

Rituximab Therapy in Adults with Refractory Symptomatic Immune Thrombocytopenia: Long-Term Follow-Up of 15 Cases

Refrakter Semptomatik İmmün Trombositopeni Tanılı Erişkinlerde Rituksimab Tedavisi: 15 Olgunun Uzun Süreli İzlemi

Fehmi Hindilerden¹, İpek Yönel-Hindilerden², Mustafa Nuri Yenerel², Meliha Nalçacı², Reyhan Diz-Küçükaya³

¹Bakırköy Sadi Konuk Training and Research Hospital, Clinic of Hematology, İstanbul, Turkey

²İstanbul University İstanbul Faculty of Medicine, Department of Internal Medicine, Division of Hematology, İstanbul, Turkey

³İstanbul Bilim University Faculty of Medicine, Department of Internal Medicine, Division of Hematology, İstanbul, Turkey

Abstract

Objective: This paper prospectively evaluates the long-term follow-up [mean \pm standard deviation (SD) duration: 89.7 \pm 19.4 months] data of 15 patients (13 females and 2 males) with refractory symptomatic immune thrombocytopenia (ITP) treated with rituximab.

Materials and Methods: Rituximab was administered at 375 mg/m² weekly for a total of 4 doses. Complete response (CR) was defined as a platelet count of \geq 100,000/mm³ and partial response (PR) as a platelet count of \geq 30,000/mm³ but less than 100,000/mm³. Early response (ER) and late response (LR) were defined as response within 42 days and after 42 days of initiation of rituximab therapy, respectively. Sustained response (SR) was defined as response lasting for at least 6 months.

Results: Mean age (\pm SD) at the start of rituximab was 46.6 \pm 11.3 years. Mean platelet count (\pm SD) prior to rituximab treatment was 17,400 \pm 8878/mm³. The mean time (\pm SD) between rituximab therapy and response to rituximab in early responders and late responders was 1.8 \pm 1.3 weeks and 10 \pm 2.8 weeks, respectively. Mean durations (\pm SD) of ER and LR were 51 \pm 47.2 months and 6 \pm 4.2 months, respectively. Seven of the 15 patients (46.7%) showed an initial response to rituximab (5 ER and 2 LR). The rate of SR over 6 months was 26.7% (4/15). Among the responders to rituximab, 3 (3/7, 42.9%) maintained their response 1 year after rituximab treatment and 2 (2/7, 28.6%) had ongoing response 5 years after initiation of rituximab. Two of the 7 patients (28.6%) still maintained their response 98 months after initiation of rituximab. All 5 initial responders with subsequent relapse achieved response from subsequent treatment modalities (3 CR, 2 PR).

Conclusion: Our data confirm, over a long period of observation, that rituximab is safe and effective in the management of patients with chronic refractory primary ITP.

Keywords: Immune thrombocytopenia, Rituximab, Early response, Late response, Sustained response

Öz

Amaç: Çalışmamızda rituksimab ile tedavi edilen refrakter semptomatik immün trombositopeni (İTP) tanılı 15 olgunun (13 kadın ve 2 erkek) uzun süreli izlemi sonucundaki [ortalama \pm SD (standart deviasyon) süresi: 89,7 \pm 19,4 ay] verileri incelenmiştir.

Gereç ve Yöntemler: Rituksimab haftada bir 375 mg/m² dozunda toplam 4 doz uygulanmıştır. Tam yanıt (TY) trombosit sayısının \geq 100,000/mm³ olması ve parsiyel yanıt (PY) trombosit sayısının \geq 30,000/mm³ olması fakat 100,000/mm³'ün altında olması olarak tanımlanmıştır. Erken yanıt (EY) ve geç yanıt (GY) ise sırasıyla rituksimab başlangıcından 42 gün içinde ve 42 gün sonra yanıt elde edilmesi olarak tanımlanmıştır. Sürekli yanıt (SY), yanıtın en az 6 ay sürmesi olarak tanımlanmıştır.

