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Review

Diagnosis and treatment of autoimmune hemolytic anemia in adults: Recommendations from the First International Consensus Meeting

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ABSTRACT

Autoimmune hemolytic anemias (AIHAs) are rare and heterogeneous disorders characterized by the destruction of red blood cells through warm or cold antibodies. There is currently no licensed treatment for AIHA. Due to the paucity of clinical trials, recommendations on diagnosis and therapy have often been based on expert opinions and some national guidelines. Here we report the recommendations of the First International Consensus Group, who met with the aim to review currently available data and to provide standardized diagnostic criteria and therapeutic approaches as well as an overview of novel therapies. Exact diagnostic workup is important because symptoms, course of disease, and therapeutic management relate to the type of antibody involved. Monospecific direct antiglobulin test is considered mandatory in the diagnostic workup, and any causes of secondary AIHA have to be diagnosed. Corticosteroids remain first-line therapy for warm-AIHA, while the addition of rituximab should be considered early in severe cases and if no prompt response to steroids is achieved. Rituximab with or without bendamustine should be used in the first line for patients with cold agglutinin disease requiring therapy. We identified a need to establish an international AIHA network. Future recommendations should be based on prospective clinical trials whenever possible.

1. Introduction

Currently, there is no licensed treatment for autoimmune hemolytic anemias (AIHAs), although some national guidelines do exist [1,2]. Furthermore, a number of new treatment approaches are being designed that may target the underlying mechanisms of hemolysis in these disorders. Given the chronic nature of most AIHAs and the need to provide a more quantitative assessment of its treatment and outcomes, a group of international experts representing study groups, registries, and centers with large basic scientific and clinical experience, convened in Vienna in November 2017. Our goals were to:

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- Review published data on epidemiology, pathophysiology, classification, diagnostics, transfusion policies, as well as standard and novel treatment options in AIHA.
- Provide international guidelines for the diagnosis of AIHA.
- Create a framework for current treatment of AIHA.
- Establish standardized criteria for diagnosis and outcomes that will instruct clinical studies.
- Provide an overview of novel treatment approaches.

Additional meetings were held in Stockholm (June 2018 during the EHA Meeting), San Diego (December 2018, ASH Meeting), and Amsterdam (June 2019, EHA Meeting) before the manuscript was finalized.

The aim was to provide an international consensus for the diagnosis and clinical management of all major forms of AIHA. The issues and sections on which the recommendations should focus were proposed by the chairs (U.J. and S.B.) and then accepted after an open discussion by the panel. Landmark basic science and diagnostic papers, randomized, controlled and uncontrolled trials, expert opinions and national guidelines were included. Recommendations were scored on a 1–10 scale, and percentage of agreement was calculated from the ratio between sum of scores and highest possible sum of scores by all participants. With only one exception, agreement was greater than 80%.

Due to the paucity of randomized trials, this consensus is not regarded a guideline with evidence levels or a systematic review in a strict sense, but represents the first comprehensive harmonized action in the field which should improve the global level of training, management and provide a basis for clinical trial planning.

2. Background

AIHAs are usually classified as either warm antibody (wAIHA) or cold antibody-mediated AIHA (cAIHA). The exact incidence of AIHA in adults is unclear but in a French study of children under age 18 was estimated to be 0.81/100,000 (95% CI 0.76–0.92) per year [3]. AIHA may occur in 10% of patients with systemic lupus erythematosus (SLE) and 5–10% of patients with chronic lymphocytic leukemia (CLL) [4–7]. wAIHA accounts for 48–70% of patients with AIHA and cAIHA for 15–25% [8–10]. The remaining cases are mixed disorders. All general types of AIHA can be acute and transient, or chronic.

wAIHA is characterized by binding of polyclonal immunoglobulin (often IgG) to RBC antigens (Rh proteins or glycophorins A–D). This binding is referred to as “warm” in that it occurs at most temperatures but is maximal at 37 °C. The density of these RBC antigens is usually not high enough to fix complement, but in some instances complement also becomes attached to the RBC. The opsonized RBC are then modified (becoming spherocytes) and eventually cleared by FcγRIII or C3b receptors on macrophages. Much of this occurs outside of the circulation (extravascular RBC destruction). RBC agglutination is rarely visible on the peripheral blood film [11,12].

In contrast, cold agglutinin disease (CAD, the “least uncommon” subtype of cAIHA) is caused by a clonal or oligoclonal IgM antibody that binds to RBC antigens (usually branched chain [“I”, adult] polymers of aminyl-lactose disaccharides) by weak van der Waal’s forces and is maximal at cold temperatures [13]. Analysis of some clonal IgM proteins has identified a heavy chain variable region encoded by the *IGHV4-34* gene segment that produces anti-I and anti-i specificities with cross-idiotypic specificity [14]. Crystal structure analysis of these clonal IgM molecules has identified a hydrophobic patch on the IgM that accounts for this weak binding interaction. Complement binding occurs in most patients due to the IgM structure and high antigen density on RBC, leading to RBC aggregation (RBC aggregates on peripheral blood smear and clinical acrocyanosis) as well as complement activation [15]. Complement activation may be complete with the entire pathway to C9, causing intravascular RBC hemolysis or it may be incomplete with C3 tagged RBC being cleared by macrophages in extravascular sites [16].

Given the ability of the body to increase RBC production up to eightfold, some patients with both forms of AIHA will have compensated hemolysis or mild anemia with no symptoms. For others, symptoms (e.g., fatigue, dyspnea, palpitations) and signs of anemia (e.g., pallor, icterus) may be present. Splenomegaly may accompany AIHA but is rarely symptomatic unless accompanied by a lymphoproliferative process.

The natural history of AIHA has not been thoroughly detailed. AIHA due to infection or drug exposure is often clinically mild and short-lived. Chronic AIHA is sometimes related to underlying lymphoproliferative or autoimmune diseases and presents a more intractable course. Of 539 patients with chronic wAIHA, 45% were idiopathic, 50% associated with autoimmune (26%) or lymphoproliferative disorders (24%), and 5% miscellaneous [17]. Most, if not all, subjects with chronic CAD have an underlying lymphoproliferative disorder [18,19]. Several studies and clinical experience of the authors attests to the chronic nature of AIHA and the difficulty of obtaining durable treatment-free remissions [11,20–23].

The risk factors for chronic AIHA include underlying autoimmune (especially SLE) and lymphoproliferative disorders. While there is a high association of AIHA with non-Hodgkin lymphoma, especially CLL, AIHA is rare in patients with Hodgkin’s lymphoma [24]. Allogeneic hematopoietic stem cell transplantation (HSCT) may also increase the risk for AIHA. Of 265 pediatric patients undergoing allogeneic HSCT, 6% developed AIHA [25]; and in 272 adults undergoing allogeneic HSCT, 4.4% developed AIHA (66% CAD, 33% wAIHA) [26]. While up to 29% of patients with immune thrombocytopenia (ITP) have a positive direct antiglobulin test (DAT, direct “Coombs test”), clinically significant Evans syndrome affects 3–5% of patients with ITP [27–29]

The classification of AIHA is summarized in Table 1. Before applying this classification based on the DAT, it is important to show that hemolysis is present. Evidence for hemolysis includes reticulocytosis, elevated lactate dehydrogenase (LDH) levels, decreased haptoglobin, elevated indirect bilirubin, positive serum free hemoglobin, positive urine hemosiderin. Of these, haptoglobin and LDH appear to be the most helpful. Using a Boolean analysis for the separation of hemolytic from nonhemolytic disorders, an increased LDH and haptoglobin $<$ 25 mg/dL is 95% specific for hemolysis while a normal LDH and haptoglobin $\geq$ 25 mg/dL are 92% sensitive for the absence of hemolysis [30,31].

The DAT alone does not define AIHA. It may be positive in many healthy subjects as well as in those without evidence of hemolysis; and negative in up to 10% of patients with clear evidence of AIHA. DAT may remain positive in patients with AIHA in remission.

3. Definitions

A recent systematic review of the literature found that important terminology used for the diagnosis and treatment of AIHA was either omitted, or inconsistent between studies [32]. This variability makes it difficult to compare studies and to apply published evidence to clinical practice. Such definitions should also take into consideration the need for harmonization with the immune thrombocytopenia (ITP) nomenclature [32–34]. Definitions proposed by the consensus group are listed in Tables 2 and 3.

Table 1
Types of AIHA.

Type	Mechanism	DAT	RBC Eluate	Specificity
wAIHA	IgG (IgA)	IgG +/- C3	IgG	Panreactive
CAD	IgM	C3	Nonreactive	I > i > Pr
Mixed type	IgG, IgM	IgG + C3	IgG	Panreactive
				I/i reactive
PCH	IgG	C3	Nonreactive	P

Table 2
Disease definitions in AIHA.

Hemolytic anemia	Anemia related to a reduction of red blood cell (RBC) lifespan due to increased destruction. In the appropriate clinical context, this can be defined by lactate dehydrogenase (LDH) > upper limit of normal (ULN) and a haptoglobin < lower limit of normal (LLN).
Intra- or extravascular haemolysis	RBC destruction is termed intravascular if it occurs within the general vasculature, or extravascular if mediated by the mononuclear phagocytic system in the spleen or liver.
Autoimmune hemolytic anemia (AIHA)	Hemolytic anemia caused by the destruction of RBCs through autoantibodies directed against antigens on their surface.
Diagnostic criteria for AIHA	Evidence for hemolysis accompanied by a positive direct antiglobulin test (DAT) and exclusion of alternative causes, such as a delayed hemolytic transfusion reaction.
DAT-negative AIHA	DAT-negative AIHA is usually due to non-IgG autoantibodies or levels of RBC-bound antibody below the sensitivity threshold. This diagnosis can be made when there is clear evidence of hemolysis, alternative causes of both hereditary and acquired hemolysis have been excluded, and the diagnosis is supported by a more sensitive test at a reference center, or there is a clear response to corticosteroid treatment.
Severe AIHA	AIHA is considered severe when the unsupported hemoglobin level falls below 8.0 g/dL and transfusion is required with an interval ≤7 days. It is characterized by severe symptoms of anemia and hemoglobin instability.
Primary versus secondary AIHA	AIHA is considered primary in the absence of an associated disorder and secondary when one is present. Drug-induced immune hemolytic anemia is a distinct category of secondary immune hemolysis.
Warm AIHA (wAIHA)	wAIHA is diagnosed in patients lacking cold associated symptoms with a DAT positive for IgG, IgA (rarely), or C3d ± IgG when a clinically significant cold reactive antibody has been excluded.
Cold agglutinin disease (CAD)	AIHA, a monospecific DAT strongly positive for C3d (and negative or weakly positive with IgG) and a cold agglutinin (CA) titer of 64 or greater at 4°C. We recognize that there may be occasional cases with CA titer < 64. Patients may have a B-cell clonal lymphoproliferative disorder detectable in blood or marrow but no clinical or radiological evidence of malignancy.
Cold agglutinin syndrome (CAS)	AIHA, a monospecific DAT strongly positive for C3d (and negative or weakly positive with IgG) and a CA titer of 64 or greater at 4°C. Patients have an associated condition, for example infection, autoimmune disorder, overt evidence of a B-cell lymphoma (clinical or radiological), or other malignancy.
Paroxysmal cold hemoglobinuria (PCH)	PCH is diagnosed in patients with hemolysis and a positive Donath-Landsteiner test.
Mixed AIHA	Mixed AIHA is diagnosed in patients with a DAT positive for C3d and IgG, a cold antibody with a thermal amplitude ≥30°C and evidence of a warm IgG antibody by IAT or IAT eluate.
Disease phase	The use of disease phase terms such as acute/chronic are not usually applied to AIHA and there is currently insufficient evidence of differences in disease biology or treatment response to justify an arbitrary threshold.

4. Diagnostic evaluation

Immune hemolytic disease is defined by a shortened red blood cell (RBC) survival and serologic evidence of an immune response directed against autologous red blood cell antigens. Physiological and pathological removal of red blood cells occurs in the mononuclear phagocytic system, e.g., liver and spleen (extravascular hemolysis). Occasionally, RBC destruction may occur in the circulation (intravascular hemolysis).

4.1. Direct and indirect antiglobulin test

Serological evidence of an autoimmune response against autologous RBCs can be detected by the DAT and the IAT. In the DAT, autoantibodies bound to the patients RBC *in vivo* are detected by adding a polyspecific antihuman globulin reagent, which will detect IgG and complement (C3d), but not IgA or IgM. To further determine the autoantibody isotypes or complement, the DAT is repeated using monoclonal antibodies specific for IgG, IgM, IgA as well as for complement fractions C3c and C3d. IgM, being a potent activator of the complement classic pathway, often escape detection since it may detach from the RBC during washing procedures [35–37]. However, complement deposition in the form of C3c and C3d is an indirect sign of prior IgM binding or a hidden IgM. It is important to realize that although IgG1 and IgG3 isotypes are able to activate complement, the majority of complement positivity detected by DAT is caused by a (hidden) IgM [38].

