	High titer

* Warm-antibody type is secondary to immunologic or lymphoproliferative disorders (e.g., chronic lymphocytic leukemia, systemic lupus erythematosus, or common variable immunodeficiency), and secondary cold agglutinin syndrome is secondary to infection (e.g., *Mycoplasma pneumoniae* or Epstein–Barr virus infection) or cancer (e.g., aggressive lymphoma).
† The designation (+) denotes weak or absent, (++) moderate, and (+++) strong.

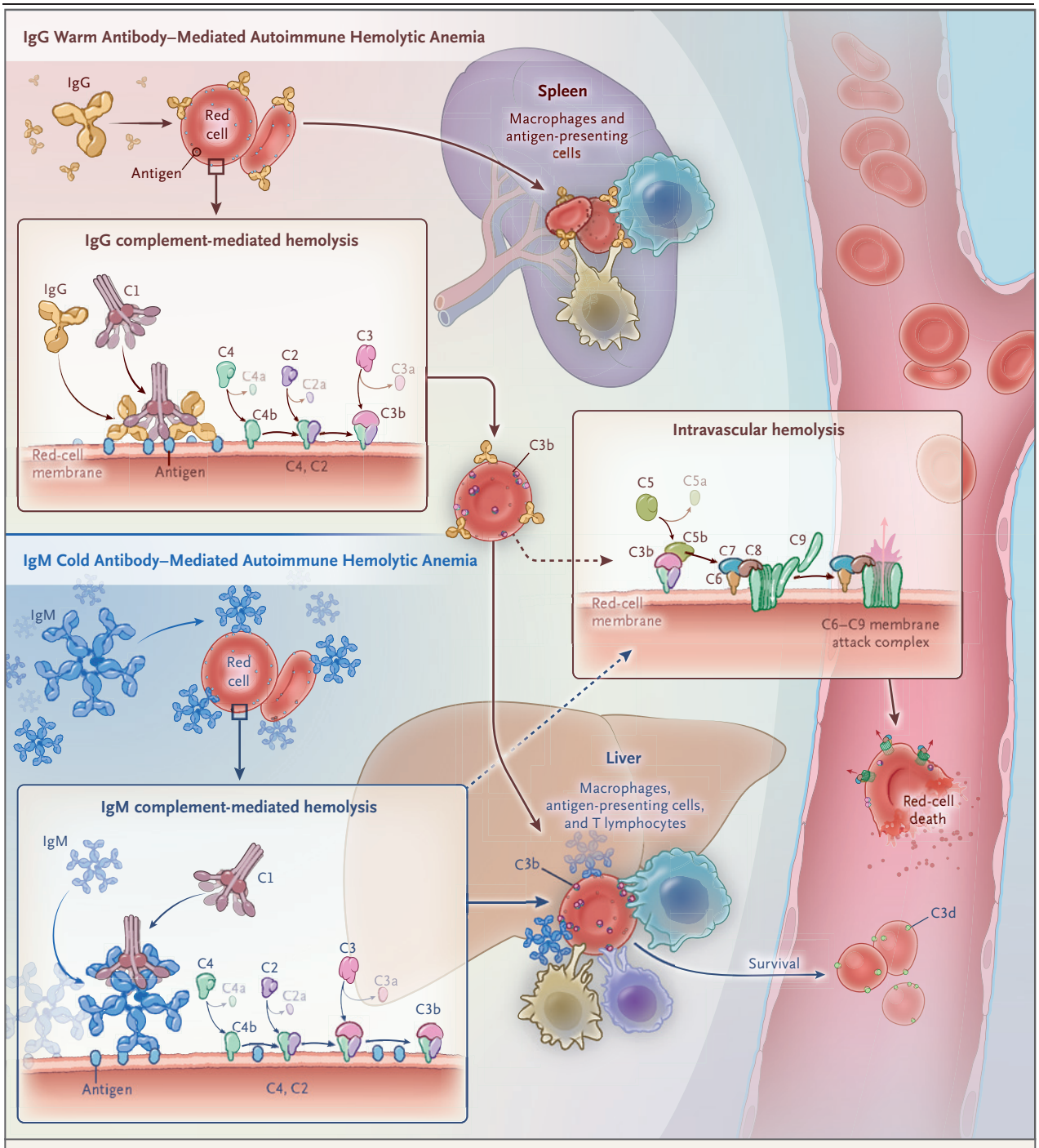

Figure 1. Pathogenesis of Warm Antibody–Mediated and Cold Antibody–Mediated Autoimmune Hemolytic Anemia.

In warm autoimmune hemolytic anemia (AIHA), erythrocytes opsonized with immunoglobulin (typically IgG) are destroyed by the mononuclear phagocytic system, mainly in the spleen and partly after transformation to spherocytes. Complement-mediated hemolysis, which can take place to a variable extent, occurs as phagocytosis of C3b-labeled cells (extravascular hemolysis) and occasionally even as intravascular hemolysis. In cold agglutinin disease, the autoantibody is typically IgM and the hemolysis is complement-dependent, mainly mediated by extravascular hemolysis of C3b-opsonized cells in the liver.

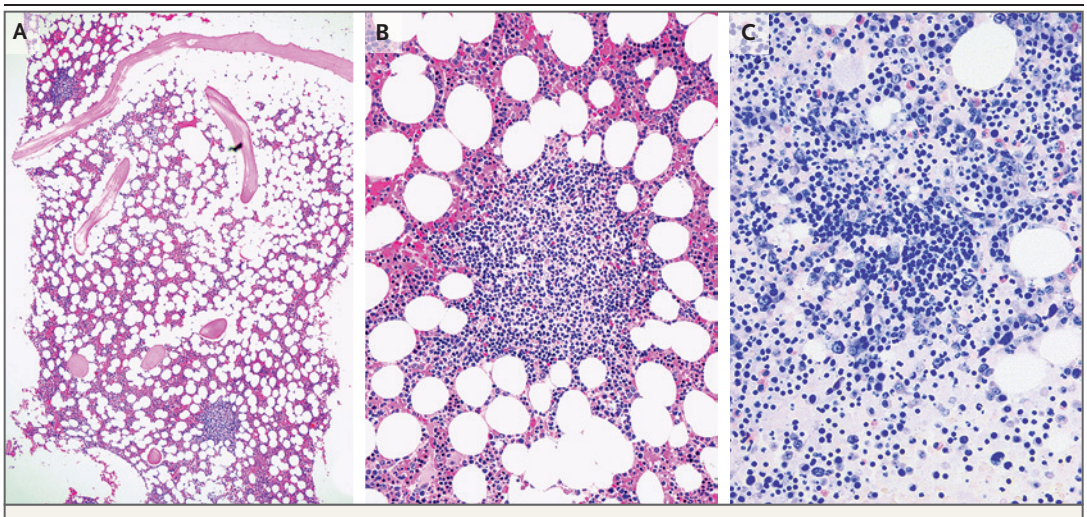

Figure 2. Histologic Features of Cold Agglutinin–Associated Lymphoproliferative Bone Marrow Disorder.
Nodular lymphoid infiltrates in a sample from a trephine biopsy of bone marrow are characterized by an intraparenchymal rather than paratrabecular location (Panels A [low-power view] and B [high-power view], hematoxylin and eosin). Scarce plasma cells can be seen, but mast-cell infiltration is not present (Panel C, Giemsa staining).

