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REVIEW

Current Approaches for the Treatment of Autoimmune Hemolytic Anemia

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Abstract Autoimmune hemolytic anemia (AIHA) is an infrequent group of diseases defined by autoantibody mediated red blood cell destruction. Correct diagnosis and classification of this condition are essential to provide appropriate treatment. AIHA is divided into warm and cold types according to the characteristics of the autoantibody involved and by the presence of an underlying or associated disorder into primary and secondary AIHA. Due to its low frequency, treatment for AIHA is largely based on small prospective trials, case series, and empirical observations. This review describes in detail the different treatment approaches for autoimmune hemolytic anemia. Warm antibody type AIHA should be treated with steroids, to which most patients respond, although relapse can occur and maintenance doses are frequently required. Splenectomy is an effective second line treatment and can provide long-term remission without medication. Rituximab is a useful alternative for steroid refractory patients, those requiring high maintenance doses and unfavorable candidates for surgery. Promising therapeutic modifications with this monoclonal antibody are emerging including drug combinations, lower doses, and long-term use. Primary cold agglutinin disease has been recognized as having a lymphoproliferative monoclonal origin. It is unresponsive to both steroids and splenectomy. Rituximab is currently the best therapeutic alternative for this condition, and several treatment regimens are available with variable responses.

Keywords Hemolytic anemia · Autoimmune · Cold agglutinin disease · Treatment · Rituximab

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Introduction

Autoimmune hemolytic anemia (AIHA) is an infrequent group of diseases defined by autoantibody mediated red blood cell (RBC) destruction. It affects mostly, but not exclusively, adults, with an estimated annual incidence of 1–3/100,000 persons (Bottiger and Westerholm 1973; Gehrs and Friedberg 2002; Klein et al. 2010). RBC destruction is associated with the presence of immunoprotein molecules on the erythrocyte surface, including immunoglobulins, most frequently IgG, with antibody activity directed against self-RBC antigens, with or without complement fractions. Subsequent cell damage mediated by diverse immune mechanisms causes shortened RBC survival. The etiology underlying the production of such autoantibodies is not completely understood and its explanation remains one of the most challenging goals in investigational hematology today. Several mechanisms have been proposed to explain AIHA development, including, but not limited to, a breakdown of immunologic tolerance with possible roles of RBC autoantigens, the complement system, and defective autoantigen presentation. Likewise, abnormalities of B- and T cell function leads to polyclonal lymphocyte activation with consequent abnormal cytokine production, a loss of central or peripheral immune tolerance and altered regulation (Barros et al.

2010; Logue and Rosse 1976; Semple and Freedman 2005). In addition, a significant role has been proposed for activated immunoregulatory T cells (Fagiolo 2004; Mqadmi et al. 2005). The purpose of this article is to review in detail the current treatment of the most frequent types of AIHA.

Classification

AIHA can be classified according to the temperature reactivity of the responsible autoantibody into warm, cold, and mixed types. It can also be further subdivided into primary or secondary, depending on the presence of an associated condition. Warm AIHA (wAIHA) explains 75 % of cases and is caused by IgG antibodies that react optimally at 37 °C binding to erythrocytes and leading to their phagocytosis by Fc and C3b receptor-expressing reticuloendothelial cells located primarily in the spleen (Gehrs and Friedberg 2002; Petz 2004). Primary or idiopathic wAIHA is an autoimmune disorder in which no underlying pathological process can be recognized, whereas secondary wAIHA can be due to a variety of subjacent conditions. Approximately, half of wAIHA cases are secondary. Neoplasias are a common cause, often of B cell origin, including non-Hodgkin's lymphomas (NHL) and chronic lymphocytic leukemia (CLL), the latter being the most commonly associated disease. Autoimmune disorders are also the important causes, particularly systemic lupus erythematosus (SLE). Other autoimmune diseases such as antiphospholipid syndrome, Sjogren's syndrome, and rheumatoid arthritis (RA) are also causes of wAIHA (Gomard-Mennesson et al. 2006; Hauswirth et al. 2007; Hodgson et al. 2011; Kokori et al. 2000).

