# Tratamiento de la anemia hemolítica autoinmune

## Treatment of AIHA

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**Abstract**

Autoimmune hemolytic anemia (AIHA) is a heterogeneous disease usually classified as warm, cold [cold agglutinin disease, paroxysmal cold hemoglobinuria] or mixed, according to the thermal range of the autoantibody. Diagnosis is based on the direct antiglobulin test (DAT), although atypical cases (DAT-negative AIHA, "warm" IgM AIHA) are reported with increasing frequency.

The treatment of AIHA is still not evidence-based. Corticosteroids are the first-line therapy for warm AIHA. For refractory/relapsed cases the choice is between splenectomy (effective in ~70% of cases but with a presumed cure rate of 20%) and rituximab (effective in ~70-80% of cases), which is becoming the preferred second-line treatment, and thereafter any of the immunosuppressive drugs (azathioprine, cyclophosphamide, cyclosporin, mycophenolate mofetil). Additional therapies are intravenous immunoglobulins and danazol. For severe or refractory cases, last option treatments are plasma-exchange, high-dose cyclophosphamide and alemtuzumab.

As regards cold agglutinin disease, rituximab is now recommended as first-line treatment. Complement inhibitors may have a potential role in the treatment of complement-mediated severe forms of AIHA.

Autoimmune hemolytic anemia (AIHA) is a relatively uncommon and heterogeneous disorder caused by autoantibodies directed against red cell self-antigens, more frequent in adults than in children (estimated incidence 1-3 per $10^5$/year vs. 0.2 per $10^5$/year, overall prevalence 17:100,000). It can be idiopathic or secondary to lymphoproliferative syndromes, infections, immunodeficiency and tumors; it is also reported to occur following hematopoietic stem cell transplantation.[1,2] AIHA is commonly classified as "warm", "cold" (which includes cold hemagglutinin disease and paroxysmal cold hemoglobinuria) and "mixed" according to the thermal range of the autoantibody and the direct antiglobulin test (DAT) positivity. In warm AIHA, DAT is typically positive with anti-IgG antisera (and anti C3d in some cases); cold forms are usually due to IgM, and the DAT is positive for C3d, since IgM antibodies are often lost or only present in small amounts on the red blood cells at 37°C. Albeit the diagnosis is usually simple, atypical cases are reported with increasing frequency: about 10% of AIHA remains DAT negative, and the diagnosis is made after exclusion of other causes of hemolysis and on the basis of the clinical response to therapy.[1,3] These atypical cases (that also include "warm" IgM AIHA which are often fatal) may represent a critical diagnostic problem and cause delays in therapy.[4, 5]

AIHA may develop gradually or have a fulminant presentation with rapid onset of severe, life-threatening anemia. Although the extent of hemolysis depends on the autoantibody pathogenicity (class, thermal amplitude, affinity, efficiency in activating complement), the degree of anemia is also determined by the efficacy of the erythroblastic response: in fact, AIHA with reticulocytopenia (about 20% in adults) may often represent a clinical emergency with extremely high transfusion needs. A retrospective investigation on 308 cases with primary AIHA has shown that mixed and atypical forms are more frequently associated with a severe onset (hemoglobin ≤6 g/dL) along with reticulocytopenia.[6] The mortality rate of primary AIHA in adults is 11% in older series[1] and ~4% in a more recent large study;[6] in children, mortality rate is 4%, but rises to 10% if the hemolytic anemia is associated with immune thrombocytopenia (Evans syndrome).[7] Secondary cases are usually more severe, with an increased mortality, particularly in post-transplant forms. The treatment of this disease is still not evidence-based as there are no randomized studies and only a few prospective phase 2 trials.[8-10]