disease develops several years after the diagnosis of AIHA.[16] Recently, several cases have been described in patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.[20] The Evans syndrome, characterized by the simultaneous or sequential combination of AIHA and immune thrombocytopenia, is often associated with more severe anemia and a worse prognosis than primary warm AIHA.[6,21,22]

COLD AGGLUTININ DISEASE

Cold-reactive antibodies bind to the antigen at a temperature of 0 to 4°C but may also react at higher temperatures.[7,23] Cold autoantibodies that can agglutinate erythrocytes are termed cold agglutinins. The thermal amplitude is the highest temperature at which agglutination can be detected, and cold agglutinins with a thermal amplitude higher than 28 to 30°C are pathogenic.[7,24] The cold agglutinin titer is the inverse of the highest plasma or serum dilution at which agglutination can be seen at a given temperature.[1,25]

Cold agglutinin disease is an AIHA in which the autoantibody is a cold agglutinin and no underlying clinical disorder is present.[1,2,23,26] Newer studies have shown that affected patients, who previously would have received a diagnosis of primary or idiopathic cold agglutinin disease, have a clonal lymphoproliferative bone marrow disease that can be difficult to recognize.[27-32] The histopathological picture has often been interpreted as several types of low-grade non-Hodgkin's lymphoma, such as lymphoplasmacytic or marginal-zone lymphoma, but a relatively uniform disease, cold agglutinin–associated lymphoproliferative bone marrow disorder, is now thought to be the underlying condition (Fig. 2).[27,29,32] The *MYD88* L265P mutation, present in nearly all cases of lymphoplasmacytic lymphoma (the bone marrow disorder seen in Waldenström's macroglobulinemia), is usually not found in cold agglutinin disease.[29,31,33]

In affected patients, the cold agglutinins are monoclonal, usually of the IgM class, and are produced by clonal lymphocytes in the bone marrow.[27] Cooling of the blood in acral parts of the circulation allows for binding of cold agglutinin to its antigen on the erythrocyte surface, resulting in agglutination and impaired passage through the capillaries. Thus, about half of patients with cold agglutinin disease have cold-induced circulatory symptoms such as acrocyanosis or Raynaud-like phenomena.[27] Gangrene is a rare complication.

Hemolysis in cold agglutinin disease is complement-dependent.[25,34] Activation of the classical pathway results in coating of erythrocytes with the split product C3b. C3b-opsonized cells are prone to phagocytosis by the mononuclear

phagocytic system, mainly in the liver (extravascular hemolysis).[13,34] C3b can also react to form C5 convertase, which initiates the terminal complement cascade.[7,13] Terminal complement activation, in turn, leads to formation of the membrane attack complex and intravascular hemolysis, at least in some patients and situations.[26,28]

OTHER AIHA TYPES

SECONDARY COLD AGGLUTININ SYNDROME

The distinction between cold agglutinin disease (a well-defined entity, as explained above) and secondary cold agglutinin syndrome has been increasingly accepted.[2,5,23,26,35] The latter is a rare, heterogeneous group of cold agglutinin–mediated AIHA disorders that are secondary to other diseases — most often, specific infections (*Mycoplasma pneumoniae* infection, Epstein–Barr virus infection, cytomegalovirus infection, SARS-CoV-2 infection, and others) or cancers (typically, aggressive B-cell lymphoma).[1,23,36]

PAROXYSMAL COLD HEMOGLOBINURIA

In paroxysmal cold hemoglobinuria, the autoantibody is a biphasic IgG hemolysin called Donath–Landsteiner antibody.[13,37] It binds to its antigen at temperatures below central body temperature, but activation of the classical complement pathway, beyond the initial steps, occurs after warming to 37°C in the central circulation. The terminal complement cascade is activated, and hemolysis is predominantly intravascular. Today, this disease occurs almost exclusively as a rare, temporary, postviral complication in children.[1,13,37]

MIXED AIHA

Mixed warm antibody–mediated and cold antibody–mediated AIHA is defined by the presence of warm IgG autoantibodies combined with high-titer cold agglutinins.[6,38] The mixed form of AIHA is often characterized by lower hemoglobin levels and a worse prognosis than warm AIHA, and two or more lines of therapy are frequently needed.[6]

DRUG-INDUCED HEMOLYTIC ANEMIA

Hemolytic anemia induced by drugs is usually mediated by immunologic mechanisms.[5,39] More than 150 drugs have been implicated as causes of this condition, which nonetheless remains rare.[1,5] Cases can be classified into two subtypes.[1,40] In the drug-dependent subtype, autoantibodies are produced in response to a neoantigen formed by the binding of a drug to a cell-surface structure. In the drug-independent subtype, the offending drug can induce an autoimmune response that persists even in the absence of the drug. Historically, methyldopa and large doses of penicillin were the most frequent causes of drug-induced hemolytic anemia. Today, most cases are caused by ceftriaxone, other cephalosporins, piperacillin, or nonsteroidal antiinflammatory drugs.[5,39] Fludarabine, chlorambucil, and even bendamustine can increase the risk of autoimmune hemolysis when they are used to treat chronic lymphocytic leukemia, especially as monotherapy.[5,41] Not unexpectedly, checkpoint inhibitors that are used to treat cancer, in particular PD-1 inhibitors, can also induce AIHA.[18]

AIHA AND THROMBOSIS

Warm AIHA is associated with a risk of thrombosis.[6,42-45] Three studies have reported thrombotic events in 11 to 20% of patients with AIHA, mostly deep venous thrombosis, with or without pulmonary embolism, but also venous thrombosis in atypical locations and arterial thromboembolism.[6,42,44] Recent studies indicate an increased occurrence of thrombosis even among patients with cold agglutinin disease, at a relative risk of 1.7 to 2.4 for affected patients as compared with the general population.[27,46]

DIAGNOSIS

A diagnostic algorithm is shown in Figure 3, and Table 2 provides a summary of diagnostic procedures. Hemolytic anemia is diagnosed on the basis of the hemoglobin level and serum levels of lactate dehydrogenase, unconjugated bilirubin, and haptoglobin.[1,4,47] Absolute reticulocytosis supports the presence of hemolysis, but the reticulocyte count may be normal or low,[1,47] probably because of autoantibody activity against erythrocyte precursors or a coexisting bone marrow disorder.