Cold reactive types of AIHA include cold agglutinin disease (CAD) and paroxysmal cold hemoglobinuria (PCH). CAD is responsible for most of the remaining cases of AIHA. In this disease, hemolysis is usually mediated by IgM autoantibodies that bind more strongly to RBCs at temperatures between 0 and 4 °C (Dacie 1975; Schubothe 1966). Primary, chronic CAD is a monoclonal B cell disorder with a close relationship to some lymphoproliferative diseases such as Waldenstrom's macroglobulinemia, marginal zone lymphoma, and lymphoplasmacytic lymphoma. Morphological and immunohistochemical features of NHL have been reported in up to 76 % of cases (Berentsen et al. 2006). This discovery has changed our knowledge of the origin of the disease from primary or idiopathic to a true lymphoproliferative disorder, with an abnormal B cell clone causing production of autoantibodies. Secondary CAD or, more appropriately, secondary cold agglutinin syndrome (CAS) is a rare disorder, associated with infectious diseases, particularly with those due to *Mycoplasma pneumoniae,* Epstein-Barr virus, cytomegalovirus (CMV), and others, as well as patients with several types of malignancies such as diffuse large B cell lymphoma and other forms of NHL (Berentsen and Tjonnfjord 2012).

Mixed cases of AIHA share characteristics of both warm and cold types, with autoantibodies reacting at a wide thermal range from 4 to 37 °C. AIHA can also be secondary to drug administration as the result of drug dependent or independent antibodies; piperacillin ceftriaxone and cefotetan are some of the most notorious responsible agents. It is important to mention the association of purine analog use for treating B cell hematological malignancies, particularly fludarabine, as it is an important cause of drug-induced AIHA that can lead to the development of severe and sometimes fatal disease (Arndt and Garratty 2005; Garratty 2010; Hoffman 2006). These drugs most probably cause a direct imbalance in patients' immune system. PCH, mixed type, and drug-induced AIHA will not be addressed further as they are very rare diseases and beyond the scope of this review.

Diagnosis

The diagnosis of AIHA is relatively straightforward. In addition to a meticulous clinical history and detailed physical examination, it can include the following laboratory findings: anemia, either macro or normocytic, usually accompanied by remarkable reticulocytosis; increased lactate dehydrogenase; increased indirect bilirubin level; low serum haptoglobin and a positive broad-spectrum direct antiglobulin (DAT); or Coomb's test (Liesveld et al. 1987; Tisdale et al. 1959; Zantek et al. 2012; Zarandona and Yazer 2006). A positive polyspecific DAT may be due to the presence on the erythrocyte surface of IgG, C3d, or both. Monospecific reagents can usually differentiate the thermal origin of the autoantibody, wAIHA generally presents with an IgG-positive and C3d-positive/-negative pattern; while cold AIHA is classically C3d-positive and IgG-negative. Mixed cases and wide thermal range autoantibodies are exceptions to this condition. The clinician must be aware that there are other causes of a positive DAT, and that this finding can even be present in healthy blood donors. Also 1–10 % of AIHA cases can be DAT-negative (Kamesaki et al. 2013). For the diagnosis of CAD, required criteria include the presence of chronic hemolysis, a positive monospecific DAT for C3d, and the determination of cold agglutinins with a $\geq$1:64 titer (Berentsen and Tjønnfjord 2012). The presence of an abnormal B cell clone can be demonstrated with serum electrophoresis and immunofixation tests as well as bone marrow biopsy and flow cytometric analysis, although they are not absolute requirements for diagnosis.

Table 1 Treatment for primary AIHA: alternatives of treatment with useful references

wAIHA	
Corticosteroids	(Allgood and Chaplin 1967; Murphy and LoBuglio 1976; Zupanska et al. 1981)
Surgical procedure	Splenectomy (Akpek et al.1999; Chertkow and Dacie 1956; Coon et al. 1985; Grant et al. 1988)
Monoclonal antibodies	Rituximab (Barcellini et al. 2012; Bussone et al. 2009; Shanafelt et al. 2003)
	Alemtuzumab (Cheung et al. 2006; Gomez-Almaguer et al. 2010; Osterborg et al. 2009; Willis et al. 2001)
Immunomodulators	IVIG (Darabi et al. 2006; Flores et al. 1993)
Steroid androgen	Danazol (Ahn 1990; Pignon et al. 1993)
Tranfusion	RBCs (Buetens and Ness 2003; Petz 2004)
	Plasma exchange (Ruivard et al. 2006)
Immunosuppressive agents	Cyclophosphamide (Moyo et al. 2002)
	Cyclosporine (Hershko et al. 1990)
	Azathioprine (Bussone et al. 2009)
	Mycophenolate mofetil (Alba et al. 2003; Howard et al. 2002; Rao et al. 2005)
CAD	
Monoclonal antibodies	Rituximab (Berensten et al. 2004, 2006, 2010; Schöllkopf et al. 2006)
	Eculizumab (Röth et al. 2009)
Immunomodulators	Alpha interferon (Hillen and Bakker 1994; Rordorf et al. 1994)
Proteasome inhibitor	Bortezomib (Carson et al. 2010; Danchaivijitr et al. 2011)
Tranfusion	Plasma exchange (McLeod 2007; Zoppi et al. 1993)