The DAT may yield false-negative results due to the presence of RBC-bound antibodies below the threshold of the test. In fact, the DAT tube test effectively diagnoses AIHA when at least 500 molecules of autoantibodies are bound to RBCs, whereas the microcolumn and solid phase tests require approximately 200–300 molecules per single RBC to yield a positive result [39,40]. More recently, the DAT tube test was reported as the most specific but least sensitive test (0.87 and 0.43, respectively), whereas the microcolumn and solid phase methods showed reduced specificity but increased sensitivity (0.70 and 0.65, respectively) [41]. Smaller amounts of autoantibodies can be detected employing even more sensitive techniques, such as flow cytometry (able to detect about 30–40 antibody molecules per RBC), enzyme-linked and radiolabeled tests, or the mitogen-stimulated-DAT (able to amplify the autoimmune reaction in culture) [42,43]. However, these methods are not routinely performed in most laboratories, and such positive tests should be interpreted with caution, given their high sensitivity and low specificity compared with DAT tube test [43].

A false negative DAT may be a consequence of low-affinity autoantibodies: this may be overcome by the use of low ionic strength solutions (LISS) or cold washings. In addition, IgM autoantibodies with a thermal range close to 37 °C (warm IgM), which can cause severe and fatal AIHA, may be DAT-negative or weakly DAT-positive for anti-C3, causing detrimental delay in diagnosis and therapy [37]. In these cases, it is advisable to perform, in a reference laboratory, the DDAT (Dual

Table 3
Criteria for assessing response to AIHA treatments.

Response definitions	*Complete response (CR):* Normalization of hemoglobin, no evidence of hemolysis (normal bilirubin, LDH, haptoglobin and reticulocytes), absence of transfusions. For CAD, additional CR criteria include disappearance of acrocyanosis, absence of clonal B cells, and absence of clonal IgM. *Response (R):* Increase in hemoglobin by > 2 g/dL or normalization of hemoglobin without biochemical resolution of hemolysis; and absence of transfusion for the last 7 days. *No response:* Failure to achieve a response.
Response duration	Measured from achievement of complete response (CR) or response (R) to loss of CR or R.
Remission	Measured from achievement of CR off all AIHA directed treatment, to loss of CR.
Steroid resistance and dependence	*Steroid resistance:* Failure to obtain hematologic response within 3 weeks on at least 1mg/kg predniso(lo)ne. *Steroid dependence:* Need to continue on predniso(lo)ne at a dose of > 10mg/day to maintain a response.
Refractory disease	Failure to respond to at least 3 lines of therapy; in wAIHA including splenectomy and/or at least one immunosuppressant.

Table 4
Recommendations for the diagnosis of secondary wAIHA in adults.

Disease or condition	Tests to be performed in every patient	Tests to be performed only in some circumstances
SLE and other autoimmune diseases	. Antinuclear Abs (ANA) and if + with titer > 1/80 : anti-dsDNA Abs and other specificities	. Lupus anticoagulant . Anticardiolipin Abs . Anti-β2gpI Abs (only for patients with overt SLE, strongly positive ANA or past history of thrombosis) . CH50, C3 and C4 in case of SLE
Lymphoma and solid tumors	. Serum protein electrophoresis . Immunoelectrophoresis . Immunophenotyping of B-lymphocytes from peripheral blood . [a]CT scan (chest/abdomen/pelvis)	. Bone marrow biopsy = > especially in the presence of monoclonal gammopathy or hypogammaglobulinemia, lymph nodes, and/or disproportionate splenomegaly on the CT scan and/or monotypic lymphocyte population . Lymph node biopsy
Primary immunodeficiency	. IgG, IgA and IgM levels	. Extended phenotype of T/ NK and memory B cells . Post vaccine (eg, tetanus toxoid, pneumococcal) serology
Infections	. HIV, HCV and (HBV)[b] tests	. CMV, EBV, Parvovirus B19 and others based on clinical and/or biological evidence

Notes: SLE = systemic lupus erythematosus; Abs = antibodies; ds = double strand.
[a] Unless an obvious case of SLE.
[b] Mostly pre-therapeutic.

Direct Antiglobulin Test) which is able to identify IgM bound to RBC [44]. Another cause of false negative DAT is the presence of IgA autoantibodies that can be detected only if the DAT is performed with an anti-IgA. In about 5% of AIHA patients, the diagnosis can only be made after extensive laboratory investigation to exclude other causes of hemolysis, and on the basis of the clinical response to steroid therapy.

It is important to know that a positive DAT may be found without clinical evidence of AIHA. This occurs in a small fraction of healthy blood donors (< 0.1%) and hospitalized patients (0.3–8%) [45]. Moreover, a false positive DAT may be observed after administration of various therapeutics (intravenous immunoglobulins, Rh immune globulins, and antithymocyte globulins, daratumumab therapy, and in diseases with paraproteins or elevated serum globulin).

Finally, the DAT may be positive due to the presence of alloantibodies in recently transfused patients, in delayed hemolytic transfusion reactions, and in hemolytic disease of the newborn [45]. The coexistence of auto- and alloantibodies has been reported in 1/3 of AIHA patients, and their presence is often masked by autoantibodies. In addition, alloantibodies possibly cause severe hemolytic reactions in case of RBC transfusion. In complex cases the distinction between allo- and autoantibody is advisable by immunoabsorbance techniques and extended RBC genotyping.

The IAT detects serum autoantibodies to RBCs, using a test panel of standardized RBC. These test RBC are first incubated with patient serum, and then, after washing to remove unbound antibodies, incubated with polyspecific antihuman globulin reagent, which will induce agglutination if serum antibodies directed to RBCs are present in patient's serum. In daily laboratory practice, DAT and IAT are performed by using fully automatized laboratory analyzing systems using column tests with gel-containing microtubes. But for some difficult patients, the old-fashion agglutination techniques in glass tubes are performed [35,36].

4.2. Assessment of peripheral blood smear, reticulocyte counts and hemolytic markers

In immune hemolysis the peripheral smear will generally reveal polychromasia and anisocytosis (indications of reticulocytosis), spherocytes and, sometimes, nucleated red blood cells. Spherocytes are commonly seen in wAIHA and RBC aggregates in CAD [11,12]. Blood smear examination is also useful to rule out thrombotic microangiopathies.

The reticulocyte count is an assessment of the production of new red blood cells by the bone marrow. They are generally elevated at the time of presentation however up to 37% of patients had an inappropriately low reticulocyte count at presentation [46]. In general, a reduced reticulocyte response can be a sign of inadequate bone marrow capacity (e.g., after chemotherapy, myelophthisis) and/or infection (e.g., parvovirus B19 infection). However, in some patients with AIHA with reticulocytopenia, the anti-RBC antibody may bind to antigens on immature RBC precursors and reticulocytes as well as to those on the mature RBC [47,48]. A decreased or absent haptoglobin can be seen in hemolysis of any cause. Haptoglobin is produced mainly in the liver and acts as a scavenger of free hemoglobin, preventing the release of toxic highly reactive heme [49]. Low haptoglobin is also observed in liver disease, prior transfusion therapy, rigorous exercise, and, rarely, in ahaptoglobinemia, a genetic condition affecting 1:1000 whites and as many as 4% of African Americans [50]. LDH is particularly elevated in intravascular hemolysis, and unconjugated bilirubin in extravascular RBC destruction, but they are not specific, being present in many other conditions [51]. Hemoglobinuria is seen only in patients who have severe intravascular haemolysis, and hemosiderinuria is a sign of subacute or chronic hemolysis.

4.3. Assessment for other underlying disorders

AIHA may be secondary to an underlying disease (Table 4, 5, and 6). Serology for *Mycoplasma pneumoniae*, EBV, CMV, hepatitis B and C, and HIV, as well as anti-nuclear, anti-DNA, anti-extractable nuclear antigens, lupus-like anticoagulant, anti-cardiolipin and anti-beta-2 antibodies are advisable. A bone marrow biopsy and flow cytometry should be performed in all CAD cases prior to therapy, and should be

Table 5
Drug induced antibody formation.

Hapten and drug adsorption mechanisms
Drugs such as penicillins, cephalosporins, tetracycline, carbromal, hydrocortisone, oxaliplatin, and tolbutamide
Immune/ternary complex mechanisms
Drugs such as stibophen, metformin, quinine, quinidine, cephalosporins, amphotericin b, rifampicin, antazolinc, thiopental, tolmetin, probenecid, nomifensine, cephalosporins, diclofenac and doxepin
Autoantibody mechanism
Drugs such as cephalosporins, tolmetin, α-methyldopa, L-dopa, mefenamic acid, teniposide, pentostatin, cladribine, fludarabine, lenalidomide, procainamide and diclofenac
Non-immunologic protein adsorption
Cephalosporins, carboplatin, cisplatin and oxaliplatin
Unknown methods of AIHA causation
Drugs such as mesantoin, phenacetin, insecticides, chlorpromazine, acetaminophen, ibuprofen, thiazides, omeprazole, carboplatin, nalidixic acid, erythromycin, and streptomycin

Table 6
Main disorders or conditions associated with secondary wAIHA in adults.

Hematologic disorders and lymphoproliferative diseases:
Chronic lymphoid leukemia
T-LGL leukemia
B-cell lymphoma/ Hodgkin lymphoma
Angioimmunoblastic T cell-lymphoma
Castleman disease
Myelodysplastic syndromes/Myelofibrosis
Other immune cytopenias e.g., Evans syndrome
Solid tumors: Thymoma /Ovarian dermoid cyst/Carcinoma
Auto-immune and inflammatory diseases
Systemic lupus erythematosus /Antiphospholipid syndrome
Rheumatoid arthritis, Sjögren syndrome
Pernicious anaemia[a]/Thyroiditis[a]
Myasthenia gravis[a]
Auto-immune hepatitis, primary biliary cirrhosis
Ulcerative colitis
Sarcoidosis
Eosinophilic fasciitis
Infections:
Virus: HIV/Ebstein Barr virus/hepatitis C/cytomegalovirus
Bacteria: tuberculosis / brucellosis/babesiosis
Drugs[c]: antibiotics (ceftriaxone, piperacillin); NSAIDs (diclofenac); antineoplastic drugs (oxaliplatin); check-point inhibitors (nivolumab).
Primary immunodeficiencies
Common variable immunodeficiency
Hyper IgM syndrome[b]/ALPS[b]
Others :
Post-allogenic bone marrow transplantation, post-liver or small bowel transplant
Rosai-Dorfman disease

Notes : LGL = large granular lymphocytes; HIV = Human Immunodeficiency Virus; ALPS = Autoimmune lymphoproliferative syndrome.

[a] These disorders are more associated diseases on a common genetic background rather than specific causes of wAIHA.

[b] Onset almost exclusively during childhood.

[c] Since drug-induced immune haemolytic anaemias are beyond the scope of this article, only some drugs are mentioned.

considered in wAIHA and mixed AIHA patients who relapse after steroid therapy [1,10]. Likewise, it is also advisable in DAT-negative cases to evaluate myelodysplastic syndromes, either congenital or acquired. Chest and abdominal/pelvic CT scans may also be helpful. Finally, a careful review of the medical history is required to identify a possible drug-associated immune hemolysis, which is relatively rare but frequently undiagnosed.

4.4. Warm AIHA (wAIHA)

Warm autoimmune haemolytic anemia displays a very wide spectrum of clinical characteristics in terms of age at onset, degree of clinical manifestations, and occurrence of associated or underlying disorders [8,9,52,53]. The main conditions associated with secondary AIHA are listed in Table 6. In females, secondary wAIHA may even be associated with ovarian cysts [54]. Infections, particularly viral, have been associated with the development of wAIHA as have prior transfusions and transplantation [55,56].

4.4.1. Drug-associated AIHA

Over 150 drugs have been associated with the development of wAIHA [57,58]. The drug associated wAIHAs have been divided into 2 categories based on mechanism (Table 5). The first is due to drug-dependent antibodies that activate an immune response only while the drug is present. This is the most common type of drug-related AIHA. There are two subtypes of drug-dependent antibodies: *hapten-mediated antibodies,* which react to a mixed epitope composed of red blood cell structures, and *drug non-covalently bound to RBC*. The binding of these antibodies might be very strong as typically seen in penicillin-induced AIHA (penicillin-type) characterized by a DAT for positive IgG and negative for complement. In contrast, the drug-binding to the RBC might also be rather weak (e.g., ceftriaxone) resulting in the formation of immune complexes (immune complex type), which is characterized by late onset days or weak after start of the drug and a DAT positive for complement and negative for IgG [58]. The second mechanism is due to drug-independent antibodies, which are capable of creating an autoimmune response in the absence of the offending drug. Various mechanisms have been suggested by which drugs (i.e., fludarabine, cladribine, methyldopa) stimulate autoantibody formation via adsorption, immune dysregulation, or other mechanisms but none of these have been fully elucidated [57].

