

Hemolytic Anemia Due to Warm Autoantibodies: New and Traditional Approaches to Treatment

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H&O What is the prevalence and pathogenesis of warm-antibody autoimmune hemolytic anemia (AHA)?

CP The annual incidence of warm-antibody AHA is 1 per 75,000 to 80,000 people.¹ It is predominantly a disease of the elderly, with peak incidence in those in their 70s.

In warm-antibody AHA, the patient's red blood cells (RBCs) are typically coated with IgG autoantibodies, with or without complement proteins. These coated RBCs are trapped by macrophages of the spleen (and sometimes by Kupffer cells in the liver), leading to the sphering, fragmentation, and ingestion of the antibody-coated RBCs.^{2,3} The macrophage has surface receptors for the Fc region of IgG—principally for IgG1 and IgG3—and surface receptors for opsonic fragments of C3. Interaction of a trapped RBC with splenic macrophages may result in phagocytosis of the entire cell, or more commonly, a part of the cell, resulting in the formation of spherocytes. In the latter case, membrane is lost in excess of contents, and the noningested portion of the RBC escapes the macrophage to assume a spherical shape. Direct complement-mediated hemolysis with hemoglobinuria is unusual in warm-antibody AHA.

Autoantibodies that mediate RBC destruction are mainly IgG globulins which bind with high affinity to RBC antigens at 37°C. They are usually specific for a single RBC membrane protein, most commonly an Rh-related epitope. This seeming specificity of autoreactivity suggests that the development of AHA is not caused by a generalized defect in immune regulation, but by an aberrant immune response to a self-antigen or to an immunogen that mimics a self-antigen.

We know that AHA autoantibodies are pathogenic. Labeled RBCs lacking the antigen targeted by the autoantibodies survive normally in patients with warm-antibody AHA.⁴⁻⁶ Transplacental passage of IgG anti-RBC autoantibodies from a mother with AHA to the fetus can induce hemolytic anemia in the fetus or the newborn baby.⁷ Lastly, there generally is an inverse relationship between the quantity of RBC-bound IgG antibody and RBC survival in a given patient.⁸⁻¹³

H&O What are the clinical manifestations and diagnosis of warm-antibody AHA?

CP Patients with warm-antibody AHA generally present with symptoms of anemia, but jaundice is sometimes the dominant symptom. Symptoms may be slow and insidious, or sudden and severe, at onset.

In patients with an underlying disease such as lupus or lymphoma (secondary AHA), its features may eclipse the hemolytic anemia. Splenomegaly is frequent, but modest in degree. In severe cases of acute onset, patients may present with fever, pallor, jaundice, hepatosplenomegaly, tachypnea, tachycardia, angina, or heart failure.

The severity of anemia may range from life-threatening to mild (ie, hematocrit levels below 10% or near normal). In these latter cases, the main laboratory features are an increased reticulocyte count and a positive direct antiglobulin test. Polychromasia on the blood film reflects the reticulocytosis; spherocytes are seen in patients with moderate to severe hemolytic anemia. Spherocytes in concert with a positive direct antiglobulin test (DAT) are characteristic of an immune hemolytic process. RBC fragments, nucleated RBCs, and occasionally erythrophagocytosis by monocytes, may be seen. Many patients exhibit mild leukocytosis and neutrophilia. Reticulocytosis is frequent but early in the presentation; about one-third of patients may have transient reticulocytopenia. Unconjugated hyperbilirubinemia is highly suggestive of hemolytic anemia. Usually, serum haptoglobin levels are low, and lactate dehydrogenase (LDH) levels are elevated.

H&O How should warm-antibody AHA be treated?

