

ORIGINAL ARTICLE

Iran J Allergy Asthma Immunol

October 2025; 24(5):593-606.

DOI: [10.18502/ijaa.v24i5.19743](https://doi.org/10.18502/ijaa.v24i5.19743)

A Report on the Clinical Efficacy of Rituximab Administration in Patients with Inborn Errors of Immunity and Autoimmune/Autoinflammatory Manifestations

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Received: 14 April 2024; Received in revised form: 19 December 2024; Accepted: 10 February 2025

ABSTRACT

It can sometimes be very difficult to control the manifestations of autoimmunity and lymphoproliferation in patients with primary immunodeficiency diseases, and there is no adequate response to first-line treatments. Rituximab (RTX), as a second-line treatment, is efficacious and well-tolerated for the management of these clinical manifestations.

This retrospective study was conducted to analyze the clinical, immunological, and genetic findings together with the response rate to RTX therapy in subjects with inborn errors of immunity (IEI) and autoimmune or autoinflammatory manifestations. In this study, 23 individuals with IEI

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and autoimmune or lymphoproliferation manifestations who received RTX between April 2008 and 2021 were evaluated.

Fifteen out of the 23 patients were female. The median age of cases was 12 years. The moderate and severe adverse reactions, including fever, diarrhea, and anaphylaxis shock, were manifested during RTX infusion in 5 patients. In total, 86.9% of patients responded to rituximab (complete response: n=14, partial response: n=6) while three failed to respond. The median response time to RTX treatment was 50 days. All patients were given monthly intravenous immunoglobulin (IVIG) therapy. Pneumonia and candidiasis occurred in one patient a week after receiving the second injection of RTX. Eight patients expired during follow-up.

In conclusion, the response rate of RTX could be improved through administering monthly IVIG for hypogammaglobulinemia treatment following RTX infusion. Early use of rituximab leads to a better response rate in comparison with late use of rituximab in multitreated refractory patients. The efficient cumulative dose of rituximab remains undefined.

Keywords: Autoimmune diseases; Primary Immunodeficiency diseases; Rituximab

INTRODUCTION

Inborn errors of immunity (IEI) are critical immunologic disorders resulting from genetic mutations and resultant loss or abnormal function of the expressed proteins.¹ The increased incidence of IEI in recent decades is probably attributed to improved diagnosis and the increasing accessibility of high-throughput DNA sequencing.² The clinical phenotypes of IEI have a broad spectrum from infection susceptibility to autoimmune diseases and malignancies.¹ The risk of autoimmune diseases, especially autoimmune cytopenia, considerably increased in IEI patients.³ Traditional treatments such as immunosuppressive agents and prophylactic antibiotics for the management of patients with IEI may be associated with short- or long-term consequences⁴. Rituximab (RTX), a chimeric anti-CD20 monoclonal antibody directed against maturing B cells, has made innovations in IEI patients' treatment because of more accurate direct immunomodulation.⁴⁻⁷ FDA has approved RTX for many diseases, such as non-Hodgkin's lymphoma, chronic lymphocytic leukemia, rheumatoid arthritis, and pemphigus vulgaris. Rituximab indications have been expanding due to its effectiveness in different conditions, including autoimmune hematologic syndromes, rheumatologic diseases, and immune dysregulation disorders.^{4,5,7-10} The market of RTX has been expanded following its successful function as a corticosteroid-sparing therapy and off-label uses in other specialties. Moreover, some studies reported more adverse events of RTX in patients receiving higher doses.^{10,11} The most prevalent adverse reactions related to RTX infusion usually occur within 2 hours after the

infusion and are dose-dependent. These reactions are mostly developed during the first infusion. They could be a wide spectrum of mild to severe reactions, such as fever, chills, skin rash, cold-like symptoms, to breathlessness, shock, and death.⁹ Hypogammaglobulinemia before receiving RTX increased the chance of severe infections and could be exacerbated after RTX therapy.⁵

In this retrospective study, we analyzed the clinical findings, immunologic features, molecular findings, and response to RTX therapy in subjects with IEI and autoimmune manifestations.