4.4.2. Evans syndrome

wAIHA presenting either simultaneously or sequentially with thrombocytopenia is known as Evans syndrome [29]. This diagnosis, particularly in young adults and pediatric patients, should be accompanied by a basic immunologic workup including screening for common variable immunodeficiency and autoimmune lymphoproliferative syndrome (ALPS) [59].

4.5. Cold agglutinin disease

CAD is mediated by CAs, which are IgM autoantibodies that are able to agglutinate red blood cells upon binding to the I surface antigen. Primary CAD is defined by chronic hemolysis, a significant CA titre (most often defined as $\geq$64) at 4 °C, typical findings by the DAT, and the absence of an underlying clinical disease (Table 2) [60–62]. The typical DAT pattern is a positive monospecific test for C3d only. However, DAT can be weakly positive for IgG in addition to C3d in up to 20% of the patients [60,63]. The group recommends giving the titre as a number, defined as the inverse value of the highest dilution at which agglutination occurs. We recognize that there may be occasional cases of CAD with CA titre < 64. Determination of the thermal amplitude is time-consuming and, in most cases, not required for reliable diagnosis, but may be useful in selected patients to rule out normally occurring low-titre CA as a cause of false positive findings.

The same laboratory criteria apply to secondary cold agglutinin syndrome (CAS) complicating aggressive lymphoma or specific infections (Table 2). Clinical and histological assessment, supplemented by radiological examinations as needed, will rule out cases of CAS secondary to a malignant disease [64].

Serum monoclonal IgMκ can be found by capillary or agarose electrophoresis and immunofixation in more than 90% of the patients, while IgG, IgA, or λ light chain phenotype are rare findings [60]. Clonal $CD20^+$, κ^+ lymphocytes can usually be detected by flow cytometry of bone marrow aspirates. The frequency of positive findings depend on the lengths undertaken to identify a small clone of B cells [65,66].

Primary CAD has been shown to display a specific bone marrow histopathologic pattern, termed “primary CA-associated lymphoproliferative disorder (LPD)” and found to be distinct from lymphoplasmacytic lymphoma (LPL), marginal zone lymphoma (MZL) and other previously recognized lymphoma entities [18]. Typical findings have been described as nodular B-cell aggregates (or, in some patients, only scattered B-cells) without characteristic features of LPL, such as paratrabecular growth, fibrosis, lymphoplasmacytoid cell morphology, or an increased number of mast cells surrounding the lymphoid aggregates. Differences between CAD and LPL have also been demonstrated by immunohistochemical and flow cytometric methods. The *MYD88* L265P mutation, present in almost all cases of LPL [67], is absent or infrequent in CA-associated LPD [18,68,69]. We recognize that occasional patients in whom the bone marrow histology has been interpreted as other low-grade LPDs, such as LPL, MZL, small lymphocytic lymphoma, or unclassified B-cell lymphoproliferation, should also be classified as having primary CAD [60,63].

CA binds to RBC antigens at low temperatures, followed by red

blood cell agglutination. Circulatory symptoms resulting from RBC agglutination are present in a majority and can be a problem for patients even without anemia [60,70]. The antibodies dissociate from the cells at central body temperature. Upon classical complement pathway activation, C3b-coated erythrocytes are removed by macrophages of the mononuclear phagocytic system (extravascular hemolysis) [15,71,72]. To a varying extent, terminal complement activation may also result in the formation of the membrane attack complex and intravascular hemolysis [63,73].

4.6. Mixed AIHA with diagnostic and clinical features of warm and cold antibody-mediated disease

Mixed AIHA is characterized by a DAT positive for IgG and C3, with coexistence of high titre (> 40) cold agglutinins. Such patients have both a warm and a cold AIHA and should be classified separately from wAIHA patients having a DAT positive for IgG and C3. Mixed forms represent about 8–10% of all AIHAs and are generally characterized by a severe onset, about 2/3 with Hb < 6 g/dL and ¼ with Hb 6–8 g/dL.

In a large study of 308 AIHA patients, mixed AIHA patients had a significantly lower median hemoglobin at onset (median 5.8 g/dL) compared to other types of AIHA (~7 g/dL in wAIHA and ~8 g/dL in CAD) and more reticulocytopenia. Moreover, mixed AIHAs more frequently required 2 or more therapy lines, including corticosteroids, rituximab, immunosuppressants and/or splenectomy [53,74].

4.7. Paroxysmal cold hemoglobinuria

Paroxysmal cold hemoglobinuria (PCH) is a rare form of AIHA mediated by a temperature-dependent IgG autoantibody with specificity against the P antigen on RBCs, first described by Donath and Landsteiner [75]. The autoantibody binds to patient RBCs in the cold. When the temperature rises towards 37° C, the antibody detaches from RBCs but the initially bound complement is now activated and causes hemolysis [71,76]. This biphasic antibody was first described in conjunction with syphilis in adults. Today, PCH occurs mainly in children with a recent history of a viral (“flu-like”) infection [76]. It presents with intravascular hemolysis, which can occur even in the absence of identifiable cold exposure.

In the diagnostic work-up, a positive DAT (for C3 alone), negative antibody screen together with typical symptoms in the absence of wAIHA, CAD, DIHA or PNH is suggestive of PCH. Visual inspection of a centrifuged blood sample for intravascular hemolysis can be helpful, although in vitro hemolysis due to poor blood sampling should be considered as a cause of a false positive result. Definitive diagnosis is still based on the biphasic in vitro Donath-Landsteiner (DL) test. Patient’s serum is incubated with normal red blood cells (RBCs) in the cold for 30 minutes, followed by warming to 37°C. Hemolysis in this "biphasic" test indicates a diagnosis of PCH. Depending on which testing method is used, this test can be time-consuming, resource-intensive, costly, and susceptible to false-negative results [76–78]. In a recent survey of Canadian laboratories, 17 positive tests (predominantly in children) were reported in 124 testing years, indicating that the disease is very rare or that the test is not often requested given the estimated frequency of PCH of 1–3 in 100,000 [77]. There was still poor agreement among experts on the interpretation of a positive DL test in adults, suggesting that this test is best performed in reference laboratories.

4.8. Atypical AIHA

DAT negative, IgA-driven, and warm-IgM AIHA are generally defined as atypical forms. These cases represent a diagnostic challenge, usually resulting in treatment delay. Moreover, whereas first line steroid therapy is commonly given without concern, the requirement of further treatment may yield uncertainty and difficulty. This is particularly troublesome as atypical cases often display a severe onset and are associated with an increased risk of relapse [53,74].

Recommendations for diagnosis of AIHA:

- All patients should have baseline values of LDH, bilirubin, haptoglobin, reticulocyte count, and review of peripheral smear (looking for spherocytes with wAIHA, RBC aggregation with CAD, and schiztocytes to exclude thrombotic microangiopethies) (99% agreement).
- The direct antiglobulin test (DAT) should be performed with monospecific antisera (anti-IgG, anti-IgA, anti-IgM, anti-C) (96% agreement).
- It is advisable to consider that about 5–10% of AIHAs may be DAT negative (85% agreement).
- In case of DAT negativity, other causes of congenital or acquired haemolysis should be considered, but if no alternative is found, the DAT should be performed with more sensitive methods in a reference center (99% agreement). If sensitive tests are not available, a trial of corticosteroids may be considered.
- All wAIHA patients should be evaluated for underlying autoimmune diseases (ANA, RF, antiphospholipid antibody), lymphoproliferative disorders (flow cytometry of peripheral blood, review of peripheral blood smear, possible CT scan assessing for lymphadenopathy and splenomegaly) or immune deficiency (serum protein electrophoresis with immunofixation) disorders (93% agreement).
- Diagnosis of CAD should include DAT (C3d+), a cold agglutinin titre and bone marrow examination (at least by histology and flow cytometry) (87% agreement).
- Primary CAD should be differentiated from secondary CAS (88% agreement).
- All patients with suspected CAD should be evaluated for clonal B cell disorder with SPEP, immunofixation, peripheral blood and bone marrow flow cytometry, bone marrow biopsy, and, if indicated, CT scans looking for lymphadenopathy and splenomegaly (96% agreement).
- Baseline assessment for signs of acrocyanosis in patients with CAD (93% agreement).
- In patients with DAT positive for IgG and C3 and presence of symptoms of CAD, mixed AIHA should be considered and diagnosed by performing CA test and titer (92% agreement).
- Diagnostic uncertainty after AIHA evaluation in a patient with fitting history should elicit the performance of a Donath–Landsteiner biphasic test in an experienced laboratory (77% agreement).
- In a patient with hemolysis and DAT negativity, other non-immune types of haemolysis should be excluded; if none are found, atypical AIHA should be considered and pursued with appropriate diagnostic tools in reference centers. Clinical severity and increased relapse risk should be taken into consideration (90% agreement).

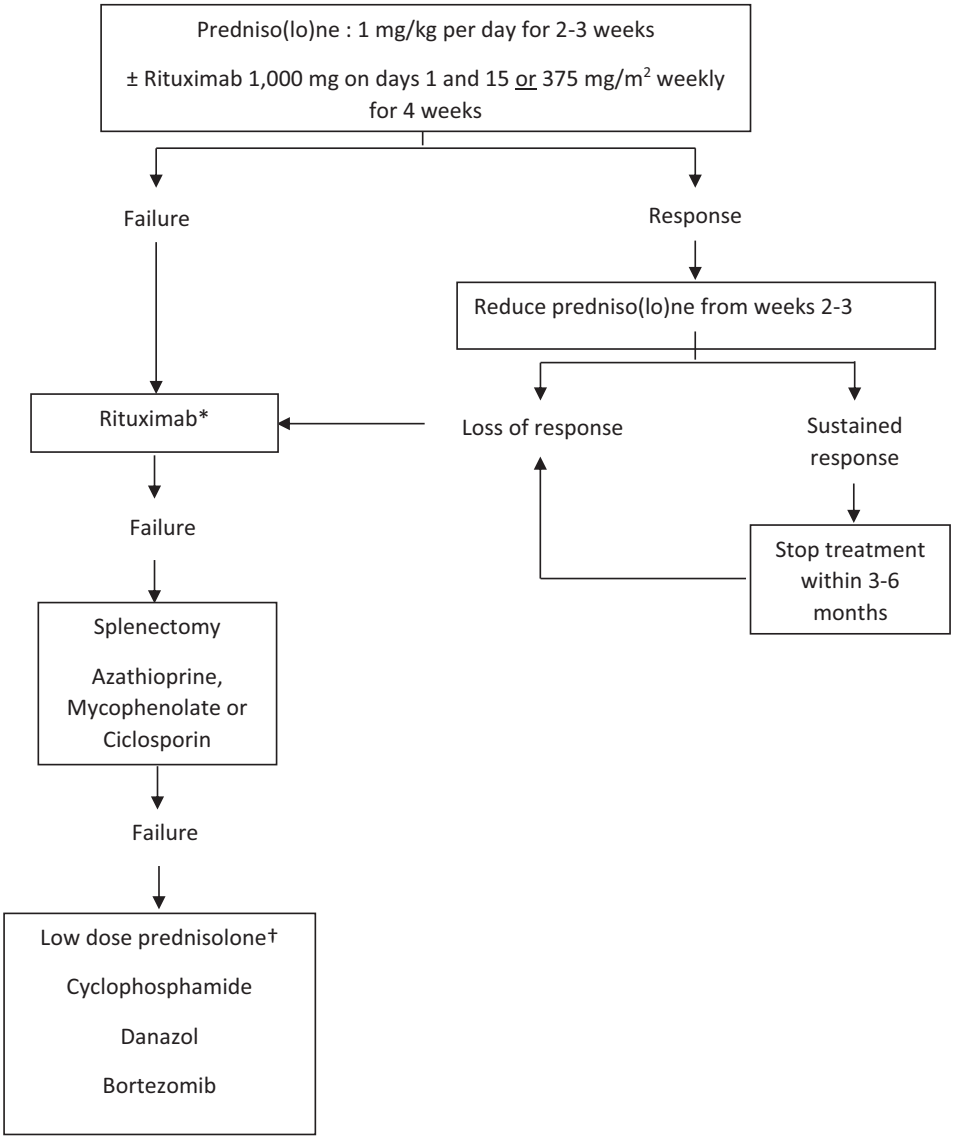
5. Treatment recommendations

5.1. Warm autoimmune hemolytic anemia (wAIHA)

An algorithm for treatment of wAIHA is provided in Fig. 1.

5.1.1. Primary warm AIHA

The principle indication for first line or subsequent therapies is symptomatic anemia. Although the majority of patients will require treatment, as disease onset is usually acute and severe and spontaneous remission is very uncommon in primary wAIHA, many patients do not achieve or sustain a complete remission [21,22]. In patients with milder and partially compensated hemolytic anemia (e.g., > 10 g/dL), it therefore may sometimes be appropriate to monitor without treatment, i.e., “watch and wait” (W&W) strategy, after weighing its potential burdens and benefits, taking into consideration individual patient factors such as symptoms, frailty and co-morbidities. In view of the limited evidence base, clinicians should consider discussion of available clinical



*If rituximab given first line, re-treatment may be considered if a sustained response was achieved. Otherwise, move to third line options.