wAIHA warm autoimmune hemolytic anemia, *CAD* cold agglutinin disease, *IVIG* intravenous immunoglobulin

For opportune and proper management of AIHA, establishing the correct diagnosis is essential. This involves defining early on if the AIHA process is primary or secondary. It is important to note that in secondary cases typical laboratory features are not always documented, which can lead to a delay in diagnosis. A fraction ranging from 20 to 80 % of cases can be secondary, depending on the authors (Bell et al. 1973; Dausset and Colombani 1959; Evans and Weiser 1957; Sokol et al. 1981). For this reason, secondary AIHA must be excluded and a work-up should be performed. A thorough history and physical examination can exclude some causes of secondary AIHA, such as overt malignancy (i.e., CLL in wAIHA) and infections (i.e., atypical pneumonia in CAS). Additional laboratory testing should include antinuclear antibodies, anticardiolipin antibodies and lupus anticoagulant to detect SLE, and antiphospholipid syndrome, respectively. Also a computed tomography scan of the thorax, abdomen, and pelvis can be performed if an occult neoplasia is suspected.

Treatment

Implementation of the most appropriate treatment for AIHA will depend on the severity of anemia, associated symptoms, and underlying conditions. The classical therapeutic schemes remain in use, although they are primarily based on empirical data. New therapeutic approaches lack the support of sufficiently powered studies based on large groups. Most are based primarily on small series of patients and case reports. A list of treatment alternatives can be found in Table 1, along with some useful references. Different approaches should be taken depending on the main characteristics of AIHA, considering a specific management for primary wAIHA, CAD, and secondary forms of cold and warm AIHA.

Warm Autoimmune Hemolytic Anemia

Primary wAIHA

Steroids

First line therapy for treating this type of AIHA is corticosteroids, usually in the form of prednisone for moderate hemolysis at a dose of 1.0 mg/kg/day for 1–3 weeks. Also, methylprednisolone 100–200 mg/day in two IV doses for 10–14 days can be used in the presence of severe hemolysis (Murphy and LoBuglio 1976; Packman 2008). Response to treatment occurs mainly during the second week, and if none or minimal improvement is observed in the third week, failure of this form of therapy is assumed. If the hematologic status improves, as it does in approximately 80 % of patients, steroid weaning can be initiated until reaching a maintenance dose of <15 mg/day for as long as 12–18 months (Allgood and Chaplin 1967; Zupanska et al. 1981). This is referred to as the highest

tolerable dose for chronic administration, although one-fifth of patients will require higher doses, indicating the need for second line therapy. If relapse occurs, another form of treatment must be implemented. Only 15–20 % of patients will reach long-term steroid-free remission (Gehrs and Friedberg 2002; Lechner and Jager 2010; Murphy and LoBuglio 1976). When remission is sustained for at least 3 months on a daily dose of 5 mg, withdrawal can be attempted and if successful, careful monitoring should be established. It has been suggested that some patients should be supplemented with folic acid and anti-osteoporosis medication.