Bulgular: Rituksimab tedavisinin başladığı sırada ortalama yaş (\pm SD) 46,6 \pm 11,3 yıldır. Rituksimab tedavisi öncesinde ortalama trombosit sayısı (\pm SD) 17,400 \pm 8878/mm³'dür. Erken ve geç yanıt edilen olgularda rituksimab başlangıcı ile yanıt kadar geçen ortalama süre (\pm SD) sırasıyla 1,8 \pm 1,3 hafta ve 10 \pm 2,8 hafta olarak saptanmıştır. EY ve GY elde edilen olgularda ortalama yanıt süresi sırasıyla 51 \pm 47,2 ay ve 6 \pm 4,2 aydır. On beş olgunun 7'sinde (%46,7) rituksimab tedavisine başlangıçta yanıt elde edilmiştir (5 EY, 2 GY). SY oranı %26,7'dir (4/15). Rituksimab tedavisine yanıt veren olgular arasında 3'ü (3/7, %42,9) yanıtını bir yıldan fazla ve 2'si (2/7, %28,6) yanıtını 5 yıldan fazla sürdürmüştür. Yedi olgunun ikisi (%28,6) rituksimab başlangıcından 98 ay sonra halen yanıtını korumaktadır. Başlangıçta yanıt veren 5 olgunun hepsi relaps sonrasında ardışık tedavilere yanıt vermiştir (3 TY, 2 PY).

Sonuç: Çalışmamız uzun bir gözlem sonucunda kronik refrakter primer İTP olgusunda rituksimab tedavisinin güvenilir ve etkili olduğunu desteklemektedir.

Anahtar Sözcükler: İmmün trombositopeni, Rituksimab, Erken yanıt, Geç yanıt, Sürekli yanıt



Introduction

Immune thrombocytopenia (ITP) is an autoantibody-mediated disorder characterized by a platelet count of less than 100,000/mm³ and increased risk of bleeding [1]. B cells play an important role in the pathophysiology of ITP, making B-cell depletion with rituximab a rational therapeutic option [2].

Glucocorticosteroids still remain the standard initial therapy for patients with symptomatic disease. Second-line treatment options include splenectomy, azathioprine, cyclosporine A, cyclophosphamide, danazol, mycophenolate mofetil, rituximab, and thrombopoietin-receptor (TPO-receptor) agonists [3]. In approximately 80% of patients, splenectomy results in response maintained at 10 years in 70% of patients, but it is associated with a life long infection risk in 1%-3% of patients [4]. Chronic refractory ITP has been defined as failure to respond to splenectomy [5]. There is no standard of care for patients with refractory or relapsing ITP after splenectomy. Although spontaneous remissions may occur in some cases, these patients carry a significant risk of bleeding and have increased morbidity and mortality [5]. Further treatment is considered in chronic refractory ITP patients with low platelet counts and bleeding symptoms [5].

Over the last decades, rituximab has been widely used to treat primary ITP patients resistant to one or more treatment lines [6,7,8,9,10,11,12,13,14,15]. Rituximab is still used off-label as a second- or third-line option in many countries. Only a few systematic reviews on the efficacy of rituximab for adult ITP patients have been published [6,16]. In the meta-analysis by Arnold et al., overall response (OR) and complete response (CR) rates with rituximab were 62.5% and 46.2%, respectively, with a median response duration of 10.5 months and a median follow-up of 9.5 months [6]. In a recent systematic review and meta-analysis including non-splenectomized ITP patients treated with rituximab, a CR rate of 46.8% was reported after a median follow-up of 6 months [16]. Khellaf et al. conducted a prospective multicenter registry of adult patients with ITP who were refractory to corticosteroids (97%), IVIG (71%), and splenectomy (10%) and were treated with rituximab [17]. After a median follow-up of 24 months, 61% showed an overall initial response and 39% had sustained response (SR) [17]. Data on the long-term efficacy of rituximab in adult ITP are limited [13,18,19,20,21]. Several studies reported SR rates ranging from 21% to 40% after a median follow-up period ranging from 2 to 5 years [13,18,19,20,21]. Here we prospectively assess the overall initial response and SR rates to rituximab in 15 chronic refractory symptomatic ITP patients with a follow-up duration of 7 years.

Materials and Methods

We prospectively evaluated 15 patients (13 females and 2 males) diagnosed with chronic refractory ITP, all of whom

had been treated with corticosteroids and splenectomy and received various immunosuppressive agents. Rituximab was offered to the patients as an off-label treatment following the approval of the Ministry of Health. Informed consent for study participation was obtained from all patients. Rituximab was administered intravenously at 375 mg/m² once weekly for 4 weeks between November 2007 and March 2008. Selective spleen scintigraphy was performed to rule out accessory spleens. Baseline platelet count prior to initial administration of rituximab and before each weekly infusion were recorded. During the follow-up period, platelet counts were obtained at the 1st, 3rd, 6th, 12th, 18th, 24th, 32nd, 40th, 48th, 56th, 64th, 72nd, 80th, 88th, and 96th months of rituximab therapy. CR was defined as any platelet count of at least 100,000/mm³ and the absence of bleeding, partial response (PR) as any platelet count between 30,000 and 100,000/mm³ and absence of bleeding, and no response (NR) as any platelet count lower than 30,000/mm³ or the presence of bleeding [1]. Early response (ER) was defined as a response within 42 days of rituximab infusion and late response (LR) was defined as response occurring 42 days after initiation of rituximab. OR to rituximab was the summation of ER and LR. SR was defined as response lasting for a minimum of 6 months [11,22]. Loss of response was defined as losing response to rituximab with any platelet count lower than 30,000/mm³ or the presence of bleeding and need for other therapy during follow-up. Time to response was defined as time from commencement of treatment to either CR or PR. Duration of response was defined as time from CR or PR until loss of CR or PR.