CP Glucocorticoids have been a mainstay of therapy for over 50 years. Improvement in hemolysis occurs in about two-thirds of patients. About 20% of treated patients achieve complete remission, but about 10% show no

response to glucocorticoids. Most patients are treated with oral prednisone at 60–100 mg daily. Critically ill patients with severe anemia may require intravenous methylprednisolone, 100–200 mg daily in divided doses. High doses of oral prednisone may be required for up to 2 weeks. When the hematocrit begins to increase, the prednisone dose may be decreased in stepwise fashion to approximately 30 mg per day. If improvement continues, the prednisone dose may be decreased further at a rate of 5 mg per day every week, to a dose of 15–20 mg per day. These lower doses are administered for 2–3 months after the acute hemolytic episode has subsided, after which the patient is weaned from the drug over 1–2 months or treatment is changed to an alternate-day schedule (eg, 20–40 mg every other day). Alternate-day therapy reduces glucocorticoid side effects but should not be attempted until the patient achieves stable remission on daily prednisone in the range of 15–20 mg per day. Therapy should not be stopped until DAT becomes negative.

Patients who require more than 15 mg of prednisone daily to maintain an acceptable hemoglobin concentration are candidates for splenectomy. About two-thirds of patients will have a partial or complete remission following splenectomy; however, the relapse rate is high. Patients who require further prednisone therapy after splenectomy often require a lower dose than what they required prior to the procedure.^{4,14,15} Alternate-day therapy is preferable to daily therapy in these cases.

Rituximab (Rituxan, Biogen Idec), a monoclonal antibody directed against the CD20 antigen on B-lymphocytes, has also been used in AHA. Rituximab eliminates B-lymphocytes, including those presumably making the autoantibodies to RBCs. In a large study,¹⁶ 13 of 15 children with warm-antibody AHA responded to rituximab 375 mg/m² weekly for 2–4 weeks. Findings from many other case reports and small series support the use of rituximab in adults, although the actual response rate is not known.¹⁷ Although rituximab appears to be safe and effective in AHA, it must be used with caution in light of its long-term effect on B-lymphocyte numbers, as well as recent reports of progressive multifocal leukoencephalopathy associated with its use in several patients.

Cytotoxic immunosuppressive therapy has been used to suppress the synthesis of autoantibodies in refractory cases of AHA, but direct evidence of such an effect is currently lacking. Beneficial responses to immunosuppressive drugs have been observed in some patients who did not respond to glucocorticoids.^{1,18}

The most successful approach used high-dose cyclophosphamide (50 mg/kg ideal body weight per day for 4 consecutive days with granulocyte colony stimulating factor support).¹⁹ Of 9 patients, 8 of whom had warm autoantibodies, all became transfusion independent. All patients had prolonged severe cytopenias and required hospitalization for a median of 21 days. For patients who

cannot tolerate prolonged cytopenias, cyclophosphamide 60 mg/m² or azathioprine 80 mg/m² may be given daily. It is reasonable to continue treatment for up to 6 months while waiting for a response. Both drugs suppress hematopoiesis; blood counts including reticulocyte counts must be monitored during therapy. Both agents increase the risk of subsequent neoplasia, and cyclophosphamide may cause severe hemorrhagic cystitis. Cyclosporin and mycophenolate mofetil have also been successfully used in a handful of patients.^{20–21} In general, immunosuppressive therapy should be reserved primarily for those patients who do not respond to glucocorticoids and splenectomy or for those patients who are poor surgical candidates.¹⁸

Plasmapheresis has been used to remove autoantibodies in patients with warm-antibody AHA. There have been reported improvements in a few cases, but its use is controversial.^{22,23} Since warm IgG autoantibodies are distributed with normal IgG in the extravascular space, it seems unlikely that plasmapheresis can remove antibodies efficiently enough to slow hemolysis.

Danazol—a nonvirilizing androgen—may be useful in patients with AHA, based on uncontrolled studies.^{24,25} Danazol may eliminate the need for splenectomy when combined with prednisone and may allow for a shorter duration of prednisone therapy.

H&O Are there any cautions that need to be taken for certain comorbidities?

CP The prognosis in secondary warm-antibody AHA (ie, in patients with an underlying disease) is largely dependent on the course of the underlying disease. The approach to management of warm autoantibody AHA in patients with lupus does not differ from that in patients with idiopathic warm-autoantibody AHA.