Splenectomy

Because the spleen is a major organ for antibody synthesis and immune destruction of immunoprotein-coated erythrocytes, splenectomy has classically been proposed as the logical second line approach for patients with no response or intolerance to corticosteroids (Barros et al. 2010; Petz and Garraty 2004a). This procedure is also an effective alternative for patients who require maintenance doses of prednisone >15 mg/day or those with multiple relapses. At least half of the patients respond initially to splenectomy (Akpek et al. 1999; Allgood and Chaplin 1967; Chertkow and Dacie 1956; Coon 1985; Grant et al. 1988; Zupanska et al. 1981). However, post-splenectomy relapses are common, presumably because there is still an elevated synthesis of autoantibodies, and the liver takes over the spleen's function of RBC destruction. Special care should be taken for splenectomized patients during febrile episodes. The risk of overwhelming pneumococcal sepsis can be reduced by preoperative vaccination (Davies et al. 2011). The lifetime risk of this catastrophic complication is 5 % with a mortality rate of up to 50 % (Brigden 1992; Davidson and Wall 2001; Davies et al. 2011; Gopal and Bisno 1977). Vaccination should be completed at least 3 weeks before the procedure is performed. Small, but not marginal additional risks include thromboembolism and pulmonary hypertension (Condliffe et al. 2009; Krauth et al. 2008; Pepke-Zaba et al. 2011; Vecchio et al. 2011). It is essential to reassess the diagnosis of primary AIHA in refractory patients before proceeding to splenectomy, as individuals with occult malignancies and warm IgM antibodies can be steroid non-responders. Factors favoring splenectomy as the best second line therapy include its considerable short-term efficacy and good long-term results, as 40–80 % of patients will obtain a partial or complete remission. An important consideration when evaluating this figure is the variability in the fraction of secondary AIHA cases in published studies (Akpek et al. 1999). Weighing in favor of surgical removal of the spleen is the fact that some splenectomized patients can be in remission for years without requiring pharmacological support, with a low perioperative risk. The procedure has a 0.5 % mortality rate when performed through laparoscopy (Casaccia et al. 2006); however, higher figures of 1.3 % for adults and 1.7 % for children have also been reported (Ram et al. 2010). It is also important to emphasize that splenectomy is the only proven form of AIHA therapy to offer long-term control of hemolysis without medication, with an assumed cure rate of up to 20 % (Lechner and Jager 2010), although this has never been confirmed by a prospective trial.

Rituximab

Rituximab is used for relapsed or refractory AIHA as a second line alternative instead of splenectomy, or after its failure. It is a useful treatment option especially for patients who refuse or are not suitable candidates for surgery. Rituximab is a monoclonal antibody against the CD20 antigen present on pre-B cells and mature B cells. It is used for therapy of B cell cancers such as Hodgkin and non-Hodgkin's lymphomas, as well as several autoimmune diseases including RA, SLE, mixed cryoglobulinemia, immune thrombocytopenic purpura, and myasthenia gravis; thus it can be useful in secondary AIHA associated to any of these conditions (Gupta et al. 2002; Shanafelt et al. 2003; Trape et al. 2003; Zaja et al. 2003; Zecca et al. 2003). For wAIHA, a dose of 375 mg/m^2/week for 4 weeks has been used, the same dose used to treat B cell neoplasms. Several retrospective studies and case series have been published regarding its use in AIHA showing variable response rates ranging from 40 to 100 % with a median of 60 % and disease-free survival of 50 % in 2 years (Bussone et al. 2009; D'Arena et al. 2006, 2007; Narat et al. 2005; Penalver et al. 2010; Quartier et al. 2001; Shanafelt et al. 2003; Zecca et al. 2003). Although it is unknown if refractoriness to its administration will develop, re-treatment with rituximab has been reported to be effective for relapsing cases, and extended rituximab therapy has also been used (D'Arena et al. 2006). It has been noted that a low risk of neutropenia (<2 %) and infection (<7 %) exists (Garvey 2008). Importantly, although few secondary effects have been reported, usually mild infusion reactions, progressive multifocal leukoencephalopathy has been referred as a potential long-term complication of rituximab administration (Carson et al. 2009). Recently, a prospective phase-II study was performed using low-dose rituximab in AIHA (Barcellini et al. 2012). The dose of rituximab used was 100 mg/week for 4 weeks instead of the usual "lymphoma doses" combined with prednisone 1 mg/kg for 30 days in newly diagnosed and steroid-relapsed patients ($n = 14$ wAIHA cases). In this study, a 100 % overall response (OR) rate at 2 months was achieved, with 78.6 %

complete responses and a median time to OR of 16 days. Duration of response was 100 % at 1 year of follow-up (median follow-up 15 months), with no adverse events related to rituximab reported. Although more studies are needed, these results suggest that lower doses of rituximab may be sufficient to produce long-term responses in most patients, with a lower incidence of side effects and a lower cost.