Statistical Analysis

Data were processed using SPSS 21 (University of Sussex). Characteristics of patients were described with mean ± standard deviation. Comparisons between groups were performed by chi-square test and Fisher's exact test. The analysis of continuous variables among the groups was performed using the Mann-Whitney U test. Odds ratios are accompanied by Cornfield 95% confidence interval limits (CIs). A curve showing the proportion of patients with continuing response to rituximab was constructed by the Kaplan-Meier method. A general linear model for repeated measures was used to compare platelet values after the initiation of rituximab in responders vs. non-responders. Probability values of p<0.05 were considered significant.

Results

Patient characteristics are summarized in Table 1. There was no response to initial corticosteroid treatment in 7 patients (46.7%) and 8 patients (53.3%) lost their response to corticosteroids during follow-up. All patients were already splenectomized before rituximab therapy but 5 (33.3%) had an accessory spleen prior to rituximab infusion. The mean number of previous treatments

Table 1. Characteristics of the patients.	
Characteristics	Mean \pm SD
Number of patients	15
Sex	
Female	13 (86.7%)
Male	2 (13.3%)
Age at diagnosis (years)	29.6 \pm 15.8
Age at the time of splenectomy (years)	31.7 \pm 16
Age at rituximab infusion (years)	46.6 \pm 11.3
Actual age (years)	54 \pm 11.6
Number of previous therapies	3.6 \pm 1.04
Previous therapies, n (%)	
Steroids	15 (100%)
Splenectomy	15 (100%)
Azathioprine	11 (73.3%)
Vincristine	5 (33.3%)
IVIg	2 (13.3%)
Other therapies	5 (33.3%)
Accompanying diseases, n (%)	
Diabetes mellitus	3 (20%)
Hypertension	3 (20%)
Response to initial corticosteroid treatment, n (%)	
NR	7 (46.7%)
R	8 (53.3%)
Presence of accessory spleen before rituximab, n (%)	5 (33.3%)
Initial response to rituximab therapy, n (%)	
NR	8 (53.3%)
ER	5 (33.3%)
LR	2 (13.3%)
Loss of response to rituximab, n (%)	5/7 (71.4%)
SR to rituximab therapy, n (%)	4 (26.7%)
Disease status at final observation, n (%)	
CR	5 (33.3%)
PR	6 (40%)
NR	2 (13.3%)
Deceased	2 (13.3%)
Platelet count at diagnosis (/mm ³)	10100 \pm 4251
Hemoglobin level at diagnosis (g/dL)	11.2 \pm 1.01
WBC count at diagnosis (/mm ³)	8513 \pm 2146
Platelet count before rituximab treatment (/mm ³)	17,400 \pm 8878
Platelet count at 1 st month after the initial dose of rituximab (/mm ³)	68,733 \pm 95,213
Time from diagnosis to splenectomy (months)	24.6 \pm 19.3
Time from splenectomy to rituximab therapy (months)	179 \pm 103.8
Time from diagnosis to rituximab therapy (months)	204 \pm 106.2
Time to response to rituximab of early responders (weeks)	1.8 \pm 1.3
Time to response to rituximab of late responders (weeks)	10 \pm 2.8
Duration of ER (months)	51 \pm 47.2
Duration of LR (months)	6 \pm 4.2
Duration of OR (ER+LR) (months)	38.1 \pm 44.4
Follow-up period after rituximab treatment (months)	89.7 \pm 19.4
Death	2 (13.3%)

NR: No response, R: response, ER: early response, LR: late response, SR: sustained response, CR: complete response, PR: partial response, OR: overall response, SD: standard deviation.