Autoimmune pathogenesis is detected by means of the direct antiglobulin test (DAT, also termed Coombs' test). A positive test shows immunoglobulin, complement, or both on the erythrocyte surface. A positive polyspecific (simple) DAT must be followed by a monospecific

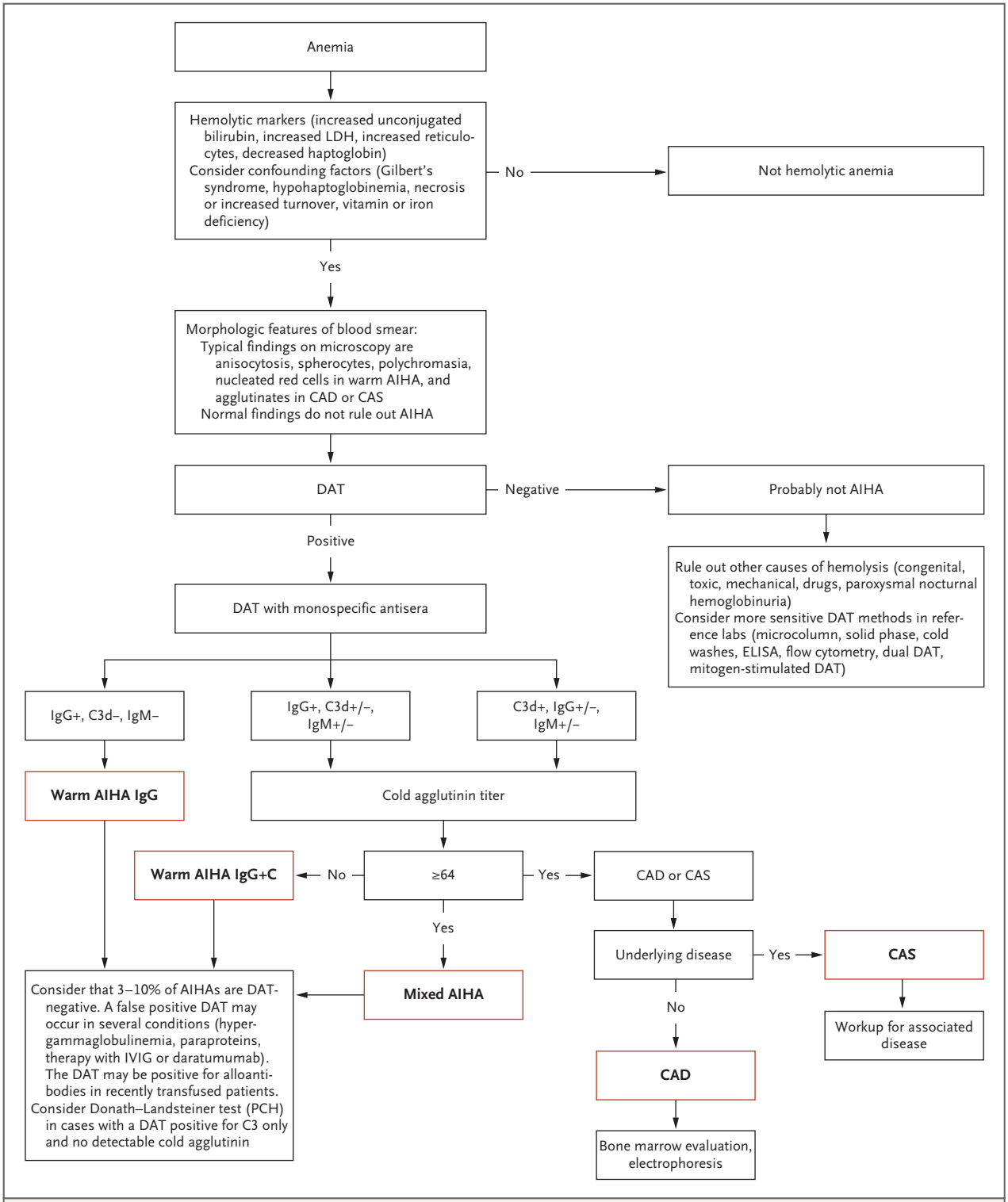

Figure 3. Algorithm for a Diagnostic Workup of Autoimmune Hemolytic Anemia (AIHA).

The cold agglutinin titer (≥64) is expressed as an integer and represents the activity of the antibody and the inverse of the dilution. C denotes complement, CAD cold agglutinin disease, CAS cold agglutinin syndrome, DAT direct antiglobulin test, ELISA enzyme-linked immunosorbent assay, IVIG intravenous immune globulin, LDH lactate dehydrogenase, and PCH paroxysmal cold hemoglobinuria.

Table 2. Diagnostic Tests for Autoimmune Hemolytic Anemia (AIHA).*

Test or Analysis	Specimen	Purpose	Comments
Hemoglobin	Blood	Detect anemia	—
LDH, unconjugated bilirubin, haptoglobin	Serum or plasma	Detect hemolysis	Elevated LDH and bilirubin levels, low or undetectable haptoglobin levels, or both indicate hemolysis
Absolute reticulocyte count	Blood	Verify increased erythrocyte turnover	Counts above normal support hemolysis, but the count may be normal or low
Free hemoglobin	Urine, or serum or plasma	Detect hemoglobinuria or free hemoglobin in blood	Shows intravascular hemolysis
Polyspecific (simple) DAT	Blood	Detect immunoglobulin, complement, or both on the erythrocyte surface	Positive test supports autoimmune pathogenesis; test is negative (DAT-negative AIHA) in 3-10% of affected patients
Monospecific (extended) DAT	Blood	Identify immunoglobulin class or type of complement protein on the erythrocyte surface	Required for classification and for diagnosis of purely IgA-mediated AIHA; positive test for C3d indicates IgM involvement
Cold agglutinin titer	Serum or plasma	Semiquantitative assessment of cold agglutinins	Specimen must be kept at 37–38°C from sampling until separation of serum or plasma from clot or cells
Donath–Landsteiner test	Blood	Detect biphasic autoantibody in paroxysmal cold hemoglobinuria	Indicated mainly for acute hemolytic anemia in children
Immunoglobulin class quantification	Serum or plasma	Abnormal levels occur in common variable immunodeficiency and, often, in monoclonal gammopathies	If cold agglutinin is present, specimen must be kept at 37–38°C from sampling until separation of serum or plasma from clot or cells
Serum protein electrophoresis	Serum or plasma	Detect monoclonal immunoglobulin or hypogammaglobinemia	—
Immunofixation	Serum or plasma	Identify low-concentration monoclonal immunoglobulin, in particular in CAD	—
Bone marrow biopsy	Bone marrow	Detect and classify bone marrow disorders	Indicated in CAD; indicated in warm AIHA if treatment fails or if bone marrow disorder is suspected
Flow cytometry	Bone marrow aspirate	Detect clonal bone marrow disorder	—

* CAD denotes cold agglutinin disease, DAT direct antiglobulin test, and LDH lactate dehydrogenase.