**Treatment of "warm" AIHA**

Corticosteroids represent the first-line treatment, although their use is based on experience rather than evidence, since published information on their effectiveness is scanty, being not supported by clinical trials.[1] Coritcosteroids, usually prednisone, should be given at the initial dose of 1-1.5 mg/kg/day for 1 to 3 weeks until hemoglobin levels greater than 10 g/dL are reached. After stabilization of hemoglobin, prednisone should be gradually and slowly tapered off at 10-15 mg weekly to a daily dose of 20-30 mg, then by 5 mg every 1-2 weeks until the dose of 15 mg, and subsequently by 2.5 mg every 2 weeks with the aim of withdrawing the drug.. AIHA patients should be treated for a minimum of 3 or 4 months with low doses of prednisone (≤10 mg/day):[1] in fact, patients receiving low dose of corticosteroids for more than 6 months have lower incidence of relapse and longer duration of remission than those discontinuing the medication within 6 months;[11] moreover, an earlier onset of steroid therapy correlates with lower probability of relapse.[12] In patients with particularly rapid hemolysis and very severe anemia, or complex cases such as Evans syndrome, intravenous methylprednisolone at a 100-200 mg/day for 10-14 days or 250 to 1000 mg/day for 1 to 3 days may be indicated. First-line therapy with corticosteroids provides a response in 70-85% of patients, however it is curative in 20-30% of cases only.[13,14] Patients unresponsive to first-line therapy, early relapsed, or requiring inacceptable high (more than 10 mg prednisone per day) and protracted steroid doses require second line therapy. These patients should undergo a diagnostic re-evaluation for a possible underlying disease, since AIHA associated with malignant tumors, benign ovarian teratomas, or with IgM warm autoantibodies are often steroid-refractory.[13]

Splenectomy is commonly considered the most effective second-line treatment of warm AIHA, albeit its efficacy has never been compared to that of other second-line approaches and the remission duration after surgery is not very clear. Splenectomy has an early response rate in ~70%, and a presumed curative effect in ~20% of cases.[13] It is performed in ~10-15% of both adult and pediatric cases, particularly in Evans syndrome.[1,4,7,15,16] The drawbacks of this option are the lack of reliable predictors of outcome, the associated surgical complications, and above all overwhelming sepsis, with a reported risk of 3-5% and a mortality rate up to 50% even after the

introduction of pre-operative vaccination against pneumococci, meningococci, and hemophilus.[17-20] The role and efficacy of antibiotic prophylaxis remains unclear, and it is not recommended by all investigators.[1,21] Splenectomy is associated with an increased risk of thromboembolism and pulmonary hypertension.[6,22-24] Thrombotic events occur in 10-15% of AIHA, independently from the presence of antiphospholipid antibodies whose role is still a matter of debate.[2,5,24] In older and small series 9% of patients with AIHA (all splenectomised) died from pulmonary embolism,[25] but more recent series do not entirely confirm these data.[6] In any case, we should be aware of the increased thrombotic risk, particularly in severe cases associated with intravascular hemolysis and/or splenectomised.

In the recent years rituximab is increasingly preferred among second line treatments (~60% of patients).[6,13,14] Several studies indicate that the drug is associated with an overall response rate of about 70-80% (half of cases complete responses), with a median duration of response of 1-2 years and a disease free survival of ~70% at one and ~55% at two years.[26-28] In a recent multicenter study the time to response was 1 month post-initiation of rituximab in 87.5% and 3 months in 12.5% of patients.[29] Although fewer patients treated with rituximab seem to maintain a long term response compared to splenectomy, most of them respond to additional courses of rituximab at relapse. A recent prospective randomized trial showed that ~70% of patients treated with glucocorticoids and rituximab were still in remission at 36 months, compared with ~45% of those treated with steroids alone,[8] strongly suggesting that rituximab combined with steroids as first-line treatment is superior to steroid monotherapy. Moreover, a prospective pilot study has shown that first line treatment with low-dose rituximab (100 mg weekly x 4) plus a short course of steroids compares favourably with conventional doses and has a steroid-sparing effect.[9,10] The drug has a well-established safety profile (infectious events in roughly 7%), although rare cases of progressive multifocal encephalopathy, mostly in onco-hematologic conditions, hepatitis B reactivation and other viral infections have been reported.[26,27] To prevent hepatitis B reactivation both after rituximab and prolonged steroid therapy antiviral prophylaxis is now recommended.[30] It is worth mentioning that a recent retrospective study has shown that the association of rituximab and bendamustine gave an overall response rate of 81% in 26 patients with LLC-associated AIHA.[31]

