SEVERE/REFRACTORY AUTOIMMUNE HEMOLYTİC ANEIMIA İN AN INFANT SUCCESSFULLY TREATED WITH RITUXIMAB

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Abstract

Autoimmune hemolytic anemia (AIHA) is a rare condition in children which differs from the adult form. It is defined by immune-mediated destruction of red blood cells caused by autoantibodies. Treatment depends on the type of AIHA, its severity and whether there is an underlying disorder. Rituximab is now the preferred second-line treatment for primary warm autoimmune hemolytic anemia (Waiha). Here, we present a 2-monthboy with wAIHA, resistant to steroids and cyclosporin A, who was successfully treated with rituximab. Our patient is one of the youngest cases in the literature.

Abbreviations	Abbreviations
AIHA	Autoimmune hemolytic anemia
SLE	Systemic lupus erythematosus
WBC	white blood cell
RBC	red blood cell
Hb	hemoglobin
Htc	hematocrit
MCV	mean corpuscular volume
MCH	mean corpuscular hemoglobin
SGOT	serum glutamic-oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
GGT	Gamma-glutamyl transferase
LDH	lactic dehydrogenase
DAT	direct antiglobulin test
IVIG	intravenous immunoglobulin
DNT	Double negative t cell
CsA	Cyclosporine-A
CMV	cytomegalovirus
PCR	polymerase chain reaction
APC	antigen-presenting cells
ORR	overall response rate
RFS	relapse-free survival

Severe/Refractory Autoimmune Hemolytic Anemia in an Infant successfully treated with Rituximab

Introduction

Autoimmune hemolytic anemia (AIHA) in childhood is an uncommon condition caused by the presence of auto-antibodies directed against antigens on the surface of red blood cells, leading to premature destruction of the cells (1). The overall annual incidence is reported to be 1-3 cases/100,000 people and approximately 0.2 cases/1,000,000 individuals under 20 years of age. Within the pediatric population, AIHA is primarily a disease of young children,10,11 with a median age of diagnosis of 3.8 years in a recent national observational study (1-4). In infancy, it is mostly due to viral and bacterial infections. However, in teenagers and young adults, there is an increased association with an underlying systemic illness, most commonly immunodeficiencies and autoimmune disorders (eg, collagen vascular disorders, systemic lupus erythematosus [SLE]) (5). Corticosteroids are the mainstay of treatment for persons with newly diagnosed primary idiopathic warm AIHA. The majority of children with W-AIHA are corticosteroid-responsive with published response rates from 50-80% in larger series but with an estimated cure rate in 20–30% only (6-8). Most published guidelines recommend rituximab as the agent of choice for corticosteroid refractory patients or for children unable to wean off corticosteroids (9). The patients aged less than one year have better response to rituximab than those over one year (10).

Here, we present a 2-month-male with w-AİHA; resistant to steroid and cyclosporin A, who was successfully treated with rituximab. Our patient is one of the youngest cases in the literature.

Case report:

The healthy and term-born patient was admitted to a hospital on the postnatal 61^{st} day with complaints of skin turning yellow, darker urine, and impaired sucking. His physical examination revealed pale and slightly icteric appearance on the skin and sclera, while other system examination findings were normal. In the lab examination, namely complete blood count: WBC 9930/ml, erythrocyte 1.53 million/ml, Hb 4.7 g/dL, Htc 13.6%, MCV 88.3 fl, MCH 30.5 pg, platelet 354.000/ml, in blood biochemistry: SGOT, SGPT, GGT normal, LDH 325 U/L, total bilirubin 11.9 mg/dL, direct bilirubin 0.6 mg/dL, indirect bilirubin 11.3 mg/dl, direct antiglobulin test (DAT) IgG 3 positive, DAT C3 negative, Ig G, M, A were within normal limits. With the positive data obtained, the patient was diagnosed with AIHA and the treatment was started. Pulse steroid and intravenous immunoglobulin (IVIG) were given for ten days. As the patient's erythrocyte transfusion requirement was every 2 days, plasmapheresis was applied for 2 days. The patient was referred to our center after 2 months and 20 days for further examination and treatment.