Warm-antibody AHA patients with chronic lymphocytic leukemia (CLL) or lymphoma respond well to the usual treatments employed in these lymphoproliferative disorders. The use of rituximab seems especially warranted in these patients, in combination with the usual chemotherapy drugs. Patients with CLL treated with the purine analogs fludarabine, pentostatin, or cladribine, sometimes develop autoimmune hemolysis.^{26,27} The hemolysis can be severe, sometimes fatal. Risk factors for autoimmune hemolysis include previous therapy with a purine analog, a positive DAT prior to therapy, and hypogammaglobulinemia. The risk of severe hemolysis is particularly high in patients who are rechallenged with a purine analog after a previous hemolytic episode.

Patients with lymphoproliferative disorders have long been recognized to develop AHA with increased frequency. More recently, it has been reported that patients with AHA develop lymphoproliferative disorders with increased frequency; in one study, 18% of patients did at a median of 26 months after onset of AHA.²⁸

Early investigators have reported an unusually high incidence of venous thrombosis, frequently fatal, in patients with AHA. There is a strong association between AHA, venous thrombosis, and antiphospholipid antibodies.²⁹ Therefore, until we have data to the contrary, lacking a contraindication to anticoagulation, it seems prudent to prophylactically anticoagulate patients with AHA and an antiphospholipid antibody.

H&O What are the approaches to selecting blood transfusion for patients with warm-antibody AHA?

CP Many patients develop anemia over a period sufficient enough to allow for cardiovascular compensation, and hence do not require RBC transfusions. However, RBC transfusions may be necessary and should not be withheld from a patient with an underlying disease complicating the anemia such as symptomatic coronary artery disease, or one who rapidly develops severe anemia with signs and/or symptoms of circulatory failure. Transfusion of RBCs in immune hemolytic anemia presents 2 difficulties: one is the problem of cross-matching, and the other is the short half-life of the transfused RBCs. It is nearly always impossible to find truly serocompatible donor blood, except in rare cases when the autoantibody is specific for a defined blood group antigen. It is therefore important to carefully test the patient's serum for an alloantibody that could cause a severe hemolytic transfusion reaction against donor RBCs, especially in patients with a history of pregnancy or prior transfusion.³⁰ For patients with an incompatible crossmatch, it is important to infuse blood slowly while watching carefully for evidence of an acute hemolytic transfusion reaction such as fever, chills, dyspnea, or pain in the chest or back. The transfusion should be discontinued immediately in the face of these symptoms or signs. Consultation between the clinician and the blood bank physician is useful.

H&O What avenue of research do you think is necessary for finding better treatment approaches for patients with warm-antibody AHA?

CP AHA is a rare disease, and hence clinical trials to test new treatments will necessarily be multi-institutional. Treatments directed at receptor function (eg, prednisone, splenectomy) or antibody formation (prednisone, splenectomy or rituximab) will undoubtedly be replaced in the future by novel agents whose activity is directed at the molecular defect leading to autoimmunity.

References

- Petz, L, Garratty, G. Immune hemolytic anemias. Churchill Livingstone, New York, 2004.
- Abramson N, LoBuglio AF, Jandl JH, Cotran RS. The interaction between human monocytes and red cells: Binding characteristics. *J Exp Med.* 1970;132:1191-1206.