Conventional immunosuppressive drugs (such as azathioprine, cyclophosphamide, cyclosporine), were reported to have 40-60% response rates in the early literature, but subsequent analysis demonstrated that a response had been obtained in less than one-third of patients[1,13] Although their use may be associated with serious side effects, immunosuppressants are still used in the clinical practice,[6] and may be considered mostly as steroid-sparing agents when splenectomy is not feasible and/or rituximab unavailable. Mycofenolate has raised increasing interest because of proven effectiveness in ~90% of refractory immune cytopenias in children,[32] and in post-hematopoietic stem cell transplant AIHA together with rituximab,[33] although larger confirmatory studies are needed to give precise recommendations.

Concerning other treatment options, it is worth reminding danazol, with responses reported in older studies in ~40% of cases,[34,35] not confirmed in more recent series,[36] and intravenous immunoglobulins (IVIG), frequently used in children for their low incidence of adverse effects, with responses in about 40-50% of cases.[37] Finally, erythropoietin has been successfully used in patients with therapy-refractory AIHA, and may be indicated particularly in the presence of reticulocytopenia.[6,38]

As regards last option treatments for the few patients refractory to previous therapies, it is worth mentioning high dose cyclophosphamide (50 mg/kg/day for 4 days),[39] and alemtuzumab (particularly in CLL-associated AIHA), whose efficacy is anecdotical and needs further confirmatory studies.[40-42] The results reported in the literature about the efficacy of plasma-exchange are controversial, with favourable effects generally short-lived, so that it represents an "heroic or last–ditch efforts on behalf of a patient".[43,44] Information on hematopoietic stem cell transplantation (HSCT) is limited, this option being used in very severe and ultra-refractory cases (mostly Evans syndrome). The overall complete remission rate is approximately 60% in allogeneic and 50% in autologous HSCT, and a continuous remission is reported in 3/7 allogeneic and 1/7 autologous HSCT, with a transplant-related mortality (TRM) of approximately 15%.[45-47]

**Treatment of "cold" AIHA**

The decision to treat cold agglutinine disease (CAD) should be deserved to patients with symptomatic anemia, transfusion dependence, and/or disabling circulatory symptoms. In fact, non-severe asymptomatic forms may require only protection

against cold exposure, and occasional transfusion support during winter.[1,14,48,49]

Erythrocyte transfusions can safely be given in CAD, provided appropriate precautions; in particular, the patient and the extremity chosen for infusion should be kept warm, and the use of an in-line blood warmer is recommended.[1,15,50]

Steroids are effective in a small fraction of cases (14-35%) and usually at unacceptably high doses.[1,48,49,51,52] Therefore, this treatment, although still widely used in the clinical practice, is now discouraged. Concerning conventional cytotoxic immunosuppressive drugs, monotherapy with chlorambucil or cyclophosphamide has shown some beneficial effect in small series (16% of cases),[1,52,53] whereas no convincing responses were observed in the few patients treated with azathioprine,[52,54] interferon-α or low-dose cladribine.[55] Splenectomy is usually ineffective,[1,6,50] due to the fact that clearance of C3b-opsonized erythrocytes primarily occurs in the liver. Rituximab is now recommended as the first-line treatment of CAD,[50] although complete and sustained remissions are uncommon.[56] The drug is effective in ~60% of cases (5-10% CR), with a median time to response of 1-2 months, and a response duration of 1-2 years.[26,56-58] However, responses are observed following a second and even a third course in relapsed cases. Furthermore, combined treatment with rituximab and fludarabine orally (40 mg/m2 on days 1–5) resulted in higher response rates (76% of cases) and sustained remissions (estimated median response duration 6.5 years).[59] Since hematological toxicities and infective complications were common, this regimen is suggested for cases refractory to 1-2 courses of rituximab.[49]