(extended) DAT, which will identify the immunoglobulin class (or classes) or complement protein bound to the red cell. Complement components, most often C3d, are detected in nearly half the cases of warm AIHA because of complement activation. If warm-reactive IgM is involved, the DAT may be positive or negative for IgM because IgM may have detached from the cell before it can be detected in the laboratory. IgM is a potent complement activator and will leave the erythrocytes coated with C3. Therefore, a monospecific DAT that is positive for C3d and negative for IgG indicates IgM involvement.[9]

The DAT is negative in 3 to 10% of patients with AIHA, depending on the DAT technique used.[1,47] AIHA with a negative DAT remains a difficult diagnosis of exclusion. A false negative result can be due to a low density of immunoglobulin, complement, or both on the cell surface. A rare cause of false negative results is warm AIHA with IgA as the only autoantibody class, since most polyspecific reagents do not

contain anti-IgA and thus cannot detect IgA. This problem can be overcome by performing a monospecific DAT even when the polyspecific test is negative. More sensitive DAT methods (microcolumn, solid phase, cold washes, enzyme-linked immunosorbent assay, flow cytometry, dual DAT, and mitogen-stimulated DAT) in reference laboratories may be useful. Nonimmune causes of hemolysis (congenital, toxic, or mechanical causes, as well as drugs and paroxysmal nocturnal hemoglobinuria) should be ruled out (Fig. 3).[1,4,47]

In cold agglutinin disease and the cold agglutinin syndrome, the DAT is positive for C3d by definition and is usually negative for immunoglobulins but is weakly positive for IgG in up to 20% of cases.[27,28] This result is not sufficient for establishing the diagnosis. The cold agglutinin titer must be assessed; it will be at least 64 (an integer that represents the activity of the antibody and the inverse of the dilution [1:64]) and usually much higher.[7,25,27] Specific precautions are required for sampling and handling blood specimens, as indicated in Table 2.[1,7]

Paroxysmal cold hemoglobinuria should be suspected in children with acute hemolytic anemia and should be confirmed or ruled out with the Donath–Landsteiner test.[1,37] In a positive test, binding of the antibody to red cells occurs at a low temperature, followed by complement-mediated hemolysis on subsequent warming. Cold agglutinins are not present in this disease, and the DAT is frequently positive for C3 and negative for IgG.

Once warm AIHA has been diagnosed, an assessment for causes of secondary AIHA should be undertaken.[1,5] This includes a careful review of the medical history, a thorough clinical examination, assessment of serologic markers of autoimmune diseases, and relevant viral serologic tests. Whole-body computed tomography and flow cytometry in peripheral blood should be considered.[1] Serum protein electrophoresis and quantification of immunoglobulin classes must be performed for all types of AIHA.[7,15,27] In cold agglutinin disease, the lymphoproliferative bone marrow disorder should be identified by biopsy and flow cytometry, although in a minority of cases, it will not be detected or interpretation of the results will be difficult.[27,29] The relevant clonal bone marrow changes are most frequently identified in specialized pathology laboratories that are familiar with this entity.[27] Even in patients with warm AIHA, bone marrow examinations should be performed for a wide range of indications, at least in case of therapy failure.[1]

Cold agglutinins are sometimes incidentally detected in blood samples obtained for unrelated indications.[48] In these cases, the patients often have a polyclonal, low-titer, low-thermal-amplitude cold agglutinin without hemolysis or clinical symptoms, and they do not have cold agglutinin disease or syndrome. Likewise, persons with incidental positive DAT results and no signs of hemolysis do not have AIHA.

TREATMENT

PRIMARY WARM AIHA

Prednisolone (or prednisone) at a dose of 60 to 100 mg, or 1 mg per kilogram of body weight, per day is still recommended as initial first-line therapy for primary warm AIHA.[1,4,8,49] After 2 to 3 weeks, dose tapering should be started in patients who have a response; prednisolone should be withdrawn if no response is observed. If the dose can be reduced to 7.5 to 10 mg per day after 3 to 6 months without relapse of clinically significant anemia, a further tapering should be attempted until the drug is discontinued. Approximately 80% of patients have an initial response to this regimen; however, only 30 to 40% have a sustained remission after 1 year.[49-51]

The addition of rituximab to first-line therapy has been studied in two prospective, randomized trials.[50,51] The results were almost identical, showing a doubling of long-term responses in the group that received both drugs. Although rituximab is not specifically licensed for the treatment of AIHA, the First International Consensus Group has recommended considering the addition of rituximab as initial therapy in patients with severe disease (i.e., those with a hemoglobin level of <8 g per deciliter).[1] Atypical AIHA (IgA-mediated, mixed, or DAT-negative cases) and the Evans syndrome are also regarded as severe disease in this context.[1,6,44]

In patients who do not have a response to first-line therapy, a diagnostic reevaluation should be considered, focusing on any previously overlooked cause of secondary AIHA. The currently recommended second-line therapy for primary disease is rituximab, if it has not been added to first-line therapy.[1,4,52,53] Response rates of 70 to 80% have been reported, with a median time to a

response of 3 to 6 weeks. However, 30% of patients have a relapse within 3 years.[51,52] The conventional dose is 375 mg per square meter of body-surface area, administered weekly for four cycles,[1,4,52,54] or a 1000-mg fixed dose, administered for two cycles at a 2-week interval.[51,52] A fixed low dose of 100 mg, administered weekly for 4 weeks, appears to be equally efficacious, but comparative clinical trials have not yet been reported.[54]

Splenectomy is currently recommended for patients who do not have a response to or who have a relapse after rituximab therapy.[1,4,53] A response is seen in approximately 70% of patients, but the rate of long-lasting remission is unknown. The drawbacks of splenectomy are a risk of severe infection and a further increased risk of thrombosis.[4,55,56] Other options for third-line or subsequent therapy are azathioprine, cyclophosphamide, cyclosporine, mycophenolate mofetil, and bortezomib.[1,4] Remission has been observed after treatment with any of these immunosuppressants, but reported response rates are based mostly on data from small retrospective series and pooled case reports.[4,57] Such analyses must be interpreted with skepticism because of selection bias, publication bias, and undefined or heterogeneous response criteria. In patients who initially have a response to glucocorticoids, long-term, low-dose prednisolone (≤10 mg daily) may be an appropriate approach.[1]