† Prednisolone ≤10 mg daily ± a steroid sparing agent

HSCT; haematopoietic stem cell transplantation

Fig. 1. Therapeutic algorithm for warm-antibody mediated AIHA in adults

trials at all stages of treatment.

5.1.1.1. First line treatment. Initial therapies are usually corticosteroids and sometimes rituximab.

5.1.1.1.1. Prednisolone or prednisone. Standard first line therapy is glucocorticoids and approximately 80% of patients respond to daily doses equivalent to predniso(lo)ne 60–100 mg [79]. Although alternative glucocorticoids such as dexamethasone have therapeutic activity, unlike in ITP, data is sparse in wAIHA with no evidence of a comparable outcome [80,81]. Early reports concluded that predniso(lo)ne at a dose higher than 60 mg or 1–1.5 mg/kg does not achieve a higher response rate [45,81]. Hence most adult patients starting treatment should receive oral predniso(lo)ne 1 mg/kg daily (Fig. 1).

5.1.1.1.2. Prednisone(lo)ne plus rituximab. The only two prospective randomized trials of treatment for warm AIHA both compared prednisolone to predniso(lo)ne plus rituximab first line, with similar findings. In the first study, with 32 patients in each arm and a rituximab regimen of 375 mg/m^2 weekly for 4 weeks, the prednisolone-rituximab arm had a significantly higher response rate at 12 months (75% vs 36%; P = 0.003) [21]. In the second blinded study, prednisone-treated patients were randomized to receive either placebo or rituximab 1 g (fixed dose) on day 1 and 15 followed by a standardized prednisone taper. The prednisone-rituximab arm had a significantly higher response rate at 12 months (75% vs. 31%; P = 0.032) and 24 months (63% vs. 19%; P = 0.011) [22]. Neither study found an excess of adverse or serious adverse events in the rituximab arm [21,22].

Although rituximab is not licensed for wAIHA, neither study had sufficient power or follow-up to address whether first line addition of rituximab improved long term remission or reduced the need for other treatments. Predniso(lo)ne-rituximab may be considered in selected

patients with severe disease or as a corticosteroid-sparing strategy.

5.1.1.1.3. How should corticosteroids be tapered and what is a steroid failure?. Corticosteroids must be tapered as side effects are cumulative and most patients are symptomatic if predniso(lo)ne is continued at a dose of 1 mg/kg daily for 4 weeks or more. In a study of 53 patients with warm AIHA receiving prednisone at an initial dose of 1–2 mg/kg daily, the mean treatment duration was 15 months ± 3 months in 43 responding patients [82]. This resulted in corticosteroid-induced diabetes in 20% of the patients, worsening of pre-existing diabetes (10%), osteoporosis with fractures (10%) and osteonecrosis of the femoral head (4%). A further study of 52 patients with AIHA found that the only significant predictor of mortality was diabetes mellitus (existing or steroid induced). Eighteen patients had diabetes and 6/18 died from infection, all while receiving 5–30 mg prednisolone [83].

In an early study of 62 adult AIHA patients responding to high dose corticosteroids, the median response time was 7 days (range 2–21) [81], similar to the median response time of 9 days (range 6–28 days) in 17 children with idiopathic AIHA receiving an initial prednisolone dose of 2 mg/kg daily [84]. A longer median response time of 25 ± 15 days observed in 53 adults with warm AIHA receiving an initial prednisone dose of 1–2 mg/kg daily may reflect differences in disease characteristics and definition of response [82].

In steroid-responsive patients, the taper can begin after 14–21 days. There are no studies comparing tapering regimens but in one study of 33 primary AIHA cases, relapse was more common if corticosteroids were tapered to ≤10 mg in less than 2 months and if stopped in less than 6 months [85]. One approach would be to reduce predniso(lo)ne to 20–30 mg over a few weeks, and then by 2.5–5 mg every month [86]. Approximately 30% of patients remain in remission when corticosteroids are discontinued [21,22]. Relapse during or after a steroid wean is an indication for second-line therapy. Steroid failure is likely in patients who do not respond to predniso(lo)ne 1 mg/kg after 21 days. Predniso(lo)ne can then be tapered and second line treatment considered in such patients, who are refractory to corticosteroids.

5.1.1.2. Second-line treatment. The best studied and most efficacious medical therapy for wAIHA is rituximab. In a meta-analysis of 21 studies encompassing 154 patients, the overall response rate was 79% for patients with wAIHA [87]. Approximately half the patients received concomitant corticosteroids. Patients received the standard rituximab regimen of 375 mg/m^2 weekly for 4 consecutive weeks in 20/21 studies of AIHA identified. A regimen of rituximab 1 g day 1 and 15 delivers a similar total dose, with a similar response rate [22,82]. Median time to response in warm AIHA is approximately 3–6 weeks (range 2–16 weeks) [88–91]. Of patients responding to rituximab first-line, 30% had relapsed after 3 years [22], while longer-term response rates are unknown [89–93].

Re-treatment of relapsing patients appears to result in a similar pattern of response [82], but has not been systematically studied. Low dose rituximab (100 mg weekly for 4 consecutive weeks) with prednisolone first or second line resulted a 100% (18/18) response rate at one year and is a promising avenue for further study [74,88,94]. Although well tolerated by the majority, important side effects include infusion reactions and infection. Neutropenia and secondary hypogammaglobinemia may rarely occur. Pre-administration screening with serology for hepatitis B virus surface antigen and core antibody is recommended.

5.1.1.3. Third-line treatment. Third line options include splenectomy or alternative immunosuppression such as azathioprine, cyclosporine and mycophenolate [1,11]. Small retrospective series show that oral immunosuppressants can be effective but these studies often lack detail (e.g., dose, steroid independence, duration of response) and systematic or comparative studies are needed. Referring patients who have failed second-line to a tertiary referral center may be appropriate.

Azathioprine. Typical daily dose is 2–2.5 mg/kg [5]. Reported response rates have been 71% (22/31, all AIHA types), 60% (9/15, wAIHA), 56% (5/9, wAIHA) [53,82,95]. Thiopurine methyltransferase (TPMT) deficiency increases the risk of myelotoxicity and should be excluded prior to commencing therapy. If the test is not available, the treatment should be started at 50 mg daily and then progressively increased up to 150 mg in the absence of neutropenia

Cyclosporine. Typical initial oral dose is 2.5 mg/kg twice per day and reported response rate 58% (7/12, all AIHA types) [53,96].

Mycophenolate mofetil. Typical dose is 500 mg twice daily, titrated up to 1 g twice daily. Reported response rates in small case series were 100% (4/4, all AIHA types), 25% (1/4, wAIHA), and 67% (mixed AIHA and CAD) [53,82,97].

Splenectomy. In patients with primary wAIHA, approximately 70% respond and 40% achieve complete remission following splenectomy [1]. One-third of patients with wAIHA relapse after splenectomy, but the likelihood of long term remission (e.g., ≥10 years) is unknown [82,98,99].

After splenectomy, patients are at greater risk of severe infection, particularly in the first year [99,100]. This can be reduced through antibiotic prophylaxis and vaccination [101]. Patients should also be educated on prompt treatment of infection and avoidance of animal, tick or mosquito bites. Vaccines should be completed 2 weeks prior to splenectomy and therefore initiated 6 weeks before. A suboptimal response may occur in patients who have received recent immunosuppression and assessment of antibody titres upon B-cell recovery may be considered. Up-to-date national guidelines should be consulted for the pre-splenectomy vaccination schedule. Current United Kingdom guidelines recommend that adults receive *Haemophilus influenzae* type b (Hib), *Neisseria meningitidis* type B and C, and pneumococcal polysaccharide vaccine (PPV23), followed 1 month later by *N. meningitidis* ACWY and the second *N. meningitidis* B dose [102]. Annual flu vaccine and a 5 yearly PPV booster are also recommended. The Centers for Disease Control and Prevention (CDC) has issued similar recommendations for splenectomized patients [103].

The overall risk of post-splenectomy venous thromboembolism (VTE) is greater in patients with hemolytic anaemia [104,105], including an increased risk of portal or splenic vein thrombosis postoperatively [106–108], and an extended period of postoperative thromboprophylaxis should be considered [108].

5.1.1.4. Subsequent lines of therapy. Subsequent treatments have either a weaker evidence base or greater potential for toxicity. In this setting, novel agents and clinical trials may be appropriate (See Section 9). Splenectomy should be considered if not previously done. Other treatment strategies are listed below. Patients should generally be referred to an experienced center and included in clinical trials wherever possible.

Cyclophosphamide. Cyclophosphamide has been given as a daily oral dose e.g., 50–100 mg or 1–2 mg/kg [79,109,110]. One retrospective study reported a 72% response rate in 40 patients, but without stating the dose or whether steroid independence was achieved [53]. In another study, 4/7 patients responded but no patient achieved a steroid-independent sustained response [82]. Two studies reported success with intravenous cyclophosphamide [110,111]. All patients responding to either 50 mg/kg (ideal body weight)/day for 4 days (4 had primary warm AIHA) without autologous stem cell transplantation or 1 g monthly for 4 months (13 had primary warm AIHA). Important side effects include myelosuppression, infections, urotoxicity, secondary malignancy, and infertility [111].

Low-dose prednisolone. Due to its relatively rapid effect, patients will usually receive steroid rescue at relapse if previously responsive, at the same time as alternative immunomodulatory treatment is started. The usual goal however is to wean and stop steroid due to long-term toxicities. Doses of prednisolone ≤10 mg daily with or without steroid-sparing immunosuppression can effectively control AIHA and may be appropriate long-term therapy in refractory cases.

Danazol. The attenuated oral androgen danazol (e.g., 200 mg three times/day) may have steroid-sparing properties but appears to be less useful in refractory cases, and there are no recently published series [112–115]. At this dosage, the androgenic effects limit its use in women and in men with prostatic adenoma or carcinoma. In the long-term, liver toxicity can also be a problem.

Bortezomib. Intravenous bortezomib 1.3 mg/m^2 weekly for 1–4 weeks resulted in remission in 2/4 cases of relapsed AIHA in children following allogeneic HSCT [116]. Of four adults with refractory warm AIHA, three responded to bortezomib 1.3 mg/m^2 days 1, 4, 8 and 11 first cycle (then weekly if subsequent cycles) for 1–3 cycles [117]. A limitation is that all patients had received prior rituximab as well as concomitant immunosuppression. Another study reported the efficacy of bortezomib combined with dexamethasone in 6 out of 8 adults with multirefractory wAIAH [118]. Common side effects include neuropathy, neutropenia, thrombocytopenia, diarrhea, fatigue and rash.

Hematopoietic Stem Cell Transplantation (HSCT). There is only little evidence regarding the risk over benefit ratio of autologous HSCT for wAIHA and even less evidence regarding allogeneic HSCT) [119–121]. HSCT should be limited to carefully selected patients following multidisciplinary review.

5.1.1.5. Rescue therapy for emergency situations. Despite recurrent transfusions, patients with severe hemolysis may not maintain a satisfactory hemoglobin. Immunoglobulins and plasma exchange should be regarded as a bridging therapy and alternatives such as immunosuppression also be considered concomitantly.

Intravenous methylprednisolone. Although an early study found no benefit for parenteral administration of corticosteroids [81], by analogy with other autoimmune diseases, the use of an initial bolus dose of intravenous methylprednisolone (e.g., 500 mg) may be considered in severe, fulminant cases.

Intravenous immunoglobulin (IVIg). In the only study reported in 1993, only 12/37 (32%) patients responded to IVIg at a typical dose of 0.4–0.5 g/kg/day for 5 days, and responses usually lasted for ≥3 weeks [122]. Side effects include headache, back pain, nausea and allergic reactions. Rarely, acute renal failure and thromboembolic reactions are observed and pre-existing cardiac and renal function should be considered.

Erythropoiesis-stimulating agent (ESA). By analogy with the use of thrombopoietin receptor agonists for immune thrombocytopenia (ITP), the transient and off-label use of an ESA (recombinant erythropoietin) at relatively high dose may be useful for patients with severe wAIHA (e.g., requiring transfusions) and with a relatively low reticulocyte count [123,124].

Emergency splenectomy or partial splenic embolization. Urgent splenectomy may be considered in highly transfusion-dependent patients that are unresponsive to other treatments. If not given 2 weeks before, vaccination should be deferred until 14 days after splenectomy to improve functional antibody responses. Partial splenic embolization has been successful in some patients considered unfit for splenectomy [59].

Plasma exchange. Daily exchange with human albumin at 1–1.5 times plasma volume has been used as an emergency measure [125,126]. The goal is to remove pathogenic immune complexes, circulating autoantibodies and activated complement. Evidence is limited to case reports showing variable success in patients often receiving concomitant immunosuppression and its role yet to be established [127].