MATERIALS AND METHODS

In this retrospective study, patients' data with IEI who were referred to an academic children's center in Tehran (Mofid Children's Hospital) were analyzed. The European Society for Immunodeficiencies (ESID) Registry Working Party suggested criteria that were employed to diagnose the underlying IEI in this study.¹² Based on clinical findings, immunological examinations, and finally genetic testing, all patients were categorized based on the International Union of Immunological Societies (IUIS) expert committee (EC) on IEI.¹³⁻¹⁵ Among the patients with immunodeficiency, those who had one or more treatment-resistant autoimmunity or an autoinflammatory disorder related to the Epstein-Barr virus (EBV) and were resistant to the usual treatments for that disorder based on hematology and oncology criteria were included in the study.¹⁶

Demographic information, disease-related data, and patient outcomes were extracted from their records and follow-up visits. In addition, the results of the patients'

immunological investigations, including the percentage of T, B, and NK cells, levels of immunoglobulins, antibody titers against vaccines, and finally, the findings of genetic analysis were recorded in the questionnaires. This study was performed according to the Helsinki Declaration. The Ethics Committee at Shahid Beheshti University of Medical Sciences approved the study. Informed consent was obtained from both patients and their parents.

As the next step, the patients who had received RTX from April 2008 to April 2021 were enrolled. Patients' data were recorded in the Research Patient Database Registry. Before starting treatment with RTX, a series of baseline tests were performed, including a complete blood count (to assess hemoglobin, platelet, and white blood cell counts), and a reticulocyte count (to assess bone marrow activity), lactate dehydrogenase (LDH) and indirect bilirubin as indicators of hemolysis, direct antiglobulin test (DAT/Coombs test) to confirm immune hemolysis in autoimmune hemolytic anemia (AIHA), immunoglobulin levels (IgG, IgA, IgM) to detect hypogammaglobulinemia and viral screening including hepatitis B, C, and human immunodeficiency virus (HIV) testing to rule out contraindications.¹⁶

RTX was intravenously given as a course of four or six 375mg/m² infusions weekly (according to the patients' autoimmune or autoinflammatory disorders), and the rate of infusion rate was 50 mg/hour. In order to minimize infusion reactions, acetaminophen, diphenhydramine hydrochloride, and methylprednisolone were administered 30 minutes before the RTX infusion. The infusion-related reaction based on Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 is classified into 5 grades of toxicity.¹⁷ Considering intravenous immunoglobulin (IVIG) modulates the immune system and improves antibody production, all patients were given monthly IVIG (0.4–0.7 g/kg every 4 weeks) before and after RTX therapy.⁴

During Treatment, CBC, to monitor platelet recovery in immune thrombocytopenic purpura (ITP), and hemoglobin stabilization in AIHA, reticulocyte count, LDH, and bilirubin, evaluating signs of ongoing hemolysis (AIHA), were checked in all patients weekly or biweekly for the first 4 to 8 weeks. Periodic monitoring of immunoglobulin levels (every few months) was performed to assess hypogammaglobulinemia. Clinical Signs were watched for improvement in bruising or bleeding for ITP, reduction in jaundice or fatigue for AIHA, and new or worsening infections. Our patients had

long-term monitoring every 1 to 3 Months for the first year to ensure sustained remission.¹⁶

The criteria for a partial response in AIHI was established as an improvement in biological indicators of hemolysis (significant improvement but not fully normalized, decrease in reticulocytosis, indirect bilirubin, and LDH, and partial restoration of haptoglobin), increase in hemoglobin level by >2 g/dL from baseline but not yet fully normalized for age and sex, and noticeable improvement in clinical findings such as fatigue, pallor, and jaundice, but not full resolution.¹⁶

The criteria for a partial response in chronic ITP include platelet count $\geq 30 \times 10^9/L$ for isolated platelet count (ITP) with a minimum of a 2-fold increase and no bleeding, improvement in range of movement but without active synovitis for Juvenile idiopathic arthritis (JIA) in the absence of extra-articular complications, and incomplete recovery of visual outcomes lasted longer than 24 h for optic neuritis.¹⁸⁻²¹

The criteria for the complete response in AIHA include the restoration of the hemoglobin level to the normal level for the child's age, a normalized reticulocyte count appropriate for their age that indicates the resolution of hemolysis. Moreover, indirect bilirubin, LDH, and haptoglobin change to normal levels, signifying no ongoing hemolysis, and clinical symptoms are completely improved, with the absence of pallor, jaundice, and other anemia-related symptoms. In patients with chronic ITP, a complete response is characterized by a platelet count $\geq 100 \times 10^9/L$ ITP, the lack of bleeding, no synovitis, and a full range of movement for JIA in the absence of extra-articular complications, and full recovery of visual outcomes lasting longer than 24 h for optic neuritis.^{16,18-21}

Finally, based on the duration approved in the valid European and American hematology guidelines¹⁶ to check the response rate of patients to treatment, all files were checked in terms of laboratory values and clinical findings.