Early responders to rituximab therapy (n=5)									
Number	Sex	Actual age	Previous therapies	TTR (weeks)	Relapse	DOR (months)	FU period after first R therapy (months)	Treatment after R therapy	ITP final status
1	F	37	Steroids, splenectomy	4	Yes	52	98	Steroids, IVIG	CR
2	F	56	Steroids, splenectomy, IVIG	1	No	98	98	No	CR
3	F	56	Steroids, splenectomy	1	No	98	98	No	CR
4	F	42	Steroids, splenectomy, azathioprine, vincristine, danazol	2	Yes	2	98	Steroids, azathioprine, vincristine, eltrombopag	PR
5	F	58	Steroids, splenectomy, azathioprine, vincristine	1	Yes	5	96	Steroids	CR
Late responders to rituximab therapy (n=2)									
Number	Sex	Actual age	Previous therapies	TTR (weeks)	Relapse	DOR (months)	FU period after first R therapy (months)	Treatment after R therapy	ITP final status
1	F	48	Steroids, splenectomy, azathioprine, vincristine, danazol	8	Yes	3	96	Steroids, danazol	CR
2	M	60	Steroids, splenectomy, azathioprine	12	Yes	9	96	Eltrombopag	PR

TTR: Time to response, DOR: duration of response, FU: follow-up, R: rituximab, CR: complete response, PR: partial response, ITP: immune thrombocytopenia.

was 3.6 ± 1.04 . The mean duration of follow-up after rituximab was 89.7 ± 19.4 months. Seven of the patients (46.7%) showed an initial response (5 ER and 2 LR). The cumulative response rate was 46.7%. Four of the 15 patients (26.7%) achieved SR with a duration of more than 6 months. Patients with SR included 3 early responders and 1 late responder. During follow-up, 2 of the patients who obtained SR lost their response 9 months and 52 months after the initiation of rituximab, respectively. Durations of ER and LR were 51 ± 47.2 months and 6 ± 4.2 months, respectively. The duration of OR (ER+LR) was 38.1 ± 44.4 months. One patient succumbed to intracranial hemorrhage and another to myocardial infarction. Patient characteristics of early and late responders are summarized in Table 2.

Comparison of Immune Thrombocytopenia Patients According to Their Response Status to Rituximab Therapy

Clinical and laboratory features of ITP patients stratified by response status to rituximab are outlined in Table 3. The presence of comorbid diseases was more frequent in non-responders

compared to responders, but the difference was not statistically significant (62.5% and 14.3%, respectively, $p=0.117$).

The presence of response did not correlate with actual age, age at diagnosis, age at time of splenectomy, age at initiation of rituximab, sex, hemoglobin level and platelet count at diagnosis, initial response to corticosteroids, number of previous therapies, interval between diagnosis and initiation of rituximab, and time between splenectomy and rituximab therapy ($r<0.2$). A weak positive correlation was detected between the presence of response and the interval between diagnosis and splenectomy ($r=0.281$). A moderate positive correlation was found between the presence of response and WBC count at diagnosis ($r=0.464$). We showed a moderate inverse correlation between the presence of response and comorbid diseases ($r=-0.423$).

Clinical Course

Of the 7 patients with response to rituximab, 5 (3 in ER and 2 in LR) relapsed after a response duration ranging from 2 to

Table 3. Comparison of characteristic features of immune thrombocytopenia patients stratified by response status to rituximab therapy.

Chronic refractory ITP patients	Patients with OR, n=7 (Mean ± SD)	Patients with NR, n=8 (Mean ± SD)	p
Age, at diagnosis, years	26.1±13.9	32.7±17.7	0.602
Actual age, years	51±8.8	56.7±13.6	0.685
Age at the time of splenectomy, years	28.7±13.5	34.3±18.4	0.817
Age at rituximab infusion, years	44.2±8.4	48.7±13.7	0.772
Female, n (%)	6 (85.7%)	7 (87.5%)	1
Hemoglobin at diagnosis, g/dL	11.4±0.9	11±1	0.363
WBC count at diagnosis, /mm ³	9514±2150	7637±1837	0.083
Platelet count at diagnosis, /mm ³	10,428±4613	9812±4208	0.861
Number of previous therapies	3.4±1.2	3.8±0.83	0.435
Time from diagnosis to splenectomy, months	30±22.5	19.8±16.1	0.293
Time from diagnosis to rituximab therapy, months	217±140	192±73.6	0.728
Time from splenectomy to rituximab therapy, months	187.8±130	171.2±82.8	0.728
Presence of accessory spleen before rituximab, n (%)	2 (28.6%)	3 (37.5%)	1
Response to initial corticosteroids, n (%)	7 (100%)	8 (100%)	0.619
R (n, %)	3 (42.9%)	5 (62.5%)	-
NR (n, %)	4 (57.1%)	3 (37.5%)	-
Accompanying diseases, n (%)	1 (14.3%)	5 (62.5%)	0.117
Hypertension (n, %)	0	3 (37.5%)	-
Diabetes mellitus (n, %)	1 (14.3%)	2 (25%)	-
Follow-up period after rituximab treatment, m	97.1±30.1	83.2±25.4	0.282
Death, n (%)	0	2 (25%)	0.467

IITP: Immune thrombocytopenia, WBC: white blood cell, OR: overall response, NR: no response, R: response.