- LoBuglio AF, Cotran RS, Jandl JH. Red cells coated with immunoglobulin G: binding and sphering by mononuclear cells in man. *Science.* 1967;158:1582-1585.
- Dacie JV: The Haemolytic Anaemias, vol 3, *The Autoimmune Haemolytic Anaemias*, 3d ed. Churchill Livingstone, New York, 1992.
- Mollison PL: Measurement of survival and destruction of red cells in haemolytic syndromes. *Br Med Bull.* 1959;15:59-67.
- Holländer L: Study of the erythrocyte survival time in a case of acquired haemolytic anaemia. *Vox Sang.* 1954;4:164-165.
- Chaplin H, Cohen R, Bloomberg G, et al. Pregnancy and idiopathic autoimmune haemolytic anaemia: A prospective study during 6 months gestation and 3 months post-partum. *Br J Haematol.* 1973;24:219-229.
- Mollison PL, Crome P, Hughes-Jones NC, Rochna E. Rate of removal from the circulation of red cells sensitized with different amounts of antibody. *Br J Haematol.* 1965;11:461-470.
- Mollison PL, Hughes-Jones NC. Clearance of Rh-positive red cells by low concentration of Rh antibody. *Immunology.* 1967;12:63-73.
- Rosse WF: Quantitative immunology of immune hemolytic anemia: II. The relationship of cell-bound antibody to hemolysis and the effect of treatment. *J Clin Invest.* 1971;50:734-743.
- Schreiber AD, Frank MM: Role of antibody and complement in the immune clearance and destruction of erythrocytes: I. In vivo effects of IgG and IgM complement-fixing sites. *J Clin Invest.* 1972;51:575-582.
- Atkinson JP, Schreiber AD, Frank MM: Effects of corticosteroids and splenectomy on the immune clearance and destruction of erythrocytes. *J Clin Invest.* 1973;52:1509-1517.
- Atkinson JP, Frank MM: Complement independent clearance of IgG sensitized erythrocytes: Inhibition by cortisone. *Blood* 1974;44:629-637.
- Eyster ME, Jenkins DE Jr: Erythrocyte coating substances in patients with positive direct antiglobulin reactions: Correlation of γ G globulin and complement coating with underlying diseases, overt hemolysis and response to therapy. *Am J Med* 1969;46:360-371.
- Allgood JW, Chaplin H Jr: Idiopathic acquired autoimmune hemolytic anemia: A review of forty-seven cases treated from 1955 to 1965. *Am J Med* 1967;43:254-273.
- Zecca M, Nobili B, Ramenghi U, et al: Rituximab for the treatment of refractory autoimmune hemolytic anemia in children. *Blood.* 2003;101:3857-3861.
- D'Arena G, Califano C, Annunziata M, et al. Rituximab for warm-type idiopathic autoimmune hemolytic anemia: a retrospective study of 11 adult patients. *Eur J Haematol.* 2007;79:53-58.
- Murphy S, LoBuglio AF: Drug therapy of autoimmune hemolytic anemia. *Semin Hematol* 1976;13:323-334.
- Moyo VM, Smith D, Brodsky I, et al: High-dose cyclophosphamide for refractory autoimmune hemolytic anemia. *Blood.* 2002;100:704-706.
- Emilia G, Messori C, Longi, Bertesi M. Long-term salvage treatment by cyclosporin in refractory autoimmune haematological disorders. *Br J Haematol.* 1996;93:341-344.
- Howard J, Hoffbrand AV, Prentice HG, Mehta A. Mycophenolate mofetil for the treatment of refractory auto-immune haemolytic anaemia and auto-immune thrombocytopenia purpura. *Br J Haematol.* 2002;117:712-715.
- Shumak KH, Rock GA: Therapeutic plasma exchange. *N Engl J Med* 1984;310:762-771.
- Council Report: Current status of therapeutic plasmapheresis and related techniques. *JAMA* 1985;253:819-825.
- Ahn YS, Harrington WJ, Mylvaganam R, et al: Danazol therapy for autoimmune hemolytic anemia. *Ann Intern Med* 1985;102:298-301.
- Pignon J-M, Poirson E, Rochant H: Danazol in autoimmune haemolytic anaemia. *Br J Haematol* 1993;83:343-345.
- Weiss R, Freiman J, Kweder SL, Diehl LF, Byrd JC. Hemolytic anemia after fludarabine therapy for chronic lymphocytic leukemia. *J Clin Oncol.* 1998; 16:1885-1889.
- Chasty RC, Myint H, Oscier DG, et al: Autoimmune haemolysis in patients with B-CLL treated with chlorodeoxyadenosine (CDA). *Leuk Lymphoma* 1998; 29:391-398.
- Sallah S, Wan JY, Hanrahan LR. Future development of lymphoproliferative disorders in patients with autoimmune hemolytic anemia. *Clin Cancer Res.* 2001;7:791-794.
- Hendrick AM. Auto-immune haemolytic anaemia—a high-risk disorder for thromboembolism? *Hematology.* 2003;8:53-56.
- Petz LD. A physician's guide to transfusion in autoimmune hemolytic anaemia. *Br J Haematol.* 2004;124:712-716.