For emergencies, high-dose intravenous methylprednisolone, intravenous immune globulin, complement C1 inhibition, plasma exchange, splenectomy, and partial splenic embolization have been tried with some success in single patients or retrospective series.[1,4,58,59] In patients with refractory disease, bortezomib in combination with dexamethasone has shown promising efficacy in a small series.[60] High-dose cyclophosphamide, alemtuzumab, and even autologous or allogeneic stem-cell transplantation have been used as a last resort.[1,8,61] The evidence supporting the use of these therapies is limited, and they carry a considerable risk of serious adverse effects. Their use should be restricted to carefully selected patients at highly specialized centers and only after documentation of refractory disease.[1]

SECONDARY WARM AIHA

With some exceptions, first-line therapy for secondary warm AIHA should be the same as first-line therapy for the primary form.[1] Specific therapy for the underlying or associated disease is indicated if it requires treatment by itself or if the hemolytic anemia is resistant to first-line therapy — for example, in AIHA complicating chronic lymphocytic leukemia.[1,5]

COLD AGGLUTININ DISEASE

Glucocorticoids should not be used to treat cold agglutinin disease because response rates are low and patients who have a response often require unacceptably high doses to maintain the remission.[4,7,27] Unfortunately, however, this ineffective therapy is still prevalent in many countries.[27,62] Patients with mild anemia or compensated hemolysis should be monitored without treatment if circulatory symptoms and fatigue are absent or tolerable.[1,4,7,23] Nonpharmacologic management, which is advisable even in this group, consists of thermal protection to limit hemolysis and relieve any ischemic symptoms.[4,62]

Among treatments directed at the pathogenic B-cell clone, rituximab monotherapy (four doses of 375 mg per square meter at 1-week intervals) has become the most commonly used first-line therapy for cold agglutinin disease.[27,52,63] Prospective and retrospective studies have shown response rates of 45 to 60%, with only rare complete responses.[27,63-65] A majority of patients with a response have a relapse within 12 to 15 months, but a repeat series of rituximab infusions has a fair chance of inducing remission.[27,63] A fixed low dose of 100 mg has not shown equal efficacy in cold agglutinin disease.[54] The addition of fludarabine has yielded much higher overall and complete response rates but also more acute and late-onset toxic effects.[27,66]

Treatment with rituximab plus bendamustine, studied in a prospective trial, resulted in overall and complete response rates of 71% and 40%, respectively, which increased to 78% and 53% during long-term follow-up.[27,67] The median time to a response was 1.9 months (upper end of the range, 12 months), and the time to the best response was up to 30 months. The deepening of responses with time and the occurrence of very late responses are probably related to long-lived plasma cells.[27,29,68,69] The estimated median response duration was more than 88 months. Temporary grade 3 or 4 neutropenia (neutrophil count, $<0.5 \times 10^9$ per liter) was observed in 33% of the patients, but only 11% had infection, with

or without neutropenia.[67] The frequency of late-occurring cancers did not exceed that reported in untreated patients.[27]

In a small, prospective study of bortezomib administered as a single cycle of monotherapy, responses were observed in 32% of the participants.[70] In theory, this proportion might be increased by extending the duration of treatment or using bortezomib-based combination therapy. In a retrospective study of therapy with ibrutinib, a Bruton's tyrosine kinase inhibitor, responses were observed in all 10 study participants.[71] This finding will have to be confirmed in a larger series or a prospective trial.

Complement inhibition is a promising new therapeutic approach but cannot be expected to relieve the circulatory symptoms, which are not complement-mediated.[7,13,26,34] In a study of therapy with sutimlimab, a monoclonal antibody against complement protein C1s, patients had a rapid response and normalization of hemolysis, and the drug was safe.[72] Sutimlimab will probably be available outside clinical trials in the near future. Favorable results have also been reported with pegcetacoplan, a pegylated peptide that inhibits C3.[73] Unlike most B-cell–directed therapies, which are given for brief intervals, complement inhibition will probably have to be continued indefinitely to maintain its effect.[7,13] This approach will provide a therapeutic option for patients in whom B-cell–directed therapy has failed or is contraindicated. The much shorter time to a response may be particularly helpful in patients with severe anemia and those in acute crisis.

OTHER AIHA TYPES

In secondary cold agglutinin syndrome, the only established therapy is treatment of the underlying disease when possible. Although infection-associated cold agglutinin syndrome resolves some weeks after the infection has been eliminated, anemia can be profound, and therapy is largely ineffective.[23] A response to complement inhibition has been described in single cases,[26,35] but no systematic study has been reported.

Childhood paroxysmal cold hemoglobinuria is self-remitting, but anemia can be severe and occasionally life-threatening.[1,37] Immediate improvement was recently reported after a single dose of the complement C5 inhibitor eculizumab in a child with the postviral form of the disorder.[37]

Mixed AIHA is generally characterized by a severe onset and a relapsing or refractory course. Glucocorticoids should be given in high doses, and rituximab should be considered early. If cold agglutinin symptoms are prominent, treatments for cold agglutinin disease are advisable. Splenectomy is discouraged.[1,6]

In patients with drug-induced hemolytic anemia, the suspected medication is discontinued.[5,39,40] In the drug-independent subtype, recovery can be delayed for weeks after the causative medication has been stopped. The benefit of adding glucocorticoids is unproven, but this approach is frequently implemented.[5,39]

SUPPORTIVE MEASURES

TRANSFUSION

Transfusion in patients with warm AIHA requires specific precautions.[1,4,74] Pretransfusion screening for irregular antibodies will be positive, and cross-matching of type-identical blood will show incompatibility. Indications should be restrictive, but transfusion must not be withheld in cases of life-threatening anemia.[53] If time permits, extended phenotyping should be performed with respect to Rh subgroups and Kell, MNS, Kidd, S/s, and Duffy antigens.[1,53] Genotyping can be considered but will often be too time-consuming. Transfusion should start with a bedside in vivo compatibility test: rapid infusion of 20 ml of erythrocyte concentrate, observation for 20 minutes, and if no reaction is observed, further infusion at the usual rate.[1]

In patients with cold agglutinin disease, precautions are different. It is often possible to find compatible erythrocytes by cross-matching at 37°C, but in vivo cooling during transfusion can cause agglutination and hemolysis of patient as well as donor erythrocytes. The patient and the extremity chosen for infusion should be kept warm, and most authors recommend the use of a blood warmer during infusion.[1,4,62]

PROPHYLAXIS AGAINST THROMBOSIS

The role of prophylactic anticoagulation in patients with AIHA has not been established. In patients with acute exacerbations, hospitalized patients with marked hemolysis, and those who

have undergone splenectomy or have additional risk factors, we recommend administration of low-molecular-weight heparin in prophylactic doses or a direct oral anticoagulant.[1,4,8,62]

OTHER SUPPORTIVE MEASURES

Erythropoietin and its analogues have been shown to increase hemoglobin levels in patients with warm AIHA, as well as in those with cold agglutinin disease, and can be used as a supportive strategy.[75,76] Plasma exchange (with the use of albumin instead of plasma, to avoid complement supplementation) has been successful in single cases of both warm and cold AIHA, although systematic studies have not been reported.[4,77] Plasma exchange can be considered in critical situations, but the effect is short-lived, and specific therapy should be initiated concomitantly.