Statistical Analysis:

The statistical analysis was done by SPSS software version 26 (IBM, Armonk, NY, USA). Descriptive statistics, including mean and standard deviation (SD) as well as median and 25th and 75th percentiles (P25, P75) were calculated for variables with normal and nonnormal distributions, respectively. The categorical variables were reported as percentages. GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) was used to draw the graphs.

RESULTS

Out of 23 subjects, 15 were females (65.2%) and 8 were males (34.8%). The median (P25, P75) age of subjects at the start of the study, onset, and diagnosis were 12 (6, 14), 3 (1, 5), and 5 (3, 9) years, respectively. The median duration of follow-up following treatment with RTX was 2 years (Table 1).

Patients had a broad range of IEL diseases, including common variable immunodeficiency (CVID) (n=1, 4.34%), disease of immune dysregulation (n=7, 30.43%) including LPS-responsive Beige-like Anchor protein (*LRBA*) deficiency (n=4, 17.4%), *STAT3* gain of function (n=2, 8.7%), and *RIPK1* deficiency (n=1, 4.34%); patients with unspecified disease of immune dysregulation (n=7, 30.43%); with non-syndromic combined immunodeficiency (CID) (n=4, 17.4%); with combined immunodeficiency with syndromic features (ataxia telangiectasia) (n=3, 13%); Epstein-Barr virus-susceptibility was detected in 4 patients (17.4%) and were related to lymphoproliferative disorders; familial hemophagocytic lymphohistiocytosis (HLH) (n=1, 4.34%), Burkitt lymphoma (n=1, 4.34%), and Non-Hodgkin lymphoma (n=2, 8.7%). Besides, one of our patients had complement deficiency (C1q deficiency) (4.34%) (Table 1).

The different clinical manifestations of the patients in this study included autoimmune manifestations (n=16, 69.56%), recurrent infections (n=16, 69.6%), respiratory disorders (n=10, 43.5%), and organomegaly (n=10, 43.5%). A limited number of cases also showed atopy, neurologic manifestations, familial Mediterranean fever (FMF), polymyositis, Behçet's disease, Kawasaki disease, reactive arthritis, malignancy, skin disorders, and musculoskeletal disorders (Table 1).

Autoimmune manifestations were identified as the most frequent clinical symptoms among the study patients (n=16, 69.56%). They included ITP (n=8, 34.78%), AIHA (n=6, 26.1%), juvenile idiopathic arthritis (JIA) (n=1, 4.3%), mixed connective tissue disease (MCTD) (n=2, 8.7%), systemic lupus erythematosus (SLE) (n=2, 8.7%), Kawasaki (n=1, 4.3%), autoimmune thyroiditis (n=1, 4.3%), JIA (n=1, 4.3%), and Behçet's disease (n=1, 4.3%) (Table 1).

The RTX indications were ITP (n=8, 34.78%), AIHA (n=6, 26.1%), large B cell lymphoma (n=2, 8.6%), JRA (n=1, 4.3%), optic neuritis (n=1, 4.3%), and Evans syndrome (n=2, 8.7%) (Table 1)

The frequency of IVIG treatment, high-dose immunosuppressive agents, and antibiotics was (n=22, 95.7%), (n=18, 78.3%), and (n=16, 69.6%), respectively. Further, 1 patient reported a history of bone marrow transplantation.

Five children experienced moderate and severe adverse reactions during the infusions. Two patients had fever and diarrhea (grade 2 adverse events) during the first infusion of RTX that completely resolved after conservative treatment. The anaphylaxis shock (grade 4 adverse events) occurred in two patients during the first and fourth doses of RTX, respectively. It was resolved with cessation of the drug infusion, administration of methylprednisolone, hydroxyzine treatments, the use of salbutamol, adrenaline, and monitoring the patient.

One patient suffered an episode of pneumonia and candidiasis a week after the second infusion, and he had not encountered hypogammaglobulinemia during rituximab therapy. Pneumonia and candidiasis were resolved eventually by appropriate antibiotics and antifungal drugs.