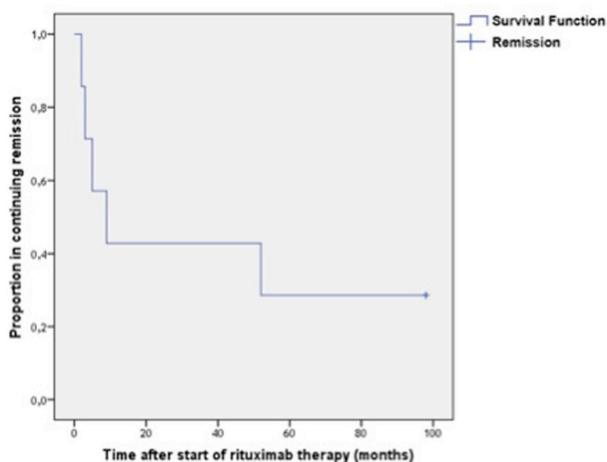


Figure 1. Proportion of patients with ongoing response during long-term follow-up. Two of the 7 patients (28.6%) still maintained their response 98 months after initiation of rituximab. 52 months (Figure 1). Figure 2 demonstrates the mean platelet counts in the whole population after initiation of rituximab. The mean platelet counts showed a trend to be higher in initial rituximab responders (n=7) compared to non-responders (n=8) (112,201±29,008/mm³ vs. 33,750±31,332/mm³, p=0.060, odds ratio: 7.8; 95% CI 35,212-176,049) (Figure 3).

Status of Patients at the Final Observation

Seven of the 15 patients (46.7%) showed an initial response (5 ER and 2 LR). However, 3 of the 5 early responders (20%) and all of the late responders lost their response, leaving 2 patients with long-lasting remissions with a mean follow-up of 89.7±19.4 months. Relapsed patients and patients with NR subsequently received various types of treatment, including steroids (n=12), eltrombopag (n=4), azathioprine (n=2), vincristine (n=1), danazol (n=1), IVIG (n=1), and accessory spleen operation (n=1). All of the initial 7 responders to rituximab achieved long-term remission even after relapse (5 CR, 2 PR), irrespective of subsequent treatment modalities (4: steroids, 2: eltrombopag, 1: azathioprine, 1: vincristine, 1: danazol, 1: IVIG) (Figure 4). In contrast, of the 8 non-responders, 2 patients still showed NR and 2 died (1 of intracranial hemorrhage and 1 of myocardial infarction). In total, 11 of the 15 patients (73.3%) achieved CR or PR during long-term observation with a mean follow-up time of 89.7±19.4 months.

Discussion

The algorithm for managing adult ITP has changed with the advent of rituximab and TPO-receptor agonists as options

Table 4. Review of the published data and our results in adults with immune thrombocytopenia treated with rituximab.

Ref.	Number of patients	ITP duration before rituximab	Previous therapies	Splenectomized patients	Overall response	Sustained response	Duration of response	Follow-up duration
Stasi et al. [11]	25	Median: 22 months (9-56)	2-5 different regimens	8 (32%)	13 (52%)	7 (28%) for more than 6 months	-	-
Cooper et al. [7]	57	Median: 34 months (3-360)	Median: 3 (2-8)	31 (54%)	31 (54%)	17 (29.8%) for more than 1 year	Median: 72.5 weeks (24-165)	-
Peñalver et al. [24]	89	Median: 31 months (1-305)	Median: 5 (2-13)	47 (53%)	49 (55.1%)	12 (13.4%) for more than 1 year	-	Median: 9 months (2-42)
Braendstrup et al. [12]	35	Median: 49 months (1-288)	Several different treatments	16 (45.7%)	17/39 treatments (40%)	5/39 treatments (12.8%) for more than 1 year	Median: 47 weeks	-
Santoro et al. [13]	19	Median: 2.1 years (0.33-33.1)	Median: 3 (2-6)	2 (10.5%)	9 (47.4%)	6 (31.6%) at last follow-up	-	Median: 53.2 months (9.2-92.9)
Zaja et al. [25]	20	-	At least one treatment	2 (10%)	13 (65%)	9 (45%) at last follow-up	-	Median: 180 days (60-480)
Aleem et al. [26]	24	-	Median: 3 (1-8)	11 (45.8%)	19/29 treatments (66%)	10 (24%) for more than 6 months	Median: 13 weeks (1 week to 55 months)	-
Zaja et al. [27]	37	Median: 34.5 months (1-264)	At least a full course of steroid therapy	5 (13.5%)	27 (73%)	15 (40.5%) at last follow-up	-	Median: 25 months (3-55) in responding patients
Pasa et al. [28]	17	-	Median: 5 (3-11)	17 (100%)	14 (82.3%)	10 (58.8%) for more than 6 months	Median: 19 months (9-41)	-
Present study	15	Mean±SD: 204±106.2 months	Mean±SD: 3.6±1.04	15 (100%)	7 (46.7%)	4 (26.7%) for more than 6 months	Mean±SD: 38.1±44.4 months	Mean±SD: 89.7±19.4 months