Recommendations for treatment of primary wAIHA:

- The indication for treatment of wAIHA is symptomatic anaemia. Exceptionally, in the rare patients with mild and stable anemia, monitoring may be appropriate (“W&W”) (Figure 1) (100% agreement).
- First line treatment for warm AIHA is oral predniso(lo)ne starting at 1 mg/kg daily. Predniso(lo)ne-rituximab may be considered frontline in selected patients with severe disease or in elderly patients with comorbidities (100% agreement).
- In patients responding to predniso(lo)ne at a dose ≥1 mg/kg, begin to taper after 14–21 days. Also begin to taper by 21 days in unresponsive patients. For patients responding well to predniso(lo)ne, consider stopping the treatment after at least 3 months after a complete response is achieved (see response criteria). Consider second line treatment in unresponsive patients and those relapsing as the steroid is weaned (100% agreement).
- The preferred second line therapy for wAIHA is rituximab 375 mg/m^2 weekly for 4 weeks. An alternative regimen of 1 g day 1 and 15 may also be considered. For patients receiving rituximab first line, re-treatment may be considered in patients with a response of meaningful duration (e.g., 1 year or more), otherwise consider third line treatments (96% agreement).
- Third line treatment options include splenectomy, azathioprine, cyclosporin and mycophenolate and should be based on the individual assessment of the benefit over risk ratio (95% agreement).
- Patients with chronic refractory wAIHA not responding to at least 3 treatment-lines (including splenectomy and or at least one immunosuppressant) should be referred to a specialized center and whenever possible treated in the setting of a clinical trial (99% agreement).
- Other lines of therapy to be considered are cyclophosphamide, continuous low dose prednisone, danazol, haematopoietic stem cell transplantation, and bortezomib (82% agreement).
- Management of transfusion-dependent life-threatening wAIHA must combine supportive therapies (ESA ± plasma exchange and/or IVIg), active immunosuppression, and emergency splenectomy/embolization. Thromboprophylaxis is also indicated in order to minimize the risk of venous thrombosis (97% agreement).

5.1.2. Secondary wAIHA

Approximately 50% of wAIHA seen in adulthood are caused by or associated with an underlying disease or condition, defining secondary wAIHAs. The most important diseases associated with secondary wAIHAs during adulthood are summarized in Table 6. In some of these patients, the associated condition is key to development of AIHA, but in others, the two may arise from a shared genetic background. Consequently, treatment of the associated condition sometimes, but not always, improves the AIHA. As a general strategy, treatment of the associated condition should be optimized and the timing and intensity of AIHA directed treatment based on the individual patient.

Three groups of diseases that are preferentially associated with wAIHA in adults are: (1) Lymphoproliferative diseases (mostly B-cell lymphomas) and especially CLL, seen almost exclusively in patients aged over 50; (2) autoimmune diseases and especially SLE affecting preferentially young women; and 3) primary immunodeficiencies and mostly common variable immunodeficiency (CVID).

5.1.2.1. How should wAIHA associated with CLL be managed?. Up to 14% of patients with CLL have a positive DAT at the time of diagnosis [6], and the prevalence of AIHA in CLL is approximately 2.9% in stable Binet stage A disease compared with 10.5% in stage B and C [128].

The management of CLL-associated wAIHA must take into account the stage of CLL. If wAIHA occurs in a patient with stage A CLL or in whom AIHA is the predominant feature, the management of wAIHA is comparable to that of primary wAIHA and the initial treatment should be corticosteroids. Rituximab can be considered second-line, with an overall response rate of 71% (10/14) in one study [129]; although responses are often not sustained [130].

Patients not responding to corticosteroids and rituximab and those with active CLL should be treated with CLL targeted therapy as per current guidelines according to age and comorbidities [131]. The management of CLL-associated wAIHA has relied on combination regimens such as rituximab, cyclophosphamide and dexamethasone

(RCD) or rituximab, cyclophosphamide, vincristine and prednisolone (R-CVP) [86,132]. Over 80% of patients with immune cytopenias respond to these regimens, with a response duration of approximately 22–24 months [132–134]. Other regimens, such as bendamustine plus rituximab, may also be effective [135]. Fludarabine carries the risk of inducing AIHA or pure red cell aplasia and should be avoided [136]. This effect seems to be less pronounced when combined with rituximab.

In multirefractory cases or patients with 17q deletion or TP53 mutations, ibrutinib should be preferred [137,138]. Other therapies that appear useful for refractory cases include alemtuzumab [139]. Due to the increased risk of severe infections in patients heavily pre-treated for CLL, the benefit over risk ratio of a splenectomy must be carefully weighted [140].

5.1.2.2. How should wAIHA associated with SLE be managed?. wAIHA occurs in up to 10% of patients with SLE, mainly in patients of African ancestry, and it is often associated with the presence of antiphospholipid antibodies [81]. There are currently no specific guidelines for the management of SLE-associated wAIHA but empirically and based on some retrospective studies, most of the patients achieve an initial response on predniso(lo)ne. For those patients who do not achieve a complete response or relapse after predniso(lo)ne tapering and have no reason to be treated with immunosuppression for other non-hematological SLE-related manifestations, rituximab seems to have a good efficacy and safety profile [141]. Alternatives to rituximab are mycophenolate mofetil or azathioprine. There is no evidence that hydroxychloroquine at the average dose of 200 mg bid has a corticosteroid-sparing effect in SLE-associated wAIHA, but by analogy with SLE-associated immune thrombocytopenia and considering that this drug is indicated in most of SLE cases, it should be given in combination with corticosteroids. Splenectomy must be avoided in SLE because it may increase pre-existing acquired immunosuppression, promotes thrombosis especially in case of positive antiphospholipid antibodies, and some data suggest that it could also worsen the disease course and/or trigger vasculitis [142].

5.1.2.3. How should wAIHA associated with CVID be managed?. wAIHA occurs in 2 to 5% of patients with CVID and can be the first manifestation of the disease [143]. Infective complications are a concern with immunosuppressive treatment and severe infections have been documented in patients treated with corticosteroids, oral immunosuppression, and rituximab [144,145]. Severe and sometimes fatal infection with encapsulated bacteria are reported post-splenectomy, for example in 5/12 [145] and 9/40 patients [146]. Maintenance immunoglobulin increases survival and reduces the severity and frequency of infective complications in CVID patients and is therefore recommended in those receiving corticosteroids, other immunosuppression and following splenectomy [147]. Patients undergoing splenectomy should receive lifelong prophylactic antibiotics.

Most patients with CVID treated for an isolated wAIHA or Evans' syndrome achieve a response to first line predniso(lo)ne. However, given the increased risk of infections, the minimal effective dose of predniso(lo)ne must be reached as soon as possible and the long-term use of corticosteroids must be avoided. Patients that are refractory or who relapse after predniso(lo)ne may be considered for rituximab, which resulted in an overall response rate of 80% (8/10) in a retrospective study [144]. Refractory patients can respond to oral immunosuppression such as azathioprine, and although responses to splenectomy appear similar to primary AIHA, splenectomy should be avoided whenever possible [145,146,148].

Recommendations for treatment of secondary wAIHA:

- Management of CLL-associated wAIHA must take into account the stage and activity of CLL. Patients with stage A CLL can be managed as primary wAIHA, whereas for patients with active CLL, combination therapies including rituximab + chemotherapy ± dexamethasone should be considered. Kinase inhibitors should be preferred in patients with TP53 aberration and considered in other refractory cases. The use of fludarabine or chlorambucil as a single agent should be avoided (100% agreement).
- Rituximab is a relevant option for the management of corticosteroid-refractory or dependent SLE-associated wAIHA. In case of rituximab refractoriness or severe non-hematologic manifestations, mycophenolate mofetil may be effective. Splenectomy should be avoided, especially for patient with an associated antiphospholipid antibody syndrome, particularly regarding splenectomy) (88% agreement).
- Rituximab can be considered as a second-line and corticosteroid-sparing strategy for the treatment of CVID-associated wAIHA. Maintenance immunoglobulin replacement is strongly recommended in patients receiving corticosteroids, other immunosuppression and following splenectomy even without a history of severe or recurrent infections (98% agreement).

5.2. Cold agglutinin disease

Fig. 2 shows an algorithm for treatment of cAIHA.

5.2.1. What are the indications for therapy?

Most patients suffer from mild to moderate anemia, with exacerbation at cold temperature or other triggers like infections, vaccination, major surgery or trauma. Once C3 is fixed to the red cell surface, C3 convertase cleaves the C3 molecule, releasing C3a and coating the red cell with C3b. C3b is further cleaved, releasing C3c and leaving the red cell coated with C3d [15,71]. There are no C3d receptors in the mononuclear phagocyte system, and these cells are resistant to further extravascular haemolysis. It is, therefore, naïve to set an arbitrary number below which therapy is recommended. It would be unusual for a patient to immediately require therapy for a hemoglobin (Hb) > 10 g/dL, although even these levels can be associated with reduced quality of life [149].

Treatment would usually not be recommended for patients whose Hb is $\geq$ 10 g/dL. Exceptions could be made for patients with significant comorbidities such as ischemic cardiomyopathy and chronic obstructive pulmonary disease that would reduce oxygen delivery to the tissues unrelated to the oxygen-carrying capacity of the blood. Although acrocyanosis is a common accompaniment of CAD, it is uncommon for this to be a driving indication for therapeutic intervention. In most patients, this can be managed with thermal protection only. Patients who have severe Raynaud's phenomenon may require treatment if thermal protection fails [70]. Those with stable baseline anemia can suffer exacerbations during febrile or bacterial infections, which can often be managed with short-term transfusion support without committing to long-term systemic therapy [60,150]. There appears to be an increased incidence of venous thromboembolism (VTE) in patients with CAD [151,152]. However, it is unclear whether treatment of the hemolytic process itself could reduce the risk of VTE.

Patients who are undergoing cardiothoracic or other surgery where there is extracorporeal circulatory circuits involved, where cooling of the patient's plasma occurs, require specialized management. Because of the narrow thermal amplitude of many CAs, cardiothoracic surgery involving bypass should occur with normothermia, and an intraoperative transfusion protocol should be in place in case agglutination occurs ex vivo in the bypass equipment [1,153]. If the risk of exacerbation is considered high, preoperative use of eculizumab might be considered [154].

For most patients without relevant symptoms or problems, watchful waiting is justified (Fig. 2). Folic acid should be supplemented (1–5 mg/d), as should Vitamin B12 if deficient. Bacterial infections should be treated early to prevent hemolytic crisis [10,150]. Blood transfusions can be given when indicated. Thromboprophylaxis is recommended in

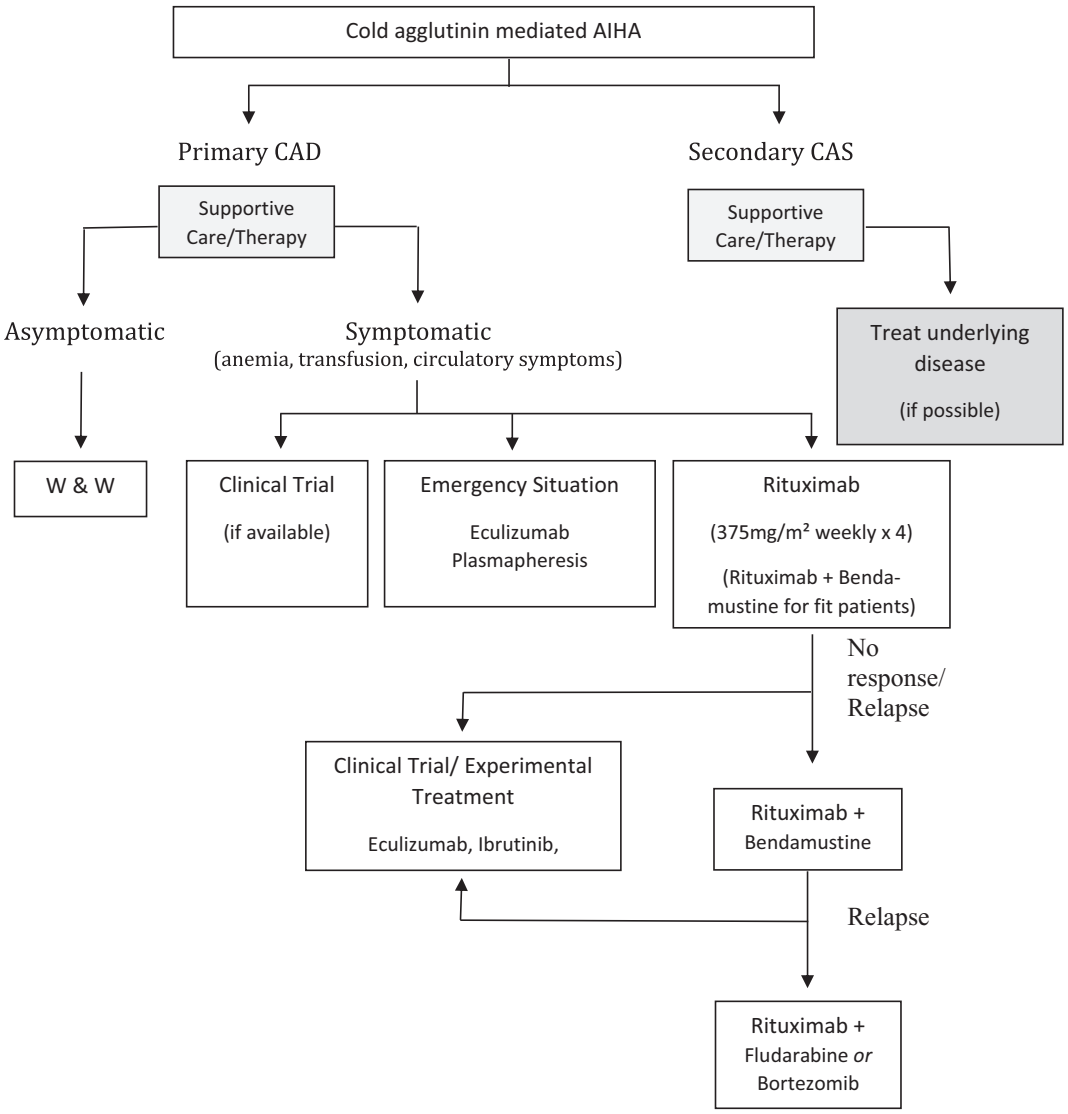


Fig. 2. Therapeutic algorithm for cold agglutinin disease.
W & W, watch and wait.

acute exacerbations or for chronic disease in risk situations (immobilization, long-distance flights, etc.).