There is no important detail in his history. In the family history; he was born from consanguineous parents, his mother was healthy, his father was treated with the diagnosis of Multiple Sclerosis and he was carrying a thalassemia trait gene. On patient's physical examination; fever 36^{o}, pulse 136 / min, respiratory rate 38 / min, blood pressure 112/72 mmHg, pale skin, slightly icteric, pale conjunctiva and oral mucosa, lymphadenopathy. Respiratory and cardiovascular systems were normal. In the abdominal examination, there was 3 cm hepatomegaly, and splenomegaly (Traube's space was positive); neurological, and other system examination findings were normal. In the lab. examinations made in our hospital; complete blood count: WBC 5.470/ml, erythrocyte 2.59 million/ml, Hb 8 g/dL, Htc 22.2%, MCV 85.7 fl, MCH 30.9 pg, platelet 295.000/ml, in blood biochemistry: SGOT, SGPT, GGT normal, LDH 418 U/L (N: 120-300), albumin 3.8 g/dL, total bilirubin 2.3 mg/dL, direct bilirubin 0.7 mg/dL, DAT Ig G and C3 negative, antinuclear antibody negative, anti-ds DNA negative, B12 and folic acid levels were normal, other biochemical tests were normal. No abnormality was detected in lymphocyte flowcytometry. The ratio of double-negative T cells (alpha + beta + CD4- CD8- T cell; DNT) was determined as <1% by flowcytometry. The reticulocyte was 265 thousand/µl (N: 0.046bin-0.112x106), haptoglobin <10 mg/dL (N: 30-200 mg/dL). Fecal occult blood was negative, urine analysis is urobilinogen negative. In blood smear; erythrocytes had anisocytosis, poikilocytosis, polychromasia; spherocytes and schistocytes were not observed. Bone marrow aspiration and biopsy were performed. Bone marrow was heterogeneous, hypercellular, myeloid/ erythroid ratio was 1/1 due to

erythroid activity. The patient's positive DAT from the previous hospital, positive hemolysis parameters and clinical findings confirmed AIHA diagnosis. After excluding secondary causes (collagen tissue diseases; hereditary immune deficiencies; lymphoproliferative diseases; infections), methylprednisolone treatment at a dose of 20 mg/kg/day was given for 3 days. IVIG was given for 3 days at 1 g/kg/day. Then, the dose of methylprednisolone was reduced to 3 mg/kg/day and continued for 12 days in total. However, as the decrease in Hb levels continued, CsA was added to the treatment and 1 g/kg/g IVIG was given. Methylprednisolone dose was increased to 20 mg/kg/day again and continued for 3 days at the same dose. Then the dose was reduced to 2 mg/kg/day. Since the CMV antigenemia was positive, which is common for the patients who were given steroids and/or immunosuppressant drugs, ganciclovir was started. 375 mg/m^2 rituximab was added to the current treatment of the patient, whose erythrocyte transfusion rate persists approximately every 4 days, and CMV positivity developed during steroid use. Plasmapheresis was applied for a total of 3 times, every other day. The dose of methylprednisolone was gradually reduced to 1 mg/kg/day and then to 0.5 mg/kg/day. Meanwhile, a total of four doses of rituximab (375 mg/m^2/dose) were administered once a week; there was no need for erythrocyte transfusion after the third dose and an increase in Hb levels was observed. The patient was discharged with oral methylprednisolone (4 mg/day) and called for outpatient control. WBC 5980/ml, erythrocyte 2.71 million/ml, Hb 8.1 g/dL, Htc 23.3%, platelet 497.000/ml, reticulocyte 107 thousand/µl, DAT were negative in the control. (Figure 1).

Hb level increased up to 13.1 g/dl during follow-up. The patient had to take IVIG every 3-4 weeks for secondary hypogammaglobulinemia, which is thought to develop due to the rituximab used in the patient. During the follow-up, cyclosporine treatment was discontinued, the disease recurred a year later, but responded well to short-term steroid therapy. He is now 24 months old and healthy.

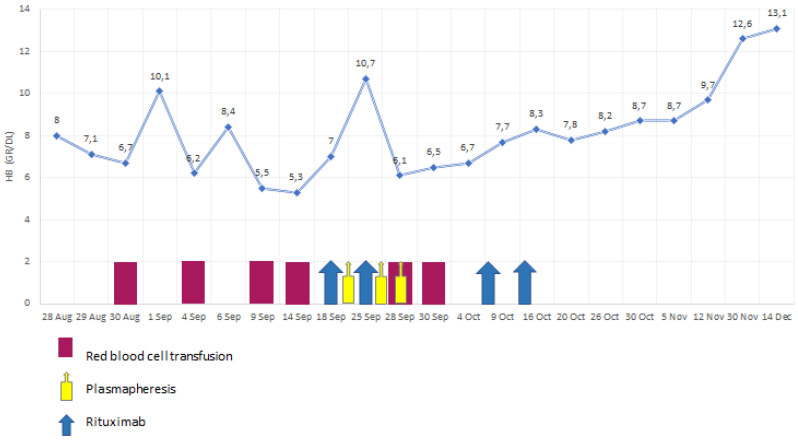

Figure 1: Treatment process and clinical course of the patient.