The platelet counts and hemoglobin level increased after RTX, and the EBV copy number decreased (Figure 1 and Table 2). The cumulative response rate was 86.9% (complete response [CR] 60.8% n=14, partial response [PR] 26% n=6), and the median time to respond was 50 days. All patients continued their therapy with IVIG monthly. Unfortunately, 8 patients died during the follow-up period (34.7%).

Patients 8, 19, and 21 had complete/partial responses to RTX. However, they died from COVID-19. Patient 10 had received high-dose corticosteroids and chemotherapy for Burkitt lymphoma. He had received 6 doses of RTX and presented a complete response. He had a large mediastinal mass lesion with a severe mass effect on the left main bronchus. The risk of surgical resection was too high; therefore, the operation was canceled. He died from exacerbation of respiratory distress and cardiac arrest due to the mass effect. Patients 5, 11, 15, and 18 expired due to bacterial sepsis, HLH, severe cytomegalovirus infection after hematopoietic stem cell transplantation, and disseminated intravascular coagulation, respectively.

Table 1 presents comprehensive information regarding the patients, the treatment indications, and their responses.

Rituximab in Patients with IEI and Autoimmune or Autoinflammatory Manifestations

Table 1. Comprehensive information regarding the patients, the treatment indications, and their responses

	Gender	Age, y	AOO, y	AOD, y	Clinical manifestations	Autoimmune/ autoinflammatory reaction	PID diagnosis/IUIS classification	Response to RTX	RTX adverse effects	Other therapies
1	F	14	1	3	Recurrent respiratory infections	FMF Chronic ITP	CID (Non-syndromic)	Complete	0	IVIG/ antibiotics Prednisolone Colchicine CellCept
2	F	17	13	15	JIA Recurrent infection	JIA	CID (Non-syndromic)	Complete	Pneumonia and candidiasis	IVIG Antibiotics MTX Prednisolone NSAID
3	F	13	7	12	Reactive arthritis Behcet's disease SLE G6PD deficiency Seizure	SLE	RIPK1 deficiency (disease of immune dysregulation)	Partial	0	IVIG Antibiotics NSAID TNF alpha blocker Cyclosporine prednisolone
4	F	5	2	3	Petechiae Purpura Bleeding ITP	Chronic ITP	CID (Non-syndromic)	No response	0	IVIG Immunosuppressive (prednisolone)
5	M	12	1	2	Hepatosplenomegaly Pancytopenia Kawasaki disease	Chronic ITP AIHA (Evans syndrome)	STAT3 gain-of-function mutation (disease of immune dysregulation)	Complete	Anaphylaxis and macular rash	IVIG Antibiotics Immunosuppressive BMT
6	F	12	4	8	Recurrent pneumonia Sinusitis Failure to thrive	Chronic ITP	ataxia telangiectasia (CID with associate or syndromic features)	Complete	0	Prednisolone IVIG

Table 1. Continued...

	Gender	Age, y	AOO, y	AOD, y	Clinical manifestations	Autoimmune/ autoinflammatory reaction	PID diagnosis/IUIS classification	Response to RTX	RTX adverse effects	Other therapies
7	F	11	5	10	Epistaxis Bruising	Chronic ITP	Unspecified disease of immune dysregulation	Partial	0	IVIG Prednisolone
8	F	7	6 months	2	Generalized edema Thrombocytopenia	Chronic ITP	LRBA deficiency (disease of immune dysregulation)	Partial	0	IVIG antibiotics Immunosuppressive (prednisolone)
9	F	12	3.5	7	Huge splenomegaly Recurrent pneumonia	Chronic ITP	CVID	Complete	Anaphylaxis and dyspnea	IVIG Antibiotics Immunosuppressive (prednisolone)
10	M	9	3	6	Respiratory distress Mediastinal mass CMV retinitis	Burkitt lymphoma (EBV-related)	STAT3 gain-of- function mutation (disease of immune dysregulation)	Complete	0	IVIG Antibiotics Immunosuppressive Chemotherapy
11	F	6	3	4	Respiratory distress Recurrent pneumonia	Non-Hodgkin lymphoma (EBV-related)	Unspecified disease of immune dysregulation	Partial	0	IVIG Antibiotics Chemotherapy dexamethasone
12	M	6	5	5	Hepatosplenomegaly Recurrent infections	AIHA	Unspecified disease of immune dysregulation	Complete	0	IVIG immunosuppressive (Prednisolone)
13	M	13	1 month	9	CMV retinitis Arthrogyriposis Lymphoma	Non-Hodgkin lymphoma related to EBV	Unspecified disease of immune dysregulation	Partial	Fever	IVIG Antibiotics Chemotherapy

Rituximab in Patients with IEI and Autoimmune or Autoinflammatory Manifestations

Table 1. Continued...