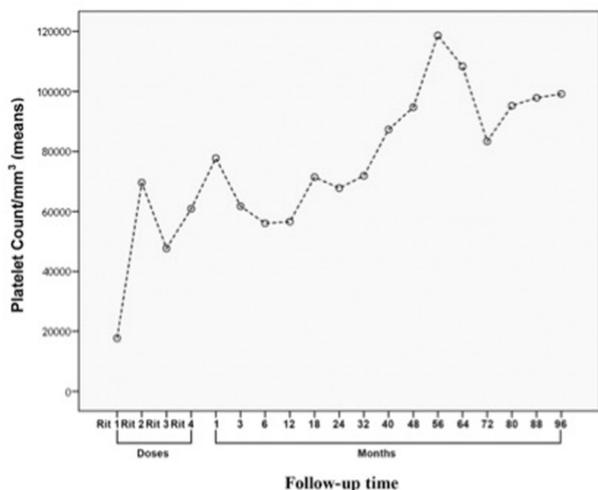


Figure 2. Mean platelet counts in the whole study group after initiation of rituximab. Relapsed patients and non-responders were treated with various other therapies. Mean platelet counts at times of first, second, third, and fourth doses of rituximab and 1st, 3rd, 6th, 12th, 18th, 24th, 32nd, 40th, 48th, 56th, 64th, 72nd, 80th, 88th, and 96th months of rituximab (\pm SD) were 17,400 \pm 8878/mm³, 70,666 \pm 122,495/mm³, 42,266 \pm 53,518/mm³, 53,533 \pm 79,974/mm³, 68,733 \pm 95,213/mm³, 54,333 \pm 81,260/mm³, 50,400 \pm 85,816/mm³, 50,266 \pm 79,408/mm³, 62,666 \pm 110,205/mm³, 59,880 \pm 116,443/mm³, 62,920 \pm 103,727/mm³, 76,746 \pm 101,374/mm³, 94,707 \pm 103,763/mm³, 118,607 \pm 128,846/mm³, 108,315 \pm 119,597/mm³, 83,384 \pm 107,987/mm³, 95,230 \pm 104,396/mm³, 97,846 \pm 98,858/mm³, and 99,153 \pm 99,049/mm³, respectively.

for second-line treatment. The lack of studies comparing splenectomy to other second-line therapy options presents an important dilemma. Rituximab may be a curative therapy with an initial response in 50%-60% of ITP patients and a SR of 3-5 years in 20% of patients [23]. The primary aim of our study was to evaluate the long-term efficacy of rituximab treatment in 15 patients with chronic refractory ITP. The patients described in this study had persistent, severe ITP and had received a mean of 3.6 \pm 1.04 previous therapies.

Table 4 summarizes our results as well the results of previous studies describing adult ITP patients treated with rituximab. Seven of our 15 patients achieved an initial response to rituximab (46.7%) (5 ER and 2 LR). Various studies have used different criteria to define response to ITP treatment. Our results are comparable with those of several studies that reported response rates between 40% and 55.1% [7,11,12,13,24]. However, our results are less favorable compared to several other reports [6,25,26,27,28]. In the systematic review by Arnold et al., the overall platelet count response to rituximab was 62.5% in adult ITP patients [6]. Zaja et al. in 2 different studies reported an initial response rate of 65% (13/20 patients) and 73% (27/37 patients), respectively [25,27]. The latter study hypothesized that earlier administration of rituximab enables higher rates of long-lasting response in adult ITP [27]. Moreover, our results are inferior to those of other studies reporting an OR of 66% and 82.3%, respectively [26,28].