Discsusion:

Autoimmune hemolytic anemia (AIHA) is an acquired and highly heterogeneous disease, caused by the increased destruction of red blood cells (RBCs) by anti-RBC autoantibodies with or without complement activation. Traditional treatment strategies for autoimmune AIHA [glucocorticoids, intravenous immune globulin (IVIG)] inhibit uptake of antibody coated hematopoietic cells by phagocytes or decrease antibody production. Corticosteroids are used as first line treatment. Second line treatment often involves immunosuppression to reduce production of the antibody, for example rituximab. An alternative to medication is splenectomy, since the spleen is a major site of red cell destruction (10-13).

Adverse effects of prolonged corticosteroid use should be considered. These adverse effects include irritability or even psychoses, sleep disturbances, hypertension, hyperglycemia that may require insulin therapy, weight

gain, impaired longitudinal growth, and osteoporosis. Patients who are treated with prolonged courses of glucocorticoids should also take calcium and vitamin D supplements and be screened for osteopenia or osteoporosis if their diet and lifestyle place them at particular risk for these conditions. Although it has been well established that large doses of corticosteroids are a prominent risk factor for CMV infection in the immunocompromised population, it is unavoidable in most patients, as they need continued corticosteroid therapy to control their underlying diseases (12). After the pulse dose steroid treatment, the number of CMV PCR copies of the patient increased to 43.056 copies/mL. Both ganciclovir treatment and rituximab treatment were started; simultaneously steroid dose was reduced. The number of CMV PCR copies became negative after 4 weeks treatment.

Plasma exchange (therapeutic apheresis) can be used to control severe symptoms by temporarily lowering the titer of cold agglutinins, but it has no effect on the underlying disease. The rationale of using plasma exchange is to remove circulating immune complexes, complement activated components and circulating auto-antibodies. It is estimated that each cycle can remove up to 65% of the circulating autoantibodies, so, it is frequently necessary to repeat the procedure. Plasma exchange is an option that should be considered only for extremely severe cases of AIHA, with no response to either transfusion or pharmacological therapy (14). Plasma exchange was performed to our patient due to treatment-resistant disease and the need for frequent transfusions. However, there was no obvious response.

It is well known that B lymphocytes are the main effectors of humoral immunity by secreting antibodies, but act also as antigen-presenting cells (APCs), produce immunoregulatory cytokines, and express surface co-stimulatory molecules. Rituximab is a chimeric human IgG1/K monoclonal antibody specific for the CD20 antigen, which is expressed on B lymphocytes. It induces rapid in vivo depletion of both normal B lymphocytes and lymphoma B cells. Its mechanism of action includes complement-mediated cytotoxicity, inhibition of B-cell proliferation, and induction of apoptosis (15,16). Rituximab, is effective in both idiopathic and secondary forms of AIHA, including those associated with autoimmune and lymphoproliferative disorders, and BM transplant. As reported in several studies and in a recent meta-analysis (16), the overall response rate (ORR) in w-AIHA is ˜80%, with a relapse free survival of ˜60% at 3 years, and a median time to response of approximately 3-6weeks (range 2-16). Rituximab has a good safety profile and re-treatment is also effective, although the risk of immunosuppression should be kept in mind, prompting adequate prophylaxis of viral infections reactivation. The drug has also been used in first line or as early second line together with steroids, particularly in severe wAIHA cases (17,18,20).

There are a few studies in the literature involving a large number of pediatric patients with AIHA treated with ritixumab. In the literature review, including seven case series, a small percentage (14 cases) of the total (132 cases) patients were under 1 year of age (10,11,13,21-23). Childhood autoimmune hemolytic anemia requires second-line immunosuppressive therapy in 30–50% of cases. Sixty-one children were given rituximab between 2000 and 2014. The median interval from diagnosis to rituximab was 9.9 (interquartile range 1,6–28,5) months. Forty-six patients responded (75%) and the 6-year relapse-free survival (RFS) was 48%. Ten out of 61 patients were infants, seven of who responded with a 6-year RFS of 71% (10).

Our case is 2 months old infant; therefore, he is one of the smallest cases in the literature. During the previous immunosuppressive treatments, we could not provide the desired response to AIHA furthermore had to give antiviral therapy due to CMV reactivation. Since the third dose of Ritixumab, our patient did not need transfusion. We did not observe CMV reactivation.

Given the high degree of efficacy of rituximab in treating refractory AIHA in pediatric patients, early use should be considered, and its use should also be considered for patients who respond to steroids but have significant adverse effects. Patients taking corticosteroids before the initiation of rituximab should continue to take steroids until a response to rituximab is clearly established. Although published data that compare rituximab or splenectomy as a second-line therapy are lacking, the trend has been to use rituximab first (12).

In conclusion Our case is 2 months old infant, one of the smallest cases in the literature. Rituximab was well tolerated and provided durable responses in pediatric patients with refractory or persistent autoimmune

hemolytic anemia especially in infantile period. Extending immunosuppressive therapies, especially steroids, can lead to the reactivation of viral infections (especially CMV), so it should not be too late for Ritixumab treatment.

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