	Gender	Age, y	AOO, y	AOD, y	Clinical manifestations	Autoimmune/ autoinflammatory reaction	PID diagnosis/IUIS classification	Response to RTX	RTX adverse effects	Other therapies
14	M	7	1	5	Recurrent infections Splenomegaly Jaundice	AIHA	Ataxia telangiectasia (CID with associate or syndromic features)	Complete	0	IVIG Antibiotics Immunosuppressive (prednisolone)
15	M	14	2	3	GI bleeding Petechiae Mucositis Pseudomonas dermatitis	Chronic ITP AIHA: (Evans syndrome)	LRBA deficiency (disease of immune dysregulation)	Complete	Diarrhea and bleeding gums	IVIG Antibiotics Immunosuppressive (prednisolone)
16	M	18	4	5	Visual loss Seizure Respiratory distress	Optic neuritis	Unspecified disease of immune dysregulation	Complete	0	IVIG Antibiotics Immunosuppressive
17	F	7	3	3	Splenomegaly Anemia Fever	AIHA	ataxia telangiectasia (CID with associated or syndromic features)	Complete	0	IVIG Antibiotics Immunosuppressive (prednisolone)
18	M	5	2	2	Fever Pancytopenia Hepatosplenomegaly	HLH due to EBV	Unspecified disease of immune dysregulation	No response	0	IVIG Immunosuppressive Dexamethasone VP16 MTX
19	F	18	6	6	Pancytopenia Huge splenomegaly Hematemesis	AIHA	LRBA deficiency (disease of immune dysregulation)	Complete	0	IVIG Antibiotics Immunosuppressive (prednisolone)

Table 1. Continued...

	Gender	Age, y	AOO, y	AOD, y	Clinical manifestations	Autoimmune/ autoinflammatory reaction	PID diagnosis/IUIS classification	Response to RTX	RTX adverse effects	Other therapies
20	F	3	1	2	Petechiae Purpura Candidiasis	Chronic ITP	Unspecified disease of immune dysregulation	No response	0	IVIG immunosuppressive Nplate
21	F	19	12	13	Respiratory distress Generalized edema Intermittent fever Generalized skin Ulcers	SLE AIHA	C1q deficiency (complement deficiency)	Partial	0	IVIG antibiotics immunosuppressive (prednisolone) TNF Alpha blocker Cyclophosphamide CellCept
22	F	4	2	4	Hepatosplenomegaly Diarrhea Fever	AIHA	LRBA deficiency (disease of immune dysregulation)	Complete	0	IVIG Antibiotics Immunosuppressive (prednisolone)
23	F	18	12	17	Thrombocytopenia Fever Seizure Rash Hypothyroidism	Polymyositis ITP	CARD11(LOF) (CID)	Complete	0	IVIG Antibiotics Immunosuppressive (prednisolone) MTX Cyclosporine

AOO: age at onset; AOD: age at diagnosis; PID: primary immunodeficiency; IUIS: International Union of Immunological Societies classification; RTX: rituximab; IVIG: intravenous immunoglobulin; MTX: methotrexate; NSAID: non-steroidal anti-inflammatory drug; BMT: bone marrow transplant; AIHA: autoimmune hemolytic anemia; ITP: immune thrombocytopenic purpura; CID: chronic inflammatory disease; FMF: familial Mediterranean fever; JIA: juvenile idiopathic arthritis; SLE: systemic lupus erythematosus; RIPK1: receptor-interacting protein kinase 1; G6PD: glucose-6-phosphate dehydrogenase; TNF: tumor necrosis factor; STAT3: signal transducer and activator of transcription 3; EBV: Epstein-Barr virus; CMV: cytomegalovirus; CVID: common variable immunodeficiency; HLH: hemophagocytic lymphohistiocytosis; IL-6RA: interleukin-6 receptor antagonist; C1q: complement component 1q; LOF: loss-of-function mutation