5.2.2. What are the treatment goals?

An obvious goal of treatment is an increase in hemoglobin levels in patients with symptom-producing anemia. This includes, but is not restricted to, achievement of transfusion independency. In some patients, treatment also aims at improvement or resolution of disabling cold-induced circulatory symptoms [66,70]. It is not yet clear whether an increased risk of thrombosis in itself justifies pharmacologic therapy for disease control [151,155,156]. Regarding therapies targeting the pathogenic B-cell clone, achievement of long-term control of the LPD is also a goal because this is associated with durable hematologic remission [70,157]. All treatment should aim at an improved quality of life. Objective response criteria, as defined in prospective studies, may also be useful in clinical practice. Response criteria defined for B-cell directed therapies [66] will be different from those used in trials of complement inhibition [71,73,158].

5.2.3. First-line therapy

Rituximab monotherapy 375 mg/m^2 for 4 weeks at 7-day intervals is the oldest and best documented therapy for CAD. In prospective uncontrolled trials, it has yielded a response rate of about 50% although with very few complete responses (CR), a median increase in Hb levels of 4.0 g/dL, with a median response duration of less than one year [159,160]. Toxicity is low and a repeat course of rituximab is often effective in relapsed disease.

In a recent trial of rituximab plus bendamustine combination therapy, 45 patients received rituximab 375 mg/m^2 day 1 and bendamustine 90 mg/m^2 day 1 and 2 for 4 cycles at 28 days interval [70]. Seventy-one per cent responded; 40% achieved CR and 31% partial response (PR). Hemoglobin levels increased by a median of 4.4 g/dL in the complete responders and 3.9 g/dL in those who had a PR. Median time to response was 1.9 months, and less than 10% of the responders had relapsed at 32 months. Grade 4 neutropenia occurred in 20% of the patients, but only 11% experienced infection with or without neutropenia.

Splenectomy is not effective in CAD as the sensitized red blood cells are mainly removed in the liver [60]. Typically, treatment options such as those used in wAIHA (corticosteroids, azathioprine or cyclophosphamide) are not effective in CAD and should not be used [60,156].

For patients who require pharmacological therapy for CAD, the choice should be individualized. Relatively fit patients with severe CAD and without contraindications to cytotoxic therapy may be treated with

rituximab-bendamustine upfront. Addition of filgrastim might be considered. For other patients, the recommended first-line treatment is rituximab monotherapy.

5.2.4. Second-line therapy

In the second-line situation, we recommend the rituximab-bendamustine combination if not given in first line or contraindicated [70]. Alternatively, one may repeat rituximab monotherapy in patients who have previously responded for at least one year [159]. Rituximab-bendamustine may be repeated in those who have previously responded if two years or more have passed after they received the same treatment.

Rituximab plus fludarabine combination therapy was investigated in a prospective, uncontrolled trial of 29 patients who received rituximab 375 mg/m^2 on days 1, 29, 57 and 85, and fludarabine orally, 40 mg/m^2 on days 1–5, 29–34, 57–61 and 85–89 [157]. Responses were observed in 76%; 21% achieved CR and 55% achieved PR. Median increase in Hb level was 3.1 g/dL in the responders and 4.0 g/dL among those who achieved CR. Median time to response was 4.0 months, and estimated median response duration was more than 66 months. Hematologic toxicity grade 3–4 was observed in 12 patients (41%), including grade 4 neutropenia in four (14%), and 59% experienced grade 1–3 infection. Fludarabine-induced warm-antibody AIHA did not occur, but three patients (10%) experienced a transient, mild exacerbation of CAD precipitated by infection [150,157]. The study was not designed to address the risk of late-occurring hematologic malignancies, which have been reported after fludarabine-based therapy for Waldenström macroglobulinemia [161]. We consider the rituximab-fludarabine combination an option for second-line treatment in fit, elderly patients.

In a recently presented, prospective trial of bortezomib therapy, six of 19 patients responded [162]. Regarding the modest response rate, it should be noted that the study drug was administered as a single cycle of monotherapy. This leads to the preliminary conclusion that bortezomib, in particular when given as an extended treatment and/or in combination, may be an option in the second line.

Patient preference will have to be taken into consideration for individualized therapy in the second-line setting. Inclusion in clinical trials should be considered a good alternative to documented regimens.

5.2.5. Third-line therapies

There is no evidence-based therapy available for the third-line situation. Patients should be included in clinical trials, if possible.

The clonal cells that produce CA usually do not express the typical *MYD88* L265P mutation [18,68,69]; however, this mutation is probably not essential for the effect of Bruton tyrosine kinase (BTK) inhibitors [163]. Therefore, the BTK inhibitors ibrutinib and acalabrutinib should have activity in reducing the production of the IgM monoclonal protein. Although not specifically tested in CAD, ibrutinib is approved for the treatment of Waldenström macroglobulinemia and acalabrutinib shows activity in the treatment of CLL [164]. BTK inhibitors are a potentially exciting class for the management of refractory CAD. A potential role for the BCL2 inhibitor, venetoclax, may also exist. [165,166].

Current clinical trials are aimed at the use of complement inhibitors that block the fixation of the complement components on the red cell surface, thereby eliminating their interaction with the mononuclear phagocyte system. Eculizumab, the monoclonal anti-C5 antibody, was the first complement inhibitor to be used in the treatment of severe CAD, and a trial in 13 patients demonstrated significant reductions in LDH, hemoglobin stabilization and transfusion avoidance [73,167]. In theory, an inhibitor of C5 should not have a profound effect since complement fixation on red blood cells occurs before the C5 component of the complement system [71,72].

More upstream, classical pathway inhibition would, theoretically, do better [168–170]. Sutimlimab (BIVV009, TNT009) is a humanized form of a monoclonal IgG4 antibody that specifically inhibits C1s and can abrogate complement-mediated haemolysis in vitro [72,158]. This agent was granted FDA breakthrough therapy designation based on data from a phase 1b trial that showed this agent normalized hemoglobin levels [158]. Seven of 10 patients with cold agglutinin disease responded with a hemoglobin increase > 2 g/dL. Sutimlimab rapidly increased haemoglobin levels by a median of 1.6 g/dL within the first week, and by a median of 3.9 g/dL within 6 weeks. Sutimlimab has now entered phase 3 trials (NCT03347422, NCT03347396). An inhibitory molecule against C1q, ANX005, blocks classical complement activation as well as haemolysis in an in vitro sheep red cell assay [171]. Pegcetacoplan (APL-2), a subcutaneously administered peptide inhibitor of C3, is assessing the preliminary efficacy in both wAIHA and CAD [16,172].

5.2.6. Rescue therapy for emergency situations

Sometimes severe and life-threatening haemolysis and anemia can occur due to acute situations such as infections, major surgery, or severe cold exposure, with the need for options for a rescue treatment [150]. Typically, treatment with rituximab monotherapy or in combination with bendamustine takes a significant time for response [70]. Blood transfusions should be given depending on the hemoglobin levels, preferentially with a blood warmer. As hemolysis in this severe situation might be more intravascular, the acute use of eculizumab is an option to block the terminal complement pathway and intravascular hemolysis [73,167]. This could be a bridging option for more long-term treatment to work.

Eculizumab is commercially available and has been reported to both reduce the level of LDH and produce modest increases in hemoglobin (median 0.9 g/dL) and an improvement in quality of life. For life-threatening cold agglutinin haemolysis refractory to other therapies, the use of eculizumab to immediately stop or prevent intravascular hemolysis has been reported [154,167,173].

Because the IgM monoclonal protein that results in complement fixation is always found intravascularly, plasma exchange is a potential therapy for reducing the level of the IgM in the plasma and could potentially provide transient benefit when other chemoimmunotherapy is not available or an immediate effect is needed [174]. There are no prospective studies, but exchange of the plasma volume with albumin daily or every other day has been recommended [127]. This effect is short-lived and should not be considered a long-term therapy. Extracorporeal column immuno-absorption apheresis has been used in cryoglobulinemia and thrombotic thrombocytopenic purpura [175]. Theoretically, there would be potential for this technique to be used in patients with CAD in an effort to remove immunoglobulin and complement proteins. Some successful case reports are published, but there is a significant reporting bypass neglecting the failed attempts to improve the clinical situation in wAIHA and CAD patients, respectively.

Plasma substitution with donor plasma supplies the patient circulation with a fresh complement source, which might have potentially harmful effects [150]. Especially in CAD the apheresis procedure has to occur at 37°C, which ask for a suitable infrastructure and is quite laborious. Therefore, plasmapheresis has a limited value and might offer a “last resort” therapy in wAIHA and CAD patients. Another approach specific approach to remove autoantibodies is immune adsorption, where autoantibodies are specifically removed without supplementation of complement-containing plasma. Although an attractive concept, there is no broad experience with this approach.

Recommendations:

- Rituximab, alone or in combination with bendamustine, is the best documented first-line treatment (100% agreement).
- Rituximab can be repeated as monotherapy or in combination with bendamustine or fludarabine as second line treatment. Bortezomib has also shown efficacy (100% agreement).
- In case of severe and life-threatening hemolytic anemia requiring repeated transfusions despite high dose of corticosteroids and rituximab, the patient should be managed in intensive care unit in a

tertiary referral center. The use of either IVIg and/or plasma exchange can be considered as a transfusion-sparing strategy (99% agreement).
- Other potentially useful agents include tyrosine kinase- or BCL2-inhibitors (94% agreement).
- Complement inhibition at various levels of the complement cascade is a promising novel approach for later treatment lines or emergencies (100% agreement).

5.3. Secondary cold agglutinin syndrome (CAS)

A cold hemolytic syndrome similar to CAD is occasionally seen in patients with aggressive lymphoma [64,176]. CAS has also been described in a variety of other cancers (although the causal association can be questioned in some of the reported cases), in systemic lupus erythematosus (SLE), and after allogenic stem cell transplantation [26,64]. A more acute clinical picture of CAS can complicate *Mycoplasma pneumoniae* pneumonia, Epstein Barr virus (EBV) infection or, even more rarely, *Chlamydia* and some other specific infections [64]. CA in patients with aggressive B-cell lymphoma are monoclonal anti-I antibodies of the IgM class. The light chain restriction can be λ as well as κ [177]. In infection-associated CAS, the CA are polyclonal and anti-I specific when triggered by *Mycoplasma*, but anti-i specific in EBV infection [64,178]. The immunoglobulin class is IgM in *Mycoplasma* pneumonia and IgG or IgM in EBV infection.

Secondary CAS is still rarer than primary CAD. The “least infrequent” form is *Mycoplasma* associated CAS, which typically occurs in adults or adolescents during the second or third week after onset of the infection [64]. In most patients the onset of hemolysis is sudden with pallor, jaundice and, sometimes, prostration. In addition to biochemical signs of hemolysis, high-titre CA are demonstrable and DAT is positive for C3d. Intravascular haemolysis, as evidenced by hemoglobinuria, has been described in several cases. The prognosis is good and the hemolytic complication is usually self-remitting within 4–6 weeks, although a lethal course has been reported.

There is no evidence-based therapy for secondary CAS except for treatment of the underlying condition when possible. Transfusions can be given when indicated; the same precautions must be observed as in primary CAD. Therapy with corticosteroids has been described in several case reports but the effect is poorly documented, as hemolysis will always improve with resolution of any underlying infection [64,179]. The theoretical rationale for therapeutic complement inhibition at an upstream classical pathway level is strong and favorable effect has been observed in one single case [73], but prospective studies will be difficult to undertake.