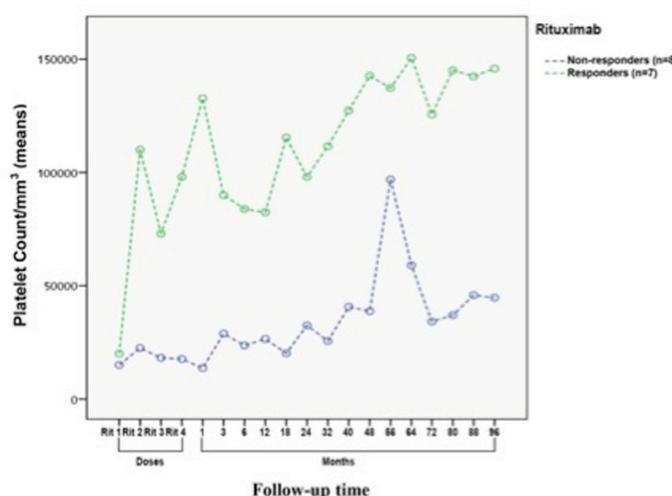


Figure 3. Comparison of mean platelet counts in responders and non-responders following rituximab therapy. There was a trend towards higher mean platelet counts (\pm SD) in initial rituximab responders (n=7) compared to non-responders (n=8) (112,201 \pm 29,008/mm³ vs. 33,750 \pm 31,332/mm³, p=0.06, odds ratio: 7.8; 95% CI 35,212-176,049). In rituximab responders mean platelet counts at times of first, second, third, and fourth doses of rituximab and 1st, 3rd, 6th, 12th, 18th, 24th, 32nd, 40th, 48th, 56th, 64th, 72nd, 80th, 88th, and 96th months of rituximab (\pm SD) were 20,000 \pm 2905/mm³, 110,000 \pm 47,594/mm³, 73,000 \pm 18,886/mm³, 98,000 \pm 28,684/mm³, 132,714 \pm 30,811/mm³, 90,000 \pm 31,195/mm³, 83,857 \pm 33,795/mm³, 82,428 \pm 31,051/mm³, 115,428 \pm 41,586/mm³, 98,028 \pm 46,959/mm³, 111,571 \pm 39,294/mm³, 127,342 \pm 37,568/mm³, 142,657 \pm 35,001/mm³, 137,228 \pm 50,189/mm³, 150,571 \pm 43,329/mm³, 125,571 \pm 38,298/mm³, 145,142 \pm 34,753/mm³, 142,428 \pm 33,638/mm³, and 145,857 \pm 33,156/mm³, respectively. In non-responders mean platelet counts at times of first, second, third, and fourth doses of rituximab and 1st, 3rd, 6th, 12th, 18th, 24th, 32nd, 40th, 48th, 56th, 64th, 72nd, 80th, 88th, and 96th months of rituximab (\pm SD) were 15,000 \pm 3138/mm³, 22,500 \pm 51,407/mm³, 18,166 \pm 20,399/mm³, 17,666 \pm 30,983/mm³, 13,666 \pm 33,279/mm³, 28,833 \pm 33,695/mm³, 23,666 \pm 36,502/mm³, 26,500 \pm 33,539/mm³, 20,166 \pm 44,918/mm³, 32,500 \pm 50,722/mm³, 25,633 \pm 42,442/mm³, 40,633 \pm 40,578/mm³, 38,766 \pm 37,805/mm³, 96,883 \pm 54,210/mm³, 59,016 \pm 46,800/mm³, 34,166 \pm 41,367/mm³, 37,000 \pm 37,537/mm³, 45,833 \pm 36,334/mm³, and 44,666 \pm 35,812/mm³, respectively.

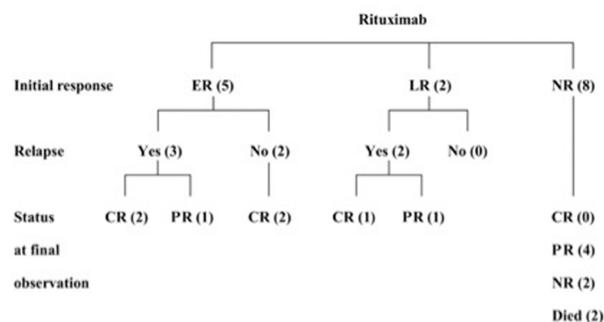


Figure 4. Algorithm of long-term outcome of 15 adults with immune thrombocytopenia after treatment with rituximab. ER, LR, CR, PR, and NR denote early response, late response, complete response, partial response, and no response, respectively.