Rituximab in Patients with IEI and Autoimmune or Autoinflammatory Manifestations

Table 2. Immunological parameters before and after treatment

Parameter (before/after rituximab)	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
WBC, cells/mm ³	7.3	9.5/7.3	4.5	8/8-5600	3.7/4.3	1.7/5.3	4.6	12.1/19.4	5/2.5	4.4/8.4	12.4/5.3	5.3
Lymphocytes, % cell count/mm ³	39 (2847)	91.3/6.6 (8673-481)	45 (2025)	37/51.9 (3300-2912)	57/53 (2109-2279)	74/41 1258/2173	48 (2208)	20/37 (2420-7178)	48/40 (2496-1000)	38/29 (1672-2436)	35/18 (4340-954)	30 (1590)
Hb	4-6.7											
PLT, cells/mm ³	8500- 78000	5000-109000				3000-60000			9400- 140000	3200- 110000		
CD3 ⁺ T cells, %	53 (1508)	81/88 (7025-423)	88 (1782)	90/0.57 (2970-17)	58-53 (1223-1117)	76 (1678)		45/89.3 (1089-6409)	83 (2072)	12.1 (202)	64/89 (2777-849)	80 (1272)
CD4 ⁺ T cells, %	31 (882)	46/28 (3989-134)	36 (729)	35/0.1 (1155-3)	28-20 (590-456)	43 (949)		19/43 (460-3086)	43 (1073)	12.5 (209)	25 (1085)	37 (588)
CD8 ⁺ T cells, %	17 (484)	40/38 (3469-182)	46 (931)	48 (1584)	32-28 (675-638)	31 (684)		34/46.1 (823-3310)	34 (849)	8.4 (140)	34 (1476)	33 (525)
CD19 ⁺ B cells, %	7.68 (218.6)	19.4/<1 (1682-<4.8)	2.9 (58)	50.2/<1 (1656-<30)	33-3 (695-68)	8.4 (185)		25/<1 (605-<72)	3.37 (84)	3.1 (52)	27/<1 (1172-<10)	3 (48)
CD20 ⁺ B cells, %	7.75 (220.6)	19.58/<1 (1698-<4.8)	5.6 (1134)	52/<1 (1716-<30)	36-2 (759-46)	8.1 (178)		22-<1 (532-<72)	3.4 (85)	15.4 (257)	26-<1 (1128-<10)	5 (79.5)
CD16 ⁺ 56 ⁺ NK cells,	6 (1110)	6.95 (602)	6.4 (130)	4.73 (156)	2.01	16.49		15.4	17.7	7	10.7	10
EBV copy number										300540- 5400	190000- 1000	
IgG, mg/dL	999	652/740	522	839/1132	670	1200	947/1032	1032/485	46/236	1100	467	766
IgM, mg/dL	261	19/14	46	114/134	32	18	73/32	163/58	860	268	389	200
IgA, mg/dL	151	14/40	10	35/53	103	7	239/182	212/161	8	35	83	165
IgE, IU/mL	8.1	18	12	3/3.2	70	1	15/23	596/1.5	2	10	8	2.7

Table 2. Continued...