As regards new experimental approaches, improvement of anemia has been observed in 2 patients following monotherapy with bortezomib,[60] and in 2 cases after administration of eculizumab (anti-C5 monoclonal antibody).[61,62] In a more recent series, eculizumab has been used in two patients refractory to five treatment lines and was effective in one, although the contribution of single drug to clinical remission was difficult to establish.[6] These observations however need confirmation in prospective trials. It is worth mentioning that TNT003, an inhibitor of the serine protease C1s, has been proven to prevent in vitro hemolysis induced by autoantibodies from a CAD patient.[63] Finally, plasmapheresis may be useful in acute hemolytic crisis and before surgery requiring hypothermia,[64,65] although its effect is transient.

Paroxysmal cold hemoglobinuria (PCH) is usually a self-resolving disease, although deaths have been reported;[66] the few severe cases may require transfusions and steroid treatment, whose effectiveness is difficult to evaluate because of the transient nature of the hemolysis.[1] Eculizumab was reported uneffective in a case of steroid-refractory PCH with associated myeloma.[67]

**"Mixed" AIHA**

About 7-8% of autoimmune hemolytic anemias have serologic findings characteristic of both warm AIHA and CAD (high-titer autoagglutinins with wide thermal amplitude), and are therefore classified as mixed forms.[1,6] Caution had been raised about this diagnosis, as sometimes it is made on the basis of inadequate serologic studies: Petz et al[1] reported that 35% of patients with warm AIHA (WAIHA) have cold agglutinins reactive at 20 °C, which were however clinically insignificant in almost all cases (only 5% reacted at 37 °C). Some authors suggest that patients with mixed AIHA have a more severe onset and more chronic course than patients with other categories of AIHA. In the GIMEMA study 8% of cases were mixed, and showed a severe clinical presentation and refractoriness to several therapy lines.[6]

**Supportive therapy**

Patients with AIHA may often require red blood cell (RBC) transfusion to achieve and/or maintain clinically acceptable hemoglobin values until specific treatments become effective. The decision to transfuse should depend not only on the hemoglobin level, but rather on the patient's clinical status and comorbidities (particularly ischemic heart or severe pulmonary disease), the acuteness of onset, the rapidity of progression of the anemia, the presence of hemoglobinuria or hemoglobinemia and other manifestations of severe hemolysis.[1,6] Blood transfusion should never be denied to patients in a critical clinical situation, even though no truly compatible units can be found since warm autoantibodies are frequently panreactive. ABO- and RhD-matched red cell concentrates can be anyway safely administered in urgent cases if alloantibodies (known to occur in 12%-40% of AIHA patients[1]) are reasonably excluded on the basis of the previous transfusion and/or pregnancy history. Some authors recommend ignoring the specificity of the autoantibody, and this indication has been demonstrated to be safe and effective in a great number of transfusions.[68,69] In less urgent

cases an extended phenotyping is advisable and the best compatible red cell units should be selected.[70] To minimize risks of febrile non-hemolytic reactions due to anti-leukocyte antibodies, leuko-depleted red cells are nowadays recommended in AIHA patients. As regards the volume to be transfused, it is worth reminding that overtransfusion (with an increased mass of RBCs available for destruction) should be avoided, particularly in elderly patients. Finally, RBCs should also be administered slowly, possibly not exceeding 1 ml/kg/h.[(1)]