Alemtuzumab

Alemtuzumab is a monoclonal antibody directed against the CD52 antigen expressed on the cell membrane of virtually all healthy as well as malignant lymphocytes, whether they are B or T cells. This drug has the ability to cause the lysis of such cells by a host-effector mechanism including complement fixation, antibody-dependent cell-mediated cytotoxicity, and the induction of apoptosis (Barros et al. 2010). The use of this antibody can result in a prolonged and profound peripheral blood lymphopenia, particularly affecting T cells (Robak 2004). Only a few case reports of alemtuzumab in refractory primary AIHA have been published, where complete remissions were achieved (Cheung et al. 2006; Willis et al. 2001). This monoclonal antibody must be used with caution as it can lead to severe lymphoid suppression, reactivation of CMV, and it can facilitate bacterial infections. We have recently reported the treatment of eight warm antibody AIHA patients refractory to first line therapy with the combination of low doses of alemtuzumab (10 mg subcutaneously for 3 days) plus rituximab (100 mg IV weekly for 4 weeks). All patients attained a response: in six patients the response was complete, with three maintaining their response to the end of follow-up (8, 11, and 22 months). The remaining three patients relapsed; two patients obtained a partial remission and relapsed later on (Gomez-Almaguer et al. 2010). From this experience, we concluded that further studies, including higher doses of this combination and/or a longer duration of treatment, or even maintenance therapy, could help define their precise role in AIHA.

Other Treatment Strategies

Cyclophosphamide, cyclosporin, azathioprine, and mycophenolate mofetil have been used with success in small studies and case reports as third line agents (Bussone et al. 2009; Hershko et al. 1990; Howard et al. 2002). Use of high doses of cyclophosphamide (50 mg/kg/day for 4 days) followed by granulocyte colony-stimulating factor was effective in achieving complete remission in 5/6 patients; however, the potential adverse effects such as bone marrow suppression, hemorrhagic cystitis, and sterility lead us to consider this alternative only in severe refractory cases (Moyo et al. 2002). Mycophenolate mofetil has been used in refractory, idiopathic, and secondary AIHA cases (Alba et al. 2003; Howard et al. 2002), particularly, due to autoimmune lymphoproliferative syndrome in children where 12 out of 13 heavily pretreated patients responded. Adverse effects were less serious compared to other immunosuppressive drugs with these effects consisting mainly of gastrointestinal intolerance and myelosuppression (Rao et al. 2005).

Intravenous immune globulin (IVIG) accelerates the clearance of autoantibodies by anti-idiotypic effect; blocks Fc receptors in the reticuloendothelial system, which is overactive in AIHA patients; and decreases or inhibits antibody synthesis (Hoffman 2009). Some studies have reported a response rate as high as 40 %; more pronounced in children and patients with hepatomegaly and a lower hemoglobin level (Flores et al. 1993). Published reports of IVIG use in wAIHA suggest that it may be necessary to use high doses, similar to those used for the treatment of patients with immune thrombocytopenia (400–1,000 mg/kg per day for 5 days) (Darabi et al. 2006), which may not be cost-effective. Its use, however, is controversial primarily because only limited, small case series have been reported.

Danazol is a synthetic derivative of ethinyl-testosterone with mild androgenic properties. Its mechanism of action for the treatment of warm AIHA is not completely understood. Based on its results in patients with immune thrombocytopenia, it has been administered at a dose of 400–800 mg with good results. Combined with prednisone in first line therapy, it can function as a steroid sparing agent, although it has a limited effectiveness in refractory or relapsed patients in whom only 43 % complete remission is reached, with 14 % partial remissions (Ahn 1990; Pignon et al. 1993). Danazol should be given with caution because of its androgenic and potentially toxic hepatic effects (Confavreux et al. 2003). The current concept is that danazol can be used for patients requiring high daily prednisone doses and in those refractory to steroids.

Autologous bone marrow or stem cell transplant (HSCT) has been proposed for life threatening and transfusion-dependent warm AIHA. In the largest study published, it was performed in 26 patients with only nine obtaining a partial or complete response, with three dying of treatment-related causes and one of progressive disease (Passweg et al. 2004). Autologous and allogeneic HSCT may induce a response in a subset of patients with severe refractory autoimmune cytopenia, and the decision to transplant should be made after a careful cost/benefit analysis in a case-by-case basis.

Transfusion Support Therapy

Autoantibodies in AIHA are directed to common RBC antigens and thus serocompatible donors can only be found

if the autoantibodies are directed to a specific blood group (Buetens and Ness 2003). Cross-matching tests are performed to rule out incompatibilities, but panreactive autoantibodies frequently lead to all cross-matched units being incompatible. This condition may mask the presence of an alloantibody, which can result in a severe hemolytic transfusion reaction. Although clinically stable patients may not require RBC transfusion, this should be employed in the presence of anemia accompanied by clinical data of limitation in tissue oxygen delivery and neurologic signs such as confusion suggest transfusion is urgently required. In this respect, it is common that the transfused red cells be hemolyzed at the same rate as the patient's own erythrocytes, but even a marginal increment in tissue oxygen availability may gain time for other therapies to exert their action (Packman 2008).