Parameter (Before/After Rituximab)	P13	P14	P15	P16	P17	P18	P19	P20	P21	P22	P23
WBC, cells/mm ³	4.8/4	6.9/3.3	6.8/7.1	10/13.5	5.3	2.2/4	7.1	6.6/5.4	7.1/5.9	7.9/2.6	8.4
Lymphocyte, cells/mm ³	22.2/20 (1066-800)	43/31 (2967-1023)	46/32 (3128-2272)	28/17 (2800-2295)	37 (1961)	52/60 (1144-2400)	29.8 (2115)	80/76 (5280-4104)	17/33 (1207-1947)	74/33 (5846-858)	38 (3192)
Hb, g/dL	5.2-9		5.9-8.6			4.6-7		5-8.4			
PLT cells/mm ³				2000-160000					8000-60000		28000- 110000
CD3 ⁺ T cells, %	88		34.1 (1066)	76 (2128)		59/76 (674-1824)	43.1 (909)	93/91 (4910-3734)	13 (253)	60 (3507)	85 (2713)
CD4 ⁺ T cells, %	63		46 (1438)	52 (1456)		33/10 (378-240)	48.2 (1019)	62/52 (3273-2134)	23 (448)	35 (2046)	17 (543)
CD8 ⁺ T cells, %	30		35 (1094)	24 (672)		26/65 (297-1560)	30 (634.5)	27/28 (1425-1149)	51 (993)	51 (2981)	65 (2075)
CD19 ⁺ B cells, %	5.6		36.6 (1144)	9.7/<1 (271-<18)		32/18 (366-432)	34.2 (723)	1.62/0.1 (86-4)	<1 (<19)	4 (234)	4.2 (134)
CD20 ⁺ B cells, %			26.2 (820)	20/<1 (560-<18)		28/17 (320-176)	28 (592)	3-0.7 (158-29)	<1 (<19)	2.4 (140)	5 (160)
CD16 ⁺ 56 ⁺ NK cells, %	4		5.6 (175)	26.7/63.4		2.57/1.06 (29-25)	6.6 (140)	5.2/4.9 (275-201)	18 (350)	10 (585)	5 (160)
EBV copy number		220905-7900									
IgG, mg/dL	738	442/301	6.47	827	108/997	620	5.4	303	812	250/685	700/1170
IgM, mg/dL	532	578/578	35	121	369/645	24	0.56	5	177	125	85/153
IgA, mg/dL	155	<10/210	0	51	11/54	74	1.72	12	44	20	49/37
IgE, IU/mL	5.6	0.6/0.6	<0.1	4	0.3/1	128	0.1	5	6.3	<1	27/4.1

WBC: white blood cells; Hb: hemoglobin; PLT: platelets; NK: natural killer; EBV: Epstein-Barr virus copy number; Ig: immunoglobulin

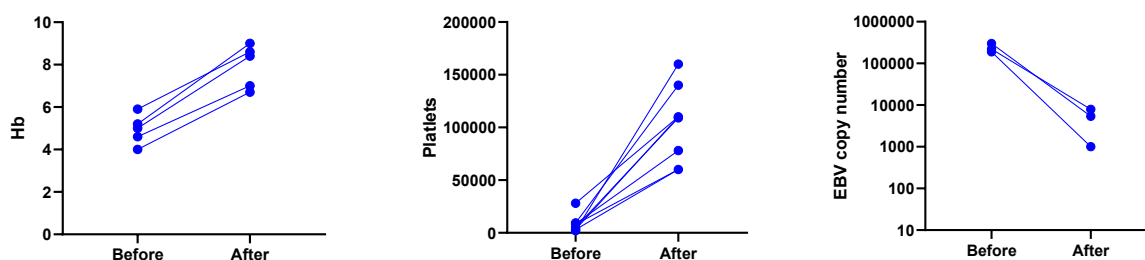


Figure 1. Comparison of hemoglobin (Hb) levels, platelet counts, and Epstein-Barr virus (EBV) copy numbers before and after rituximab treatment

DISCUSSION

In the current study, a high response rate to RTX was observed in immunodeficient individuals with a median response time of 50 days. Moreover, three patients did not respond to this medication (Table 1). A minimum of 4 RTX infusions were given to all subjects.

Responses to ITP (80%, total= 8, PR= 2, CR=6) and AIHA (100%, total=6, PR=2, CR=4) were comparable. Five children (21.7%) presented moderate to severe infusion-related reactions. Patients underwent follow-up for a mean of two years following RTX treatment.

Two main mechanisms whereby RTX can lead to the depletion of CD20⁺ B cells include antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-mediated cytotoxicity (CMC).^{7,22} This process takes place in the peripheral blood after the infusion of 2 to 3 doses of RTX.²³ B-cell recovery needs a duration of 6 to 12 months, regardless of the dose.^{24,25}

Although corticosteroids and immunosuppressive drugs are regarded as the first-line treatment for individuals with immunodeficiencies,²⁶ serious adverse reactions could occur as a result of long-term immunosuppressive regimens, such as growth impairment, fluid retention, bone vascular necrosis, and recurrence of hemolysis.²⁷ In contrast, RTX could be considered a valuable alternative to manage immunodeficient patients receiving high-dose steroids or resistance to steroids.²⁷

The successful response rate of RTX has been reported in the literature, especially in hematological diseases such as refractory AIHA.^{23,27-36} A cumulative response rate of 86.9% with a median response time of 50 days was observed in this study. Most patients with AIHA have responded after the third dose of RTX.