FUTURE DIRECTIONS

For the treatment of warm AIHA, ongoing prospective trials include studies of fostamatinib (a splenic tyrosine kinase inhibitor),[78] parsaclisib (a phosphatidylinositol 3-kinase delta [PI3Kδ] inhibitor that specifically targets B lymphocytes and a candidate drug in warm as well as cold AIHA),[79] and inhibitors of the neonatal Fc receptor (which regulates the half-life of IgG).[1,8] Drugs reported to be effective in single cases or specific situations include sirolimus and daratumumab,[1,80-82] but the use of these agents is experimental. For the treatment of cold agglutinin disease, trials of complement C1 and C3 inhibitors are ongoing, as discussed above.[72,73] Additional complement inhibitors are in the pipeline, and further studies of B-cell–targeting agents such as ibrutinib are needed.[71]

CONCLUSIONS

AIHA is a heterogeneous group of diseases, and treatment should be tailored to the individual pathophysiological features of each disease. Exact diagnosis of the type of disorder and any underlying or associated disease is critical. Glucocorticoids remain first-line treatment in patients with warm AIHA. In case of treatment failure, a diagnostic reevaluation should be considered. Therapy for cold agglutinin disease, when indicated, is directed against the pathogenic B-lymphocyte clone. An emerging alternative is therapy directed at complement inhibition. With any type of hemolytic anemia, patients who do not have a response to first-line treatment should be considered for enrollment in clinical trials.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank Dr. Ulla Randen, Akershus University Hospital, Norway, for providing histologic images.

REFERENCES

1. Jäger U, Barcellini W, Broome CM, et al. Diagnosis and treatment of autoimmune hemolytic anemia in adults: recommendations from the First International Consensus Meeting. Blood Rev 2020;41:100648.
2. Hill QA, Hill A, Berentsen S. Defining autoimmune hemolytic anemia: a systematic review of the terminology used for diagnosis and treatment. Blood Adv 2019;3:1897-906.
3. Barcellini W, Fattizzo B. The changing landscape of autoimmune hemolytic anemia. Front Immunol 2020;11:946.
4. Hill QA, Stamps R, Massey E, Grainger JD, Provan D, Hill A. The diagnosis and management of primary autoimmune haemolytic anaemia. Br J Haematol 2017;176:395-411.
5. Hill QA, Stamps R, Massey E, Grainger JD, Provan D, Hill A. Guidelines on the management of drug-induced immune and secondary autoimmune, haemolytic anaemia. Br J Haematol 2017;177:208-20.
6. Barcellini W, Fattizzo B, Zaninoni A, et al. Clinical heterogeneity and predictors of outcome in primary autoimmune hemolytic anemia: a GIMEMA study of 308 patients. Blood 2014;124:2930-6.
7. Berentsen S. New insights in the pathogenesis and therapy of cold agglutinin-mediated autoimmune hemolytic anemia. Front Immunol 2020;11:590.
8. Barcellini W, Fattizzo B. How I treat warm autoimmune hemolytic anemia. Blood 2021;137:1283-94.
9. Meulenbroek EM, de Haas M, Brouwer C, Folman C, Zeerleder SS, Wouters D. Complement deposition in autoimmune hemolytic anemia is a footprint for difficult-to-detect IgM autoantibodies. Haematologica 2015;100:1407-14.
10. Janvier D, Sellami F, Missud F, et al. Severe autoimmune hemolytic anemia caused by a warm IgA autoantibody directed against the third loop of band 3 (RBC anion-exchange protein 1). Transfusion 2002;42:1547-52.
11. Nagelkerke SQ, Bruggeman CW, den Haan JMM, et al. Red pulp macrophages in the human spleen are a distinct cell population with a unique expression of Fc-γ receptors. Blood Adv 2018;2:941-53.
12. Garratty G. The James Blundell Award Lecture 2007: do we really understand immune red cell destruction? Transfus Med 2008;18:321-34.
13. Berentsen S, Hill A, Hill QA, Tvedt THA, Michel M. Novel insights into the treatment of complement-mediated hemolytic anemias. Ther Adv Hematol 2019;10:2040620719873321.
14. Fattizzo B, Barcellini W. Autoimmune cytopenias in chronic lymphocytic leukemia: focus on molecular aspects. Front Oncol 2020;9:1435.
15. Schwab C, Gabrysch A, Olbrich P, et al. Phenotype, penetrance, and treatment of 133 cytotoxic T-lymphocyte antigen 4-insufficient subjects. J Allergy Clin Immunol 2018;142:1932-46.
16. Nearman ZP, Wlodarski M, Jankowska AM, et al. Immunogenetic factors determining the evolution of T-cell large gran-