It is important to note that appropriate transfusion support for AIHA patients requires special compatibility tests, most frequently available in immunohematology reference laboratories. If adequate compatibility testing is conducted, however, the indications for transfusion in patients with AIHA do not significantly differ from those of patients without AIHA and a similar degree of anemia. The physician in charge must understand the principles of compatibility testing needed, and fluent communication with blood bank personnel is essential to determine the best compatibility methods for selection of optimal RBC units for transfusion (Petz 2004).

Leucoreduced RBCs are recommended to avoid febrile non-hemolytic transfusion reactions that may mimic a hemolytic reaction. In warm-type AIHA, no truly matched blood transfusions are possible, but after alloantibody exclusion red cells can be safely given (Lechner and Jager 2010). In an effort to prevent the development of alloantibodies, avoid hemolytic reactions resulting from preexisting alloantibodies due to pregnancy or previous transfusions, and reduce compatibility problems, two different approaches are recommended when searching for compatible packed RBCs units. The most frequently used is the removal of autoantibodies by adsorption techniques, allowing for alloantibody detection, which has been documented to be as high as 32 % in AIHA patients (Branch and Petz 1999); the second strategy is extensive phenotyping of RBC intended for transfusion principally with respect to Rh subgroups (C, c, E, e), Kell, Kidd, and S/s with the use of monoclonal IgM antibodies (Petz and Garraty 2004b, Lechner and Jager 2010). The phenotype can be used to guide the exclusion of alloantibodies by indicating which antigen specificities the patient is at risk of developing. In some patients, the autoantibody demonstrates Rh specificity, most often auto anti-e, but it is not clear that antigen-negative RBC transfusions lead to extended erythrocyte survival (King and Ness 2005).

Apart from RBC transfusion, plasma exchange has been used as another therapeutic tool. There has been no difference, however, in the hemoglobin level between transfusion alone and the plasma exchange group (Ruivard et al. 2006).

During active and accelerated hemolysis, there is a higher probability of thrombosis. This can be manifested as deep vein thrombosis and, more severely, as pulmonary embolism. This condition can appear particularly in patients with a predisposing condition such as oral contraceptive use, prolonged bed rest, major surgery, smoking, heart failure, arrhythmia, obesity, fractures, or antiphospholipid syndrome. Heparin administration has been proposed during acute hemolytic episodes (Hendrick 2003; Petz 2001). Thromboembolism is more probable in patients with either post-splenectomy relapse or AIHA and a circulating lupus anticoagulant (Pullarkat et al. 2002). Prophylactic anticoagulation during AIHA therapy should be given if necessary.

Secondary wAIHA

Chronic Lymphocytic Leukemia

Secondary wAIHA can complicate CLL in 5–10 % of cases (Hodgson et al. 2011). Treatment of autoimmune hemolysis in this setting can be carried out with steroids in a fashion similar to primary wAIHA. Both splenectomy and rituximab may be considered second line options; however, we favor the latter. If steroids are unsuccessful or CLL-targeted treatment is required, "immunochemotherapy" regimens can be a useful approach. Two regimens have been tried with the objective of providing an antileukemic and anti-autoimmune effect: rituximab, cyclophosphamide, dexamethasone (RCD), and rituximab, cyclophosphamide, vincristine, and prednisone. In the largest retrospective series, OR rates of approximately 90 % were observed with RCD, with a duration of response of 24 months, similar to other studies (Bowen et al. 2010; Kaufman et al. 2009; Rossignol et al. 2011). It is important to mention that although fludarabine is an effective agent against CLL, its association with DIIHA precludes the use of this agent for the treatment of wAIHA secondary to CLL. Alemtuzumab has also demonstrated to be active in this particular case; successful use of this monoclonal antibody has been documented in some case reports as well as a patient series (Osterborg et al. 2009).

Other Neoplastic Diseases

The treatment of wAIHA associated with other neoplastic diseases should be aimed at both processes. Achievement of durable responses following treatment of the primary

cancer with surgery, chemotherapy, or radiation has been described in several cases and, as mentioned before, steroids can be particularly ineffective in some of these patients (Puthenparambil et al. 2010). In the case of NHL, rituximab is an attractive option, and although experience is very limited, cases of marginal zone, follicular, and diffuse large B cell lymphoma, among others, have proven to be successfully treated with this monoclonal antibody (Fozza and Longinotti 2011; Hauswirth et al. 2007). For the rare case of a splenic marginal zone lymphoma complicated with wAIHA, splenectomy is another effective option for treating both conditions simultaneously (Thieblemont et al. 2003).

Systemic Lupus Erythematosus

Treatment in the context of another autoimmune disease does not differ significantly from primary wAIHA, as attaining immunosuppression is a common objective. Regarding SLE particularly, steroids are the first line of treatment and offer good results. The lowest effective dose should be sustained for preventing relapses and other SLE manifestations. In the largest published series ($n = 26$), the initial response rate reached 96 %, with a relapse rate of 27 % in a median follow-up of 180 months (Gomard-Mennesson et al. 2006). The long-term efficacy of splenectomy is uncertain (Rivero et al. 1979). For this reason, we consider rituximab to be a better alternative for second line treatment. The use of other immunosuppressive agents is anecdotal.

Cold Autoimmune Hemolytic Anemia

Primary CAD

Primary CAD is a chronic disorder, usually with only mild anemia and stable hemoglobin levels ranging from 9 to 12 g/dL. Avoidance of low temperatures is the best way of preventing symptoms. As the stimulus for antibody binding to RBC surface is eliminated, no hemolysis takes place. It is the initial step and can be an effective form of management. Although this recommendation must be made to every patient, using warm clothing in cold weather does not address the pathological process, and other forms of treatment can be required, particularly in severe cases (Gehrs and Friedberg 2002).

Rituximab

The identification of CAD as a B cell lymphoproliferative disease has provided a different rationale for treatment. Therapy directed at the abnormal B cell clone with rituximab has shown promising results in a disease where other interventions have been previously unsuccessful. Two prospective trials using rituximab for this variety of AIHA have been carried out with the same dose as in wAIHA (375 mg/m^2/week for 4 weeks) (Berentsen et al. 2004; Schollkopf et al. 2006). The biggest study reported the use of 37 courses of rituximab administered prospectively to 27 patients with an OR rate of 54 %, an increase in hemoglobin level of 4 g/dL, a median time to response of 1.5 months, and a median duration of 11 months (Berentsen et al. 2004). Another study was performed in 20 patients with follow-up in 16; 45 % responded, with a patient still in remission at the end of follow-up. Median response duration was 6.5 months (range 2–10 months). (Schollkopf et al. 2006). These results show that although rituximab can be effective as a single agent, long-term response is yet to be achieved. The recently published study on the use of low-dose rituximab combined with steroids for the treatment AIHA, reported its results on nine CAD patients, with an OR of 55.6 % at 2 months, a stable response of 66.7 % at 12 months, and a median time to OR of 19 days (Barcellini et al. 2012). Fludarabine has been used for CAD in combination with rituximab and 76 % patients responded, 21 % of which achieved complete remission and 55 % partial remission. The median response duration was more than 66 months and hematologic toxicity occurred in 41 % of patients. No fludarabine induced AIHA was reported (Berentsen et al. 2010). These are the best results to date for the treatment of CAD; nonetheless, a risk–benefit analysis for this combined form of therapy must be made by the clinician due to its increased toxicity. Treatment with rituximab is the only well-documented effective treatment for CAD. The evidence available, although limited, has demonstrated that this monoclonal antibody can be quite an efficient therapeutic tool, making it first line therapy for this condition.

Other Treatments

Although steroids have been used to treat CAD, they are not an acceptable alternative for single agent treatment. The evidence available suggests a low response rate with a high maintenance dose to achieve long-term remission (Berentsen et al. 2006; Nydegger et al. 1991; Schubothe 1966). Few reports document success in a minority of patients with a low titer or a high thermal amplitude IgM and IgG cold agglutinin disease (Nanan et al. 1995; Schreiber et al. 1977). Splenectomy is not effective because IgM autoantibodies react with the C3 complement component activating the classical pathway leading to primary sequestration and extravascular hemolysis in the liver, not the spleen (Berentsen et al. 2006).