The strength of our study lies in the long follow-up period of our patients with a mean duration of 89.7 ± 19.4 months. We showed that 5 of the 7 responding patients (71.4%) (3/5 ER, 2/2 LR) relapsed after 2, 3, 5, 9, and 52 months, respectively. Four relapses occurred within 1 year after initial response. These findings are largely in line with previous data [9,29]. Patel et al. reported late relapses 2 years after the initiation of rituximab in adults and suggested that regular follow-up at 3-month intervals is indicated at least for the first 5 years in adults [20]. In our study, 2 adult patients showed continued response after 98 months and 1 patient relapsed 52 months after initiation of rituximab. A higher relapse rate occurred among our patients with LR compared to those with ER (100% vs. 60%). However, due to the small size of our study population, patients with ER and LR could not be compared. This issue will be the subject of our further studies.

We evaluated the relationship between clinical and laboratory variables and response to rituximab. Our results are in line with findings of several studies, which reported that splenectomy, age, sex, number of previous treatments, and pretreatment platelet count were not associated with response to rituximab [7,12]. In line with the study by Santoro et al., we demonstrated that the time between diagnosis and the start of rituximab therapy did not correlate with response to rituximab [13]. Further studies are needed to determine the best scheduling of rituximab in the course of ITP.

Considering the entire population, the SR rate in our study was 26.7% (4/15 patients) with a duration over 6 months and a mean follow-up of 89.7 ± 19.4 months. The disease-free survival of these 4 patients at 98 months was 50%. Several other authors reported SR rates ranging from 28% to 40.5% [7,11,13,19,27]. Garcia-Chaves et al. reported a SR rate of 67% over a duration of 6 months, a finding superior to our results [30]. In that study, the mean number of therapies was 5.5 and 83% of patients had failed to respond to splenectomy [30]. In the pilot study by Arnold et al., the OR rate to rituximab at 6 months in non-splenectomized adults with newly diagnosed or relapsed primary ITP was 62.5% [31]. The aforementioned study is crucial in evaluating whether rituximab could be a valuable therapeutic alternative to splenectomy [31]. Godeau et al. evaluated the efficacy of rituximab in adult chronic ITP patients who had received at least 1 previous therapy and were potential candidates for splenectomy and reported that at 2 years the response rate was 40% [19]. Patel et al., in their study on long-term outcome after initiation of rituximab, reported that 21% of adults maintained response for at least 5 years [20]. Expert consensus reported that 80% of ITP patients respond to splenectomy, and the response is sustained in approximately two-thirds of patients over 5-10 years [10,32]. Taking into account our results and literature review on efficacy and long-lasting response rate, we think that rituximab treatment has a role in a subset of chronic ITP patients.

Conclusion

To conclude, in 15 chronic refractory ITP patients, we showed an initial response rate and SR of 46.7% and 26.7%, respectively, while 71.4% of responders subsequently relapsed in a mean follow-up period of 89.7 months. The continuing effect of rituximab had declined to 13.3% (2/15 patients) at the last follow-up. Of importance in clinical practice is the observation that all initial responders to rituximab achieved long-lasting remission even after relapse, independent of subsequent therapies. Over a very long period of immunosuppression, we did not record any serious adverse events. In the era of TPO-receptor agonists, we think that rituximab still has a role in the treatment of chronic refractory ITP. Our data, based on a long period of observation, confirm the efficacy of rituximab in refractory primary ITP. Future randomized studies including large case series are needed to determine the optimal role of rituximab and which subgroup of ITP patients can most benefit from this therapy.

Ethics

Ethics Committee Approval: Rituximab was offered to these patients as an off-label treatment following the approval of the Ministry of Health; Informed Consent: Informed consent for study participation was obtained from all patients.

Authorship Contributions

Research Design: Fehmi Hindilerden, İpek Yönel-Hindilerden, Meliha Nalçacı, Reyhan Diz-Küçükkaya; Concept: Fehmi Hindilerden, Mustafa Nuri Yenerel, Reyhan Diz-Küçükkaya; Data Collection or Processing: Fehmi Hindilerden, İpek Yönel-Hindilerden; Analysis or Interpretation: Fehmi Hindilerden; Literature Search: Fehmi Hindilerden, İpek Yönel-Hindilerden; Writing: Fehmi Hindilerden, İpek Yönel-Hindilerden, Mustafa NuriYenerel, Meliha Nalçacı, Reyhan Diz-Küçükkaya.

Conflict of Interest: The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

References

1. Rodeghiero F, Stasi R, Gernsheimer T, Michel M, Provan D, Arnold DM, Bussel JB, Cines DB, Chong BH, Cooper N, Godeau B, Lechner K, Mazzucconi MG, McMillan R, Sanz MA, Imