

Sustained response to rituximab of autoimmune hemolytic anemia associated with antiphospholipid syndrome

Standard treatment for autoimmune hemolytic anemia (AIHA) due to warm antibodies includes combinations of glucocorticoids, immunosuppressive drugs (mainly azathioprine) and splenectomy. Patients who are refractory or intolerant to these therapies constitute an important therapeutic challenge. Rituximab, an anti-CD20 chimeric monoclonal antibody, can effectively deplete B-cells and is commonly used in B-cell non-Hodgkin lymphoma. In addition, it is being increasingly used in autoimmune disorders, such as idiopathic thrombocytopenic purpura, AIHA, systemic lupus erythematosus or vasculitis. We report a case of warm AIHA associated to primary antiphospholipid syndrome (APS). The patient was refractory to high-dose corticosteroids. Splenectomy was discarded in view of the high risk of thrombotic and/or hemorrhagic perioperative complications, due to the presence of APS. After treatment with four weekly doses of rituximab the patients had a rapid and sustained response which allowed progressive tapering of prednisone dose to 5 mg/d. In addition, IgM anticardiolipin titres decreased from > 600 MPL to < 100 MPL. Thirteen further cases of warm AIHA in adults treated with rituximab have been reviewed, showing excellent tolerance and high response rates. Rituximab may be considered prior to splenectomy in patients with refractory AIHA and high risk of complications following splenectomy.

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Autoimmune hemolytic anemia (AIHA) results from the production of autoantibodies directed to membrane antigens of red blood cells, leading to their premature destruction. In most types of AIHA, antibody and/or complement coated erythrocytes are removed from circulation by splenic and hepatic macrophages. The established treatment of AIHA consists of combinations of corticosteroids, other immunosuppressive drugs (mainly azathioprine), intravenous immunoglobulins and/or splenectomy.¹ When conventional treatments fail, there are anecdotal experiences with other therapies.

Rituximab is a chimeric human-murine monoclonal antibody specific for CD20 antigen, which is present on the surface of B-lymphocytes.² Rituximab is mainly used to treat patients with non-Hodgkin lymphoma. However, due to the selective depletion of B cells obtained, rituximab has also been proposed for several antibody-mediated autoimmune disorders.^{3,4} In this article, we report the case of a patient with antiphospholipid syndrome (APS)-related AIHA refractory to corticosteroids and successfully treated with rituximab. A literature review was performed limited to non-pediatric patients with AIHA due to warm antibodies. Those with underlying lymphoproliferative disorders were also excluded due to the different therapeutic approach in this group.

Case report

A 52 year old white woman was diagnosed in 1996 with microscopic polyangiitis, which progressed to end-stage renal failure. A kidney transplantation was uneventfully performed in 1999. In 2001 she had a left

hemispheric stroke, being diagnosed of primary APS after several positive determinations for anticardiolipin antibodies (aCL, IgM isotype, always higher than 600 MPL) and for lupus anticoagulant (LA). Antinuclear antibodies were always negative and clinical features of systemic lupus erythematosus were not present.

In June 2003 she was admitted because of fatigue and dyspnea. Treatment at the time of admission included low-dose prednisone (5 mg/d), tacrolimus, warfarin, and propranolol. Physical examination was normal. Laboratory investigations revealed a severe normocytic anemia, with hemoglobin levels of 6.3 g/dL and mean corpuscular volume of 94 fL. Reticulocyte count was 299000/ μ L. Platelet count was 242,000/ μ L. Biochemical evidence of hemolysis was supported by the following results: indirect bilirubin 1.8 mg/dL, lactate dehydrogenase 1508 IU/L and haptoglobin <24 mg/dL. Tests for hemoglobinuria were negative. Direct Coombs test was positive for IgA and C3d, confirming the autoimmune origin of the hemolysis.

Treatment with oral prednisone was started at increasing doses, up to 90 mg/24h due to lack of response. The patient required transfusion of 10 units of packed red blood cells. Three pulses of 250 mg methylprednisolone were given on consecutive days with no effect. Since the patient was already on immunosuppressive drugs (tacrolimus), splenectomy was the next logical step. However, the diagnosis of APS treated with oral anticoagulation, with previous stroke and very high titre-aCL plus LA, put the patient at high risk of perioperative thrombotic and/or hemorrhagic complications.

After obtaining informed consent, rituximab was started at a dose of 375 mg/m² once weekly for a total of 4 doses. Treatment was well tolerated with no side effects. Direct Coombs test soon became negative, hemoglobin levels raised to 9 g/dL and reticulocyte count decreased (see Figure 1). This response allowed the reduction of prednisone dose and the cessation of red cell transfusions soon after the first rituximab infusion. The patient was discharged after the fourth rituximab dose on 30 mg/d of prednisone. Ten months later, in June 2004, direct Coombs test remained negative and hemoglobin levels were 13.2 g/dL. The dose of prednisone had already been tapered to 5 mg/d, the lowest dose needed to prevent allograft rejection.

Discussion

AIHA can be idiopathic or secondary to several conditions such as lymphoproliferative disorders, autoimmune diseases, infections, primary immunodeficiencies and tumors.¹ Specifically, AIHA is one of the recognised clinical features of APS. In a recent multicentric series of 1000 patients with APS, 6.6% had AIHA.⁵ Warm antibodies are responsible for 48-76% of AIHA cases. Spleen is the site of destruction of erythrocytes in warm AIHA. These patients usually respond to treatment with glucocorticoids, immunosuppressive drugs and/or splenectomy.¹ Those who are refractory or intolerant to the initial therapeutic regime have usually a poor prognosis in terms of controlling hemolysis.

Rituximab is a chimeric murine/human IgG1/ κ monoclonal antibody specific for human CD20 antigen, which is present on the surface of B lymphocytes. The clearance of B cells is mediated by different mechanisms, such as complement-mediated cytotoxicity, antibody-dependent cytotoxicity, inhibition of B-cell proliferation, and induction of apoptosis.^{2,4} Rituximab can effectively deplete CD20-positive B lymphocytes for more than 6-9

Table 1. Main clinical features of adult patients with warm autoimmune hemolytic anemia treated with rituximab.

Author/ year (ref)	Sex	Age	Associated diseases	Treatments received before rituximab	Lowest Hb levels (gr/dl)	Final Hb levels (gr/dl)	Adverse effects	Last steroid dose	Response to rituximab	Duration of response
Ahrens 2001 (9)	Male	68	None	Prednisolone, azathioprine, cyclophosphamide, mycophenolate-mofetil, pulsed high-dose dexamethasone	8.4	12.3	Minor chills	NR	Response	6 months
Perrotta 2001 (10)	Female	25	SLE	Methylprednisolone, immunoglobulins, cyclosporine A.	3	14	No	Withdrawn	Response	7 months
Abdel- Raheem 2001 (11)	Male	60	Renal cell carcinoma treated with interleukin 2, Evans syndrome	Prednisone, vinblastine, cyclosporine A, 6-mercaptopurine, danazol, splenectomy, intravenous immunoglobulins, intravenous cyclophosphamide	6.1	11	No	NR***	Response	11 weeks
Zaja 2002 (12)	Male	53	None	Prednisone, azathioprine	6.1	NR	No	1 mg/Kg/d	No response	
Morselli 2002 (13)	Female	68	Cold AIHA	Prednisone	6.4	13.5	No	Withdrawn	Response	7 months
Besalduch 2003 (14)	Female	21	SLE, autologous stem cell transplantation	Prednisone, cyclophosphamide	NR	NR	No	10 mg/d	Response	NR
Shanafelt 2003 (15)*	Male	42	DVT/PE	Prednisone, methylprednisolone, intravenous immunoglobulins, splenectomy, cyclophosphamide/ vincristine/prednisone, methotrexate, danazol, cyclosporin.	5.1	14.6	No	NR	No response	
	Male	25	Hypogamma- globulinemia, chronic renal failure	Prednisone, intravenous immunoglobulins	5.8	8.8	No	NR	Death (lung haemorrhage)	
	Male	39	Acute renal failure, sinusitis, endocarditis	Prednisone, methylprednisolone, intravenous immunoglobulins, vincristine, plasma exchange	6.6	11.6	No	Withdrawn	Response	13 months
	Female	79	Hepatocellular carcinoma, hypothyroidism, SS, SC, pulmonary hypertension	Prednisone, intravenous immunoglobulins	6.6	8.9	No	NR	Death (hepato-cellular carcinoma)	
	Female	21	IBD, diabetes, SC, relapsing polychondritis	Prednisone, azathioprine, cyclophosphamide	8.3	11.5	No	Withdrawn	Response	4 months
Galor 2003 (16)**	Male	43	Evans syndrome	Prednisone, splenectomy, intravenous immunoglobulins, plasmapheresis, staphylococcal Protein A immunoabsorption, cyclophosphamide, vincristine	NR**	NR**	No	NR**	Response	9 months
Webster 2004 (17)	Male	71	Cold AIHA	Prednisone, oral cyclophosphamide	6.4	9.0	No	Withdrawn	Response	9 months
Present case	Female	51	Primary APS renal transplantation	Prednisone, methyl-prednisolone, tacrolimus	6.3	13.3	No	5 mg/d	Response	10 months

Hb: hemoglobin; SLE: systemic lupus erythematosus; CLL: chronic lymphocytic leukemia; DVT/PE: deep venous thrombosis/pulmonary embolism; SS: Sjögren syndrome; SC: sclerosing cholangitis; IBD: inflammatory bowel disease; ITP: idiopathic thrombocytopenic purpura; APS: antiphospholipid syndrome; NR: not reported * Four additional patients with Evans syndrome treated with rituximab who were reported in this series were not included in our review due to insufficient clinical data. ** Only abstract available ***Maintenance treatment with intravenous immunoglobulins and blood transfusions after response to rituximab

months.² Therefore, this monoclonal antibody offers a novel therapeutic approach to a variety of antibody-mediated autoimmune diseases.^{3,4} Published reports include patients with idiopathic thrombocytopenic purpura, AIHA, systemic lupus erythematosus, mixed essential cryoglobulinemia, Goodpasture's syndrome, and Wegener's granulomatosis.^{4,6-8}

Treatment with rituximab does not affect mature plasma cells nor memory B cells, both of which lack CD20 on their surface.⁴ This fact may explain why rituximab is not associated with a high incidence of common or opportunistic infections.⁴ On the other hand, unaffected plasma cells may continue to produce pathogenic autoantibodies leading to therapeutic failure.

Thirteen non-pediatric patients with warm AIHA non-related to lymphoproliferative disorders and treated with rituximab have been reported thus far.⁹⁻¹⁷ (Table 1). One of the papers could only be retrieved in abstract form.¹⁶ Two patients had warm AIHA and autoimmune thrombocytopenia, or Evans syndrome^{11,16} and two presented with AIHA with combined warm and cold antibodies.^{13,17} All patients were refractory to the initial therapeutic regimens, which included corticosteroids, immunosuppressive drugs, danazol, intravenous immunoglobulins, plasma exchange and/or splenectomy. Responses to rituximab were variable. Response was achieved in 9 patients, allowing the progressive tapering of corticosteroids to a low maintenance dose or complete withdrawal.^{9-11, 13, 14, 16, 17} On the other hand, two patients were resistant to rituximab, which meant the impossibility of reducing steroid dosage.^{12, 15} Two additional patients died of complications unrelated to AIHA. It is remarkable that all treatments were well tolerated, with no side effects except for minor chills during the first infusion in one case⁹ and, specifically, no infectious complications reported during follow-up.⁹⁻¹⁷

Regarding our case, it was a noteworthy finding that titres of IgM aCL, which were permanently above 600 MPL before the hemolytic event, decreased after treatment with rituximab and have remained since below 100 MPL. However, we still do not know the clinical implications of this reduction of aCL levels.

In conclusion, rituximab seems to be an effective and safe treatment for patients with AIHA, even if unrelated to lymphoproliferative disorders. Its use could be indicated prior to splenectomy in those patients at high risk for perioperative complications such as those with APS.

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