ular lymphocyte leukaemia and associated cytopenias. Br J Haematol 2007;136:237-48.
17. Ward FJ, Hall AM, Cairns LS, et al. Clonal regulatory T cells specific for a red blood cell autoantigen in human autoimmune hemolytic anemia. Blood 2008;111:680-7.
18. Khan U, Ali F, Khurram MS, Zaka A, Hadid T. Immunotherapy-associated autoimmune hemolytic anemia. J Immunother Cancer 2017;5:15.
19. Azizi G, Tavakol M, Rafiemanesh H, et al. Autoimmunity in a cohort of 471 patients with primary antibody deficiencies. Expert Rev Clin Immunol 2017;13:1099-106.
20. Barcellini W, Giannotta JA, Fattizzo B. Are patients with autoimmune cytopenias at higher risk of COVID-19 pneumonia? The experience of a reference center in Northern Italy and review of the literature. Front Immunol 2021;11:609198.
21. Hansen DL, Möller S, Andersen K, Gaist D, Frederiksen H. Evans syndrome in adults — incidence, prevalence, and survival in a nationwide cohort. Am J Hematol 2019;94:1081-90.
22. Michel M, Chanet V, Dechartres A, et al. The spectrum of Evans syndrome in adults: new insight into the disease based on the analysis of 68 cases. Blood 2009;114:3167-72.
23. Berentsen S, Randen U, Tjønnfjord GE. Cold agglutinin-mediated autoimmune hemolytic anemia. Hematol Oncol Clin North Am 2015;29:455-71.
24. Rosse WF, Adams JP. The variability of hemolysis in the cold agglutinin syndrome. Blood 1980;56:409-16.
25. Ulvestad E, Berentsen S, Bø K, Shammas FV. Clinical immunology of chronic cold agglutinin disease. Eur J Haematol 1999;63:259-66.
26. Röth A, Bommer M, Hüttmann A, et al. Eculizumab in cold agglutinin disease (DECADE): an open-label, prospective, bicentric, nonrandomized phase 2 trial. Blood Adv 2018;2:2543-9.
27. Berentsen S, Barcellini W, D'Sa S, et al. Cold agglutinin disease revisited: a multinational, observational study of 232 patients. Blood 2020;136:480-8.
28. Swiecicki PL, Hegerova LT, Gertz MA. Cold agglutinin disease. Blood 2013;122:1114-21.
29. Randen U, Trøen G, Tierens A, et al. Primary cold agglutinin-associated lymphoproliferative disease: a B-cell lymphoma of the bone marrow distinct from lymphoplasmacytic lymphoma. Haematologica 2014;99:497-504.
30. Arthold C, Skrabs C, Mitterbauer-Hohendanner G, et al. Cold antibody autoimmune hemolytic anemia and lymphoproliferative disorders: a retrospective study of 20 patients including clinical, hematological, and molecular findings. Wien Klin Wochenschr 2014;126:376-82.
31. de Tute R, Rawstron A, Evans P, Owen R. Cold agglutinin disease is a phenotypically distinct clonal B-cell disorder. Clin Lymphoma Myeloma Leuk 2015;15:Suppl 3:E184. abstract.
32. Małecka A, Delabie J, Østlie I, et al. Cold agglutinin-associated B-cell lymphoproliferative disease shows highly recurrent gains of chromosome 3 and 12 or 18. Blood Adv 2020;4:993-6.
33. Małecka A, Trøen G, Tierens A, et al. Frequent somatic mutations of KMT2D (MLL2) and CARD11 genes in primary cold agglutinin disease. Br J Haematol 2018;183:838-42.
34. Shi J, Rose EL, Singh A, et al. TNT003, an inhibitor of the serine protease C1s, prevents complement activation induced by cold agglutinins. Blood 2014;123:4015-22.
35. Tesfaye A, Broome C. A novel approach for treatment of cold agglutinin syndrome-related severe hemolysis. J Hematol 2016;5:30-3 (https://www.thejh.org/index.php/jh/article/view/242).
36. Zagorski E, Pawar T, Rahimian S, Forman D. Cold agglutinin autoimmune haemolytic anaemia associated with novel coronavirus (COVID-19). Br J Haematol 2020;190(4):e183-e184.
37. Lau-Braunhut SA, Stone H, Collins G, Berentsen S, Braun BS, Zinter MS. Paroxysmal cold hemoglobinuria successfully treated with complement inhibition. Blood Adv 2019;3:3575-8.
38. Mayer B, Yürek S, Kiesewetter H, Salama A. Mixed-type autoimmune hemolytic anemia: differential diagnosis and a critical review of reported cases. Transfusion 2008;48:2229-34.
39. Garbe E, Andersohn F, Bronder E, et al. Drug induced immune haemolytic anaemia in the Berlin Case-Control Surveillance Study. Br J Haematol 2011;154:644-53.
40. Garratty G. Immune hemolytic anemia caused by drugs. Expert Opin Drug Saf 2012;11:635-42.
41. Chan M, Silverstein WK, Nikonova A, Pavenski K, Hicks LK. Bendamustine-induced immune hemolytic anemia: a case report and systematic review of the literature. Blood Adv 2020;4:1756-9.
42. Lecouffe-Desprets M, Néel A, Graveleau J, et al. Venous thromboembolism related to warm autoimmune hemolytic anemia: a case-control study. Autoimmun Rev 2015;14:1023-8.
43. Yusuf HR, Hooper WC, Grosse SD, Parker CS, Boulet SL, Ortel TL. Risk of venous thromboembolism occurrence among adults with selected autoimmune diseases: a study among a U.S. cohort of commercial insurance enrollees. Thromb Res 2015;135:50-7.
44. Barcellini W, Zaninoni A, Fattizzo B, et al. Predictors of refractoriness to therapy and healthcare resource utilization in 378 patients with primary autoimmune hemolytic anemia from eight Italian reference centers. Am J Hematol 2018;93(9):E243-E246.
45. Audia S, Bach B, Samson M, et al. Venous thromboembolic events during warm autoimmune hemolytic anemia. PLoS One 2018;13(11):e0207218.
46. Broome CM, Cunningham JM, Mullins M, et al. Increased risk of thrombotic events in cold agglutinin disease: a 10-year retrospective analysis. Res Pract Thromb Haemost 2020;4:628-35.
47. Barcellini W, Giannotta J, Fattizzo B. Autoimmune hemolytic anemia in adults: primary risk factors and diagnostic procedures. Expert Rev Hematol 2020;13:585-97.
48. Jain MD, Cabrerizo-Sanchez R, Karkouti K, Yau T, Pendergrast JM, Cserti-Gazdewich CM. Seek and you shall find — but then what do you do? Cold agglutinins in cardiopulmonary bypass and a single-center experience with cold agglutinin screening before cardiac surgery. Transfus Med Rev 2013;27:65-73.
49. Murphy S, LoBuglio AF. Drug therapy of autoimmune hemolytic anemia. Semin Hematol 1976;13:323-34.
50. Birgens H, Frederiksen H, Hasselbalch HC, et al. A phase III randomized trial comparing glucocorticoid monotherapy versus glucocorticoid and rituximab in patients with autoimmune haemolytic anaemia. Br J Haematol 2013;163:393-9.
51. Michel M, Terriou L, Roudot-Thoraval F, et al. A randomized and double-blind controlled trial evaluating the safety and efficacy of rituximab for warm autoimmune hemolytic anemia in adults (the RAIHA study). Am J Hematol 2017;92:23-7.
52. Murakhovskaya I. Rituximab use in warm and cold autoimmune hemolytic anemia. J Clin Med 2020;9:4034.
53. Brodsky RA. Warm autoimmune hemolytic anemia. N Engl J Med 2019;381:647-54.
54. Fattizzo B, Zaninoni A, Pettine L, Cavallaro F, Di Bona E, Barcellini W. Low-dose rituximab in autoimmune hemolytic anemia: 10 years after. Blood 2019;133:996-8.
55. Patel NY, Chilsen AM, Mathiason MA, Kallies KJ, Bottner WA. Outcomes and complications after splenectomy for hematologic disorders. Am J Surg 2012;204:1014-9.
56. Thomsen RW, Schoonen WM, Farkas DK, Riis A, Fryzek JP, Sørensen HT. Risk of venous thromboembolism in splenectomized patients compared with the general population and appendectomized patients: a 10-year nationwide cohort study. J Thromb Haemost 2010;8:1413-6.
57. Roumier M, Loustau V, Guillaud C, et al. Characteristics and outcome of warm autoimmune hemolytic anemia in adults: new insights based on a single-center experience with 60 patients. Am J Hematol 2014;89(9):E150-E155.