## Conclusions

In conclusion, the therapeutic arsenal now available for steroid-refractory AIHA is certainly broader than in the past. Rituximab is undoubtedly the best option when splenectomy is contraindicated or refused. In primary warm AIHA the current opinion about the sequence of second-line treatment is splenectomy, rituximab, and thereafter any of the immunosuppressive drugs. However, in clinical practice rituximab is used with increasing frequency before splenectomy, particularly in most severe cases and children aged <5-6 years (www.AIEOP.org, Recommendations for the Management of AIHA in children). The therapeutic algorithm for warm AIHA adopted in our institution is given in **Figure 1**. As the experience with this drug evolves, it is likely that it will be recommended at an earlier point in therapy, before more toxic immunosuppressants, and in place of splenectomy in some cases or even as first-line treatment.[(71)] In any case, the choice of second-line therapies is still mostly determined by the physician personal experience, the availability of the new drugs, and the preference of the patient. Randomized clinical studies are needed to give precise recommendations on the best sequence and dosing of second-line (and hopefully third-line) therapies, based on efficacy and side effects. As regards CAD, rituximab is now recommended as first-line treatment. It is likely that complement inhibitors may have a role in selected cases with severe, life-threatening complement-mediated hemolysis.[(72.73)]

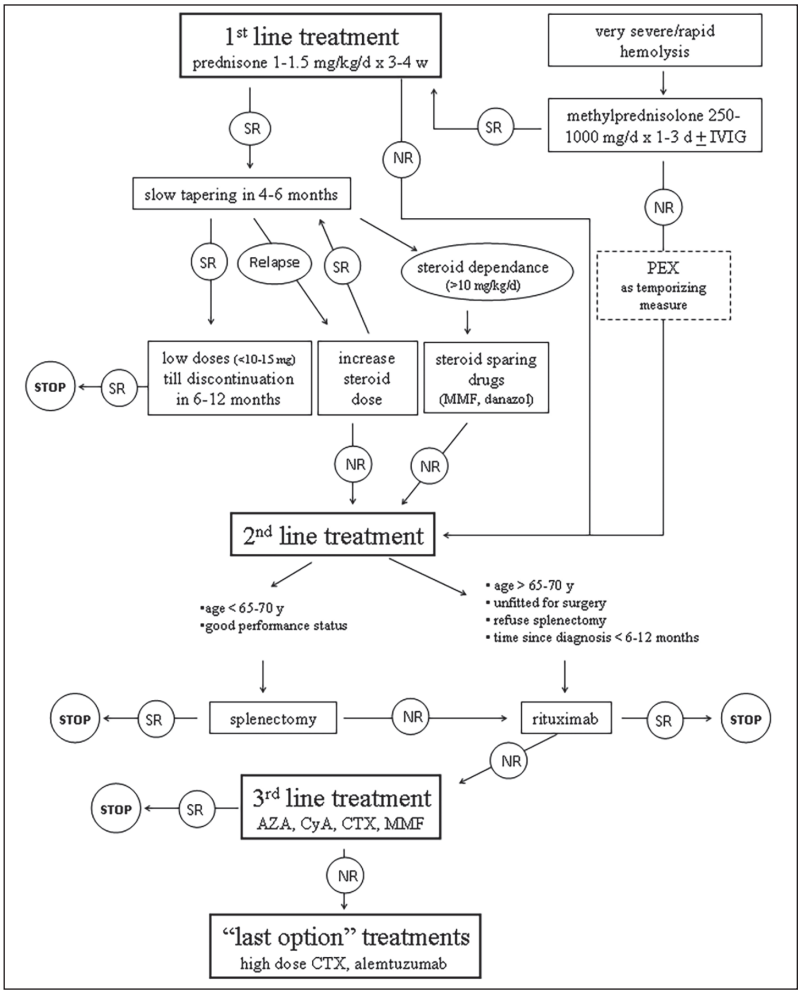


***Figure 1***
*Treatment algorithm for warm AIHA in adults.*
*Abbreviations used: SR, sustained response defined as maintenance of Hb values >10 g/dL over time;*
*NR, no response;*
*d, day; w, week;*
*y, year;*
*AZA, azathioprine;*
*CyA, cyclosporine A;*
*CTX, cyclophosphamide;*
*MMF, mycophenolate mofetil;*
*PEX, plasma exchange;*
*IVIG, intravenous immunoglobulin.*

**Declaración de conflictos de interés**
The authors have no conflict of interest and disclosures.

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