Although a sustainable response has been observed after only 2 doses of RTX in AIHA patients,^{27,37} Early starting of RTX therapy also caused a better response in primary and isolated AIHA and infants. RTX treatment resulted in a decrease in steroid use for 60% to 70 % of AIHA cases.³⁸

Long-term responses have been developed in 72% of ITP patients who initially responded to RTX.^{25,39} The severity of symptoms is attenuated in these patients following the RTX therapy, and its recovery was maintained for more than 12 months.^{11,24} ITP patients may achieve complete responses, which can sometimes last for at least a year without any further medication. Nevertheless, previous studies have revealed a high proportion of relapses in ITP patients.⁴⁰ The response rate of these patients is not affected by the total dose of RTX or prior therapies.^{25,41,42} ITP patients with splenectomized or non-splenectomized conditions demonstrated no difference between the rates of response and relapse.⁴¹ However, 1 study showed that splenectomized children and adults tended to relapse.⁴³ Therefore, RTX will replace or delay splenectomy.²⁵

Fourteen (60.8%) patients, mainly with chronic ITP and AIHA, achieved a complete response, while 6 (26%) demonstrated a partial response. Complete responses are more likely in patients who are older, both at the time they begin the study and when they undergo RTX treatment. Furthermore, a higher proportion of sustained responses was seen in pubertal ITP patients compared to younger ITP children.⁴¹ Children also responded to RTX more quickly than adults.²⁵

Most RTX side effects occurred within 1 year of therapy. Although nearly all infusion-related reactions and infections are resolved with proper treatment.⁴¹

Post-RTX hypogammaglobulinemia may occur even at normal baseline Ig levels. The most prevalent post-RTX hypogammaglobulinemia is hypo-IgM, which will not increase the risk of infection. Hypo-IgG and hypo-IgA induced by rituximab are present in patients who are inherently susceptible to RTX. For example, underlying immune defects may exacerbate post-RTX hypo-IgG, and patients with cytopenia were affected by post-RTX hypogammaglobulinemia.^{44,45}

There was a high incidence of hypo-IgG cases among patients treated with RTX (40.4%).⁴⁴ However, post-RTX hypo-IgG and hypo-IgM did not increase the frequency or severity of infections. Compared to adults, children were more prone to have post-RTX hypo-IgG and hypo-IgM.²⁵

RTX also results in unbalancing T- and B-cell homeostasis, by which ineffective Ig's are produced.^{38,45} Following treatment with RTX, patients with normal IgG levels need to be monitored for the assessment of persistent hypogammaglobulinemia.⁴⁵ Therefore, accurate monitoring and measuring of immunoglobulin levels should be given attention after RTX therapy. Prophylactic IVIG can quickly correct the impairment of creating antibodies. Higher cumulative doses of IVIG decrease the risk of infections and hypogammaglobulinemia after RTX therapy.⁵ RTX affects mature B cells, and not the long-lived plasma cells that produce autoantibodies or restore the same autoimmune clones, which explains the relapse and poor or delayed response.^{4,7,40} Although infants have better relapse-free survival (RFS) (70%) due to early RTX therapy.⁴⁶ Considering the increased rate of relapse, a follow-up of at least three years should be performed for patients after RTX therapy.⁴³

Lack of information regarding the regular measurement of immunoglobulin levels in most patients after RTX therapy was a limitation of this study. Thus, there was no possibility of determining the hypogammaglobulinemia prevalence. Furthermore, some variables, such as different therapies (such as chemotherapy or corticosteroids consumption), the heterogeneity of study samples, and the small size of the sample, may affect the results.

Rituximab, as a second-line therapy, is a well-tolerated and noteworthy option for the management of immunodeficiency diseases. Moreover, the response rate of RTX could be improved by administering monthly IVIG for the treatment of hypogammaglobulinemia. For a better response, it is suggested to use rituximab early

instead of late in multi-treated refractory patients. The efficient cumulative dose of rituximab remains undefined.

STATEMENT OF ETHICS

The Ethics Committee at Shahid Beheshti University of Medical Sciences approved the study (IR.SBMU.MSP.REC.1401.540). Informed consent was obtained from both patients and their parents.

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

This study was supported by Shahid Beheshti University of Medical Sciences.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

AI ASSISTANCE DISCLOSURE

Not applicable.

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