5.4. Mixed AIHA with diagnostic and clinical features of warm and cold antibody mediated disease

Response to different therapies in 24 cases of mixed AIHA was recently analyzed [23]. Corticosteroids were administered as first line therapy in all cases, 67% needed a second line only, and 33% a further third line; 8% displayed multi-refractoriness, requiring further therapy lines. Response to corticosteroids is similar to that observed in wAIHA (~70%, OR, but less than~30% sustained). Second line therapy with rituximab or immunosuppressant gave CR in 25% and 12.5% respectively, although rather attributable to associated corticosteroids. The few patients that underwent splenectomy, had no response or relapse after ~3 years.

Recommendations:

- Aggressive therapy with corticosteroids and rituximab should start early and inclusion in clinical trials with new drugs should be considered, since mixed AIHA are generally characterized by a severe onset and relapse/refractoriness to several therapies (100% agreement).
- Splenectomy seems ineffective and is not advisable in mixed AIHA (as with CAD) (99% agreement).

5.5. Paroxysmal cold hemoglobinuria (PCH)

Clinical symptoms in children include recurrent fever associated with hemoglobinuria (leading symptom), hemolysis, and jaundice [76,78,180]. Hepatosplenomegaly occurs in approximately 25%. Raynaud´s phenomenon, cold urticaria and kidney failure occur in some cases. The connection to cold exposure is not always evident. In adults, medical history may include syphilis, autoimmune or lymphoproliferative diseases, or several other conditions [181–185].

PCH is frequently self-limiting with spontaneous recovery after a few days or weeks with supportive measures and avoidance of cold temperatures. In severe cases, profound anemia will necessitate blood transfusions administered with warming device [76,78]. In cases with chronic or refractory disease, particularly in adults, immunosuppressive treatment with rituximab has been used successfully [182,186]. Mechanistically, there is a rationale for the use of complement inhibitors. In an adult patient with underlying multiple myeloma, eculizumab therapy failed to improve Hb levels although intravascular haemolysis was suppressed [187]. A recently published case observation of typical childhood PCH, however, reported an immediate improvement and rapid resolution after one single dose of eculizumab [188]. In theory, classical pathway modulation is also an attractive possibility, but the effect remains to be determined.

6. Supportive care

6.1. Transfusion

Because the antibody in wAIHA is directed against common blood group antigens, no truly matched blood transfusions are possible, but red cells can be safely given if alloantibodies are excluded [86,189]. In women without history of pregnancy and/or previous transfusions and in nontransfused men the risk of alloantibody is considered almost absent, thus allowing for transfusion of only ABO- and RhD-matched red cells in urgent cases. In other patients, extended phenotyping with respect to Rh subgroups (C,c,E,e), Kell, Kidd, and S/s with the use of monoclonal reagents is performed or genotyping can be considered. Warm autoadsorption or allogeneic adsorption procedures for detection of alloantibodies are used only in exceptional cases [190]. In any case a biologic “in vivo compatibility test” is done at the bedside: rapid infusion of 20 mL of blood, 20 min observation, and if there is no reaction, further transfusion at the usual rate [11,191]. Transfusion of large amounts of blood at once should be avoided.

In critical cases, transfusions should not be avoided or delayed because of uncertainty in matching, and in wAIHA, treatment with corticosteroids should begin immediately. In patients with CAD, transfused blood must be prewarmed by the use of commercial warming coils [1,10].

6.2. Preventive measures

Patients with AIHA are at greater risk of morbidity and mortality from thromboembolic events (VTE), infection, hematinic deficiency, steroid associated osteoporosis, gastrointestinal bleeding, and infection. Preventative measures are discussed below.

6.2.1. When should patients receive thromboprophylaxis?

Greater thrombotic risk has been identified in both wAIHA and cAIHA [23,53,192,193]. In two large studies a thrombotic event was recorded in 11% of patients [23,53]. Moreover, by using a USA cohort of commercial insurance enrollees (Truven Health MarketScan® Databases) the risk of VTE in AIHA was significantly increased (19 per 1,000 person-years, versus 1.9 in the control group) with an adjusted hazard

ratio of 6.30 (95% confidence interval: 4.44–8.94) [194]. Active hemolysis, indicated by a greater severity of anemia and high median LDH levels, and previous splenectomy have been associated with increased rate of thrombotic events. Whether positive anti-cardiolipin antibodies or lupus anticoagulant are risk factors is controversial. In the individual patient, disease specific risk factors should be considered in addition to general risk factors for VTE (e.g., age > 70 years, active cancer, previous VTE, reduced mobility, already known thrombophilic condition, recent trauma and/or surgery, heart and/or respiratory failure, acute infection) to determine whether the patient should receive VTE prophylaxis.

6.2.2. What treatment should be used to prevent vitamin and iron deficiency?

Folate, vitamin B12 and iron are needed for red cell production and demand rises when haemolysis increases red cell turnover. Whereas hyperferritinemia may be observed with active extravascular haemolysis, intravascular haemolysis can lead to iron loss via the renal tract. Folate deficiency has been reported in patients with chronic hemolytic anemia of various etiologies [195]. Although poorly evaluated in AIHA patients, supplementation (i.e., 1–5 mg of folic acid daily) is well tolerated and widely practiced.

6.2.3. What treatment should patients receive to prevent osteoporosis and fragility fractures while on corticosteroids?

A daily dose of ≥7.5 mg prednisolone for ≥3 months has been shown to result in an incrreased fracture risk [196,197]. Appropriate lifestyle advice for adults includes: stop smoking, limit alcohol intake to ≤2 units/day and take regular weight bearing exercise [196,198]. Adequate daily vitamin D (800 IU) and calcium (700–1200 mg) intake in adults is recommended through diet if possible or supplements if needed [196,197,199]. Bisphosphonates are effective at preventing fracture in patients receiving glucocorticoids [197,198,200,201]. Some guidelines conclude that men over 50 and postmenopausal women receiving corticosteroids for an anticipated dose and duration greater that that outlined above are high risk, and may be concomitantly considered for bone protective therapy without further assessment [198,199]. Otherwise, clinicians should refer to national guidelines where available, but most currently recommend that for adults age 40–90, the FRAX® tool (www.sheffield.ac.uk/FRAX/tool.jsp) be used, supplemented by measurement of bone mineral density if available [196,198,199].

6.2.4. Should patients receiving corticosteroids also receive anti-acid therapy to prevent gastrointestinal bleeding?

There is a 2.2–4.2 fold increase in upper gastrointestinal complications in patients receiving corticosteroids, which is comparable to the increased risk in those receiving aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) [202,203]. Other risk factors include increased age, prior peptic ulcer disease and for those on corticosteroids, concomitant use of aspirin or NSAIDs [202–204]. Most wAIHA patients on chronic corticosteroid should be assessed for anti-acid treatment (e.g., proton pump inhibitors, sucralfate).

6.2.5. Should patients receiving corticosteroids also receive Pneumocystis jirovecii pneumonia (PJP) prophylaxis?

Pneumocystis jirovecii (previously termed *Pneumocystis carinii*) pneumonia is a potentially life-threatening pneumonia in patients receiving > 20 mg corticosteroid/day for over one month [205–207]. Effective prophylaxis is trimethoprim-sulfamethoxazole, dapsone, atovaqone, or aerosolized pentamidine [208,209].

6.2.6. Supportive care for CAD patients

Supportive care for CAD patients should include avoidance of cold temperatures as well as symptomatic treatment (e.g., gloves, handwarmers) of acrocyanosis and other effects of RBC agglutination [156].

Recommendations:

- VTE prophylaxis should be given to all hospital in-patients without a contra-indication (99% agreement).
- VTE prophylaxis should be considered in ambulatory patients with marked haemolysis, particularly if there are additional risk factors for VTE (97% agreement).
- Patients with active hemolysis should receive folic acid supplementation. Hematinic should be monitored if there is a reticulocytosis, or if anemia worsens (97% agreement).
- All adults starting corticosteroids should receive dietary and lifestyle advice. Patients should also be risk assessed to determine whether they would benefit from bone protective therapy (98% agreement).
- Patients receiving corticosteroids should be considered for anti-acid therapy in the presence of additional risk factors such as prior peptic ulcer, concurrent thrombocytopenia, additional use of NSAIDs, aspirin or anticoagulants, and if age ≥60 years (100% agreement).
- PJP prophylaxis should be considered in all patients receiving > 20 mg/d corticosteroids for over one month (70% agreement).

7. Prognostic factors

7.1. Thrombosis

There is increasing awareness of the thrombotic risk in haemolytic conditions, mainly coming from the experience on paroxysmal nocturnal hemoglobinuria (PNH) and hereditary spherocytosis. In two large studies of patients with AIHA, a thrombotic event was recorded in 11% of patients and included 11 cases of pulmonary emboli, 13 deep venous thromboses, 5 splanchnic thromboses, 1 disseminated intravascular coagulation, 3 strokes, 2 transient ischemic attacks and 3 cardiac ischemic events (with 5 patients experiencing more than one event) [23,53]. A case-control single-center study reported a VTE in 8/40 patients (20%), all pulmonary embolus associated with a deep venous thrombosis in 4 [210].

7.2. Infection, other complications, and death

AIHA and infections are closely linked, either because AIHA may be triggered by a common infectious episode probably by the immunological mechanism of molecular mimicry, or because of the immunodeficiency induced by AIHA therapy. The former may complicate the diagnosis and therapy at onset but is usually self-limiting. The latter is somehow underestimated and may lead to life-threatening events. In recent large retrospective multicenter studies, infectious complications (> grade 3) occurred in ~10% cases, mostly pneumonia (of whom 11 grade 4, 8 with associated severe respiratory insufficiency, and 3 with septic shock) and 5 grade 5 (all fatal for severe respiratory insufficiency and septic shock) [23,53]. Severe infectious events were observed more frequently in splenectomized patients, whereas AIHA type and severity were irrelevant. Overall, infections, multiple treatments (4 or more lines of therapy) and splenectomy gave a hazard risk (HR) of death of 11.5, 9.1 and 3.2, respectively.

In addition concomitant thrombocytopenia (Evans syndrome) and acute renal failure occur in about 10% and 2% of AIHA patients, respectively [23,53]. Although rare, they may be associated with a fatal outcome, with a HR of 7 and 18, respectively. An update of the GIMEMA study found the overall AIHA-related mortality was 3–4%, with significant hazard ratios for Evans syndrome (8.95), acute renal failure (6.3), and infections (4.8) [23]. Thrombotic events did not result in increased risk of death.

7.3. Predictors of relapse

In the first GIMEMA study [53], predictors of relapse after first line steroid therapy were younger age at diagnosis (11% every 10 years) and

Table 7
AIHA Clinical Studies – ClinicalTrials.gov (recruitment completed).

Intervention	Mechanism of action	Study title	Condition	Study design
Rituximab vs. Placebo	CD20 antibody	Rituximab in AIHA (RAIHA study)	wAIHA	A randomized and double-blind controlled trial evaluating the safety and efficacy of rituximab for warm auto-immune haemolytic anaemia in adults
Prednisone, Low-dose Rituximab	CD20 antibody	A single-arm pilot study with low-dose Rituximab plus standard oral prednisone in idiopathic AIHA	wAIHA CAD	Single arm pilot study
Bendamustine Rituximab	Immunochemo-therapy	Bendamustine plus rituximab for chronic cold agglutinin disease: results of a Nordic prospective multicenter trial	CAD	Prospective multicenter trial
Prednisolone + Ritux. Vs. Prednisolone	CD20 antibody	Prednisolone +/- addition of Anti-CD20 AB Rituximab in patients with AIHA	wAIHA	Phase III randomized prospective trial
R-CHOP	Immunochemo-therapy	Combination chemotherapy and Rituximab in treating patients with CLL that did not respond to Fludarabine, CLL with AIHA or Richter's transformation (Langerbeins et al, 2014)	CLL ± AIHA	Phase II
Rituximab	CD20 antibody	The RITAI cohort: An observational study of Rituximab off-label use for autoimmune disorders	Pemphigus AITP AIHA	Single arm trial
Cyclophosphamide filgrastim	Cytotoxic therapy	Phase II study of high-dose cyclophosphamide in patients with severe autoimmune hematologic disorders	AIHA AITP Felty Syndrome AID	Phase II
TNT009	Inhibits C1s function in classical complement pathway	Safety, Tolerability and Activity of TNT009 in Healthy Volunteers and Patients With Complement Mediated Disorders (TNT009-01)		Open label study of 4 weekly iv treatments in patients with wAIHA

low hemoglobin values at onset (considering hemoglobin as a continuous variable, each gram of reduction yielded a 14% increased risk of relapse). A recent update of this study added another 112 cases for a total of 378 patients, allowing the evaluation of predictors of multiple refractoriness to second and further therapy lines [23]. This new analysis confirmed that hemoglobin level at onset is the only hematologic parameter associated with an increased risk of multiple relapses. Hazard ratios were 2.7, 2.4, and 2.2 for patients with Hb < 6, 6.1–8, 8.1–10 g/dl, respectively. Moreover, multivariate Cox regression analysis confirmed similar increased hazard ratios for AIHA other than wAIHA, and reinforced the negative prognostic value of Evans Syndrome. This study also found that reticulocytopenia or inadequate reticulocytosis were present in more than half of patients, particularly in cases with a severe onset (Hb < 6 g/dL). This study also found that reticulocytopenia or inadequate reticulocytosis were present in more than half of patients, particularly in cases with a severe onset (Hb < 6 g/dL). This finding indicates an insufficient bone marrow compensatory activity possibly due to an autoimmune reaction against bone marrow erythroblasts, and certainly represents a detrimental prognostic factor.