58. Wouters D, Stephan F, Strengers P, et al. C1-esterase inhibitor concentrate rescues erythrocytes from complement-mediated destruction in autoimmune hemolytic anemia. Blood 2013;121:1242-4.
59. Flores G, Cunningham-Rundles C, Newland AC, Bussel JB. Efficacy of intravenous immunoglobulin in the treatment of autoimmune hemolytic anemia: results in 73 patients. Am J Hematol 1993;44:237-42.
60. Fadlallah J, Michel M, Crickx E, et al. Bortezomib and dexamethasone, an original approach for treating multi-refractory warm autoimmune haemolytic anaemia. Br J Haematol 2019;187:124-8.
61. Snowden JA, Badoglio M, Labopin M, et al. Evolution, trends, outcomes, and economics of hematopoietic stem cell transplantation in severe autoimmune diseases. Blood Adv 2017;1:2742-55.
62. Berentsen S. How I treat cold agglutinin disease. Blood 2021;137:1295-303.
63. Berentsen S, Ulvestad E, Gjertsen BT, et al. Rituximab for primary chronic cold agglutinin disease: a prospective study of 37 courses of therapy in 27 patients. Blood 2004;103:2925-8.
64. Schöllkopf C, Kjeldsen L, Bjerrum OW, et al. Rituximab in chronic cold agglutinin disease: a prospective study of 20 patients. Leuk Lymphoma 2006;47:253-60.
65. Jia MN, Qiu Y, Wu YY, et al. Rituximab-containing therapy for cold agglutinin disease: a retrospective study of 16 patients. Sci Rep 2020;10:12694.
66. Berentsen S, Randen U, Vågan AM, et al. High response rate and durable remissions following fludarabine and rituximab combination therapy for chronic cold agglutinin disease. Blood 2010;116:3180-4.
67. Berentsen S, Randen U, Oksman M, et al. Bendamustine plus rituximab for chronic cold agglutinin disease: results of a Nordic prospective multicenter trial. Blood 2017;130:537-41.
68. El-Ayoubi A, Wang JQ, Hein N, Talaulikar D. Role of plasma cells in Waldenström macroglobulinaemia. Pathology 2017;49:337-45.
69. Castillo JJ, Gustine JN, Keezer A, et al. Deepening of response after completing rituximab-containing therapy in patients with Waldenstrom macroglobulinemia. Am J Hematol 2020;95:372-8.
70. Rossi G, Gramegna D, Paoloni F, et al. Short course of bortezomib in anemic patients with relapsed cold agglutinin disease: a phase 2 prospective GIMEMA study. Blood 2018;132:547-50.
71. Jalink M, Berentsen S, Castillo JJ, et al. Effect of ibrutinib treatment on hemolytic anemia and acrocyanosis in cold agglutinin disease/cold agglutinin syndrome. Blood 2021 July 22 (Epub ahead of print).
72. Röth A, Barcellini W, D'Sa S, et al. Sutimlimab in cold agglutinin disease. N Engl J Med 2021;384:1323-34.
73. Grossi F, Shum MK, Gertz MA, et al. Inhibition of C3 with APL-2 results in normalisation of markers of intravascular and extravascular hemolysis in patients with autoimmune hemolytic anemia (AIHA). Blood 2018;132:Suppl 1:3623 (https://ashpublications.org/blood/article/132/Supplement%201/3623/264815/Inhibition-of-C3-with-APL-2-Results-in).
74. Chen C, Wang L, Han B, Qin L, Ying B. Autoimmune hemolytic anemia in hospitalized patients: 450 patients and their red blood cell transfusions. Medicine (Baltimore) 2020;99(2):e18739.
75. Fattizzo B, Michel M, Zaninoni A, et al. Efficacy of recombinant erythropoietin in autoimmune hemolytic anemia: a multicenter international study. Haematologica 2021;106:622-5.
76. Salama A, Hartnack D, Lindemann HW, Lange HJ, Rummel M, Loew A. The effect of erythropoiesis-stimulating agents in patients with therapy-refractory autoimmune hemolytic anemia. Transfus Med Hemother 2014;41:462-8.
77. Szczepiorkowski ZM, Winters JL, Bandarenko N, et al. Guidelines on the use of therapeutic apheresis in clinical practice — evidence-based approach from the Apheresis Applications Committee of the American Society for Apheresis. J Clin Apher 2010;25:83-177.
78. Kuter DJ, Agajanian R, Arnold DM, et al. Fostamatinib, a spleen tyrosine kinase inhibitor, for the treatment of warm antibody autoimmune hemolytic anemia: initial results of the multicenter, open-label extension period of the Soar phase 2 study. Blood 2018;132:Suppl 1:3612 (https://ashpublications.org/blood/article/132/Supplement%201/3612/264686/Fostamatinib-a-Spleen-Tyrosine-Kinase-Inhibitor).
79. Forero-Torres A, Ramchandren R, Yacoub A, et al. Parsaclisib, a potent and highly selective PI3Kδ inhibitor, in patients with relapsed or refractory B-cell malignancies. Blood 2019;133:1742-52.
80. Schuetz C, Hoenig M, Moshous D, et al. Daratumumab in life-threatening autoimmune hemolytic anemia following hematopoietic stem cell transplantation. Blood Adv 2018;2:2550-3.
81. Tomkins O, Berentsen S, Arulogun S, Sekhar M, D'Sa S. Daratumumab for disabling cold agglutinin disease refractory to B-cell directed therapy. Am J Hematol 2020 July 11 (Epub ahead of print).
82. Zaninoni A, Giannotta JA, Gallì A, et al. The immunomodulatory effect and clinical efficacy of daratumumab in a patient with cold agglutinin disease. Front Immunol 2021;12:649441.