Cytotoxic agents such as cyclophosphamide have had controversial effects in small studies and case reports, with some favorable responses, although in refractory patients, bone marrow depression and temporary worsening of anemia can result (Petz 2008; Worlledge et al. 1968).

Bortezomib is a 26S proteasome inhibitor approved for the treatment of multiple myeloma. It has been used as therapy for a patient with steroid/rituximab-refractory CAD, in wAIHA as a single agent, and in combination with low-dose cyclophosphamide and dexamethasone, respectively. Three doses of 1.3 mg/m^2 were used. Bortezomib treatment resulted in transfusion independence and a stable hemoglobin level in both case reports, suggesting that this drug may be a useful addition to the therapeutic choices in severe refractory cases, although it should be noted that it has a myelosuppressive effect and carcinogenic potential (Carson et al. 2010; Danchaivijitr et al. 2011).

Alpha interferon has been used subcutaneously as single agent therapy at a dose of 3 million U/m^2 three times weekly and did not result in disease remission; however, some significant improvement of clinical condition and laboratory values was achieved (Rordorf et al. 1994). Another report on this agent contradicts these findings due to a lack of response in the whole trial group (Hillen and Bakker 1994). It seems that this drug does not have an important role in this disease.

Since in CAD the antibody involved is of the IgM class, plasma exchange can be useful because 80 % of this immunoglobulin is circulating and thus can be removed to provide temporary relief. This resource can be important in cases where the patient needs a surgical procedure that requires exposure to cold (McLeod 2007; Taft et al. 1977; Zoppi et al. 1993). Eculizumab, a humanized monoclonal antibody directed at the C5 complement fraction has been shown to reduce intravascular hemolysis and the need for transfusion in refractory or life-threatening paroxysmal nocturnal hemoglobinuria. It has shown to control hemolysis but it predisposes to meningococcal infection, possibly marrow insufficiency and extravascular hemolysis (Schrezenmeier and Hochsmann 2012). Because the activity of IgM in CAD is mostly intravascular and entirely complement-dependent, eculizumab may be useful in severe hemolysis and acute exacerbations where complement components are present in high titers. Successful use in refractory CAD has been reported in a single case (Roth et al. 2009).

Transfusion Support Therapy

In some patients, transfusion is unavoidable. Packed RBC must be warmed at body temperature to prevent hemolysis. However, RBC transfusion should be wisely indicated, because IgM is directed against the "I" surface antigen, which is expressed in most of the population. Although there is sound scientific evidence for the fear of activating hemolysis by transfusion of blood products containing complement, this is of no relevance if only packed RBC is transfused. Cold antibodies generally do not react at 37 °C, but potentially hemolytic alloantibodies do; in consequence, all testing should be carried out strictly at 37 °C (Petz and Garraty 2004b).

Secondary CAS

CAS secondary to infections or neoplastic diseases is very rare. In turn, treatment recommendations are not based on prospective trials, and only case reports are available. An adequate evidence-based approach to therapy is lacking. As in secondary wAIHA, the underlying condition should be treated as much as possible. This approach, along with cold temperature avoidance has been reported to be effective for CAS associated with malignancies that are sensitive to chemotherapy particularly lymphoproliferative diseases such as CLL and NHL (Hauswirth et al. 2007). CAS secondary to pneumonia due to *Mycoplasma pneumoniae* and other infections is usually self-remitting. Antibiotic treatment for non-viral contagions may accelerate the resolution of hemolysis. Supportive therapy can be sufficient for preventing the worsening of symptoms in these cases, and in the absence of evidence, conservative management is the best alternative. For severe cases, high-dose steroids have been successfully used in some case reports (Cherry 1993; Chu et al. 1990; Tsuruta et al. 2002) as well as plasmapheresis (Geurs et al. 1992), and although case reports are hardly optimal for guiding treatment, it is unlikely that more valuable evidence will be available in the near future.

Future Directions

The high complexity and heterogeneity in AIHA etiology, its response to therapy as well as its low incidence makes prospective evaluation in a randomized trial unlikely. In consequence, clinicians caring for AIHA patients must carefully evaluate individual cases and tailor the therapeutic approach based on the best evidence available. It is possible that combined therapy including prednisone and rituximab, as first line drugs could be an attractive option for primary wAIHA in the future, as the efficacy of both could synergize to obtain a tighter control of the immune response. The use of monoclonal antibodies directed at B and T lymphocytes in combination should be further studied, as this can result in a higher response rate in cases refractory to steroids and splenectomy.

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