Recommendations:

- The risk of thrombotic events (both venous and arterial) should be taken into account, mostly during acute haemolytic flares and in splenectomized patients (100% agreement).
- Assessment of a general thrombotic risk factor is advisable in AIHAs (age > 70 years, active cancer, previous VTE, reduced mobility, already known thrombophilic condition, recent trauma and/or surgery, heart and/or respiratory failure, acute infection, etc) (97% agreement).
- VTE prophylaxis should be considered in patients with marked haemolysis and associated risk factors (98% agreement).
- Evans syndrome, acute renal failure, infections and multi-treatment should be carefully evaluated as they are associated with increased risk of death (AIHA-related mortality is 3–4%) (99% agreement).
- AIHA with severe anemia at onset, and/or presence of Evans syndrome, should be closely monitored, since they are associated with an increased risk of multiple relapses/refractoriness to several therapy lines (84% agreement).

8. Special situations

8.1. AIHA in pregnancy

AIHA in pregnancy is rare and evidence limited. Alternative causes of haemolysis in pregnancy should be excluded. Cases are usually primary warm AIHA but secondary AIHA occurs, most often in associated with ITP (Evans syndrome) or SLE [211,212]. Maternal IgG autoantibodies can cross the placenta and when tested for, the cord DAT is usually positive, and in some cases is associated with neonatal jaundice and anaemia [213]. Later onset anemia in infants at 4–6 weeks has also been reported. The risk of late fetal loss appears to be increased. In the largest series, 4/14 had spontaneous miscarriages or intrauterine deaths at 4–9 months gestation and 2/7 had fetal loss at 12 and 28 weeks in another [211,214]. Although not documented, fetal loss could reflect in-utero hemolysis analogous to hemolytic disease of the newborn. Combined antenatal obstetric and hematology input is required with additional fetal monitoring, for example serial ultrasonography from 20 weeks to assess fetal growth and Doppler ultrasound of the fetal middle cerebral artery to screen for fetal anemia. Oral prednisolone, titrated to minimal effective dose is a first line treatment strategy for warm AIHA in pregnancy that has been successful.

Recommendations:

- Antenatal care should involve both obstetrics and hematology. Antenatal and postnatal thromboprophylaxis should be considered along with serial ultrasonography to assess fetal growth and screen

Table 8
AIHA Clinical Studies – ClinicalTrials.gov (recruiting).

Intervention	Mechanism of action	Study title	Condition	Study design
Sutimlimab (BIVV009, TNT009)		Safety, tolerability and activity of TNT009 in healthy volunteers and patients with complement-mediated disorders	BP CAD wAIHA ESRD	First in human
Fostamatinib	Inhibit syk kinase and reduce macrophage FcγRIII mediated destruction	A Safety and Efficacy Study of R935788 in the Treatment of Warm Antibody Autoimmune Haemolytic Anaemia (AIHA) (SOAR)		Open label study of oral fostamatinib 150 mg BID in patients with wAIHA and Hct < 30
INCB050465	Decreases IgG and IgM	INCB050465-206	wAIHA CAD Mixed AIHA	Phase II open label study of 1 or 2 mg QD
Orilanolimab (SYNT001)	Inhibits FcRN and reduces IgG levels	A Safety Study of SYNT001 in Subjects With Chronic, Stable Warm Autoimmune Haemolytic Anaemia (wAIHA)	wAIHA	Open label study of weekly iv treatments for 5 weeks in patients with wAIHA and Hgb < 11
Pegcetacoplan (APL-2)	Inhibits C3 and C3b in complement pathway	Study to Assess the Safety, Tolerability, Efficacy and PK of APL-2 in Patients With wAIHA or CAD	wAIHA CAD	For wAIHA patients and Hgb < 11. 270 mg/day or 360 mg/day of APL-2 treatment for up to 12 months infused by special pump
Rituximab	Reduces B cells	Rituximab in Auto-Immune Haemolytic Anaemia (RAHIA)	AIHA	Phase III randomized, double blind study of rituximab 1000 mg days 1 and 15 vs placebo in patients with wAIHA and Hgb < 10
Levamisole Prednisolone Device: Isolex300i		Efficacy and safety of Levamisole combined with standard prednisolone in wAIHA Treatment of AITP	wAIHA AID AITP AIHA	

for fetal anemia. The neonatologist should be informed of the risk of neonatal anemia and hyperbilirubinemia and monitor for up to 6 weeks in case there is late onset anemia (93% agreement).

9. Future directions

9.1. Ongoing clinical trials and novel treatment approaches

While we have described the current best clinical practice, a number of clinical trials are still ongoing in both wAIHA and CAD. Tables 7 and 8 provide an overview of completed or ongoing clinical studies. In addition, we have compiled data from case reports, small case series or personal communications regarding the use of novel drugs. These studies are intended to reduce the level of pathological anti-RBC antibody or reduce RBC clearance by inhibiting the complement pathway or reducing macrophage-mediated clearance.

Fostamatinib is an inhibitor of spleen tyrosine kinase (syk) and has been shown in animal models to reduce macrophage-mediated clearance of RBCs and platelets [215]. Syk is an important part of the pathway by which immune receptors (such as FcγRIII) signal downstream intracellular events, like receptor-ligand internalization. Fostamatinib has been studied extensively in chronic ITP and shown to produce a response (platelets $> 50 \times 10^9$/L) in 43% of patients vs 14% of those receiving placebo; 18% of patients had a durable response to fostamatinib versus 2% to placebo [216]. Fostamatinib (Tavalisse®) is now FDA-approved for the treatment of treatment of thrombocytopenia in adult patients with chronic immune thrombocytopenia (ITP) who has had an insufficient response to a previous treatment. In an ongoing study in patients with AIHA (Hb < 10), 9 of 17 (53%) patients had a rise in hemoglobin of > 2g/dL [217]. Five of the nine responders did so within the first 4 weeks and had a median hemoglobin rise over baseline of 3.0 g/dL. Responses were maintained and side effects minimal. LDH and reticulocyte counts fell and the haptoglobin levels rose [217].

Orilanolimab (SYNT001), *rozanolixizumab (UCB7665),* and *nipocalimab (M281)* are monoclonal antibodies that bind and inhibit the neonatal Fc receptor (FcRn) [218,219]. IgG normally has a half-life in the circulation of 28 days compared with 1–2 days for the other immunoglobulins. The FcRn facilitates transplacental transfer of IgG from the mother to baby but also protects IgG from catabolism by endothelial cells. In animals deficient in FcRn, autoimmune disease is uncommon and the IgG levels are about 10% of normal [220]. In other animal models of autoimmune disease, inhibition of FcRn has resulted in clinical improvement [221]. Studies with these agents are ongoing but rozanolixizumab has already shown activity in patients with ITP [218].

Plasma-derived C1-inhibitor. Red cell destruction in severe cases of AIHA with a C3d-positive DAT is at least partially mediated by activation of the classical complement pathway. A patient with warm IgM AIHA secondary to lymphoma and severe intravascular haemolysis stabilized with intravenous C1-inhibitor (Cetor) 6000 units followed by 4000, 2000 and 1000 units at 22, 38 and 50 h [168]. Four patients with severe AIHA and C3d-positive DAT (CAD or mixed, mean Hb 4.5 g/dL) responded promptly to 20 mg/kg C1-inhibitor (Berinert®) daily for 6–20 days alongside prednisolone ± rituximab [169].

Sutimlimab (*TNT009, BIVV009)* is a monoclonal antibody that inhibits the C1s subunit of C1 in the classical complement pathway (preserving the other complement pathways) [72,158]. TNT003, the murine antibody form which sutimlimab is derived, has been shown to dramatically reduce IgM mediated haemolysis in CAD [72]. Clinical improvement by a hemoglobin median of 3.9g/dl was seen in a pivotal study [158]. No data have been provided to demonstrate activity of sutimlimab in wAIHA, and not all wAIHAs are complement-mediated. Studies are underway with in both wAIHA, CAD and other complement-mediated disorders [222].

Eculizumab. Complement blockade at the level of C5 has been used in a small number of patients with CAD. Eculizumab increased

hemoglobin levels modestly from a median of 9.3–10.2 g/dL after 6 months [73,167].

Pegcetacoplan (APL-2) is a pegylated derivative of the cyclic tridecapeptide compstatin and inhibits C3 activation [172,223]. This viscous material requires a pump for subcutaneous administration and selectively binds to C3 and blocks the cleavage of C3 into C3a and C3b by C3 convertase. It is being studied in PNH, macular degeneration, and AIHA with some favorable preliminary results reported, particularly in CAD [16,172,224].

Ofatumumab, another anti-CD20 antibody also induced a response in a rituximab-refractory AIHA associated with CLL.

Alemtuzumab is an anti-CD52 antibody previously used in various B- and T-cell lymphomas and now licensed for the treatment of multiple sclerosis. Its activity against AIHA as well as pure red cell aplasia has been described [225,226].

Ibrutinib is a Bruton tyrosine kinase (BTK) inhibitor that has demonstrated marked activity in the treatment of CLL, Waldenström macroglobulinemia, and mantle zone lymphoma. Although this BTK inhibitor has significant effect on lymphocyte populations, less well appreciated is its profound effect on macrophage function. Downstream in the signaling pathway from syk, it too is vital for transmission of immunomodulatory receptors to intracellular signaling pathways. Unlike current syk kinase inhibitors that do not completely inhibit syk, the BTK inhibitors can completely block BTK and thereby reduce macrophage function. In a recent study of 306 CLL patients treated with ibrutinib, 28 were under active treatment for autoimmune cytopenias; 86% stopped treatment for cytopenias after a median of 4.7 months on ibrutinib [138]. Ibrutinib was active against secondary wAIHA in CLL in several published cases [137,227–229]. Although not yet in clinical trials for wAIHA, a trial is currently underway with another BTK inhibitor, PRN1008, in the treatment of ITP (ClinicalTrials.gov, NCT03395210).

Phosphoinositol-3-kinase (PI3K) inhibitors are also well-established drugs for treating malignant B-cell disorders such as CLL or follicular lymphoma. While PI3K inhibitors may also trigger autoimmune phenomena, partial responses of secondary wAIHA were observed when used against CLL (M. Montillo and U. Jäger, pers. communication). A clinical trial for AIHA (wAIHA and CAD) using low doses of INCB050465 is recruiting (NCT03538041).

Venetoclax is a BH3-mimetic causing inhibition of the antiapoptotic Bcl2 protein. Activity in secondary wAIHA has been described, even this is not confirmed in other reports [230,231].

Sirolimus has no published outcomes in adults with primary warm AIHA and but has been shown to be effective in children with refractory primary AIHA or Evans syndrome [232,233].

Daratumumab treatment has recently been suggested as a last resort in patients with life-threatening, refractory post-transplant AIHA. According to a case report describing 3 patients, two children responded well and 1 young adult died of refractory AIHA [234]. No prospective study has been done.

10. Practice points

- AIHA is a heterogeneous group of diseases that should be treated differently.
- A thorough diagnostic workup is required to characterize the subtype of AIHA and select the appropriate treatment.
- Corticosteroids remain first-line therapy in wAIHA. Addition of rituximab in first line should be considered in selected patients.
- In most patients with wAIHA, rituximab (if not given first-line) should be the preferred second-line therapy.
- In patients with CAD requiring treatment, first-line options are rituximab monotherapy or rituximab plus bendamustine, depending on individual patient characteristics.
- Atypical AIHAs (mixed and DAT-negative forms or AIHA driven by IgA or warm-IgM) remain a therapeutic challenge, and some guidance for best practice are provided in this review.
- Complement-directed therapies remain investigational but are promising.
- Patients with wAIHA not responding to first-line treatment, patients with CAD requiring therapy, and patients with rarer AIHAs should be considered for clinical trials if available.

11. Research agenda

- The pathogenesis and predictive factors in different types of AIHA should be further investigated, not least in wAIHA.
- Future treatment options for AIHA should be based on prospective clinical trials as far as possible.
- The potential and safety of classical complement pathway-directed therapies should be further explored, particularly in complement-mediated wAIHA and CAD, if possible also in CAS and PCH.
- Treatment with other novel agents, listed in Section 9.1, should also be further investigated.
- An international network should be established to stimulate basic research, clinical studies, and update of guidelines in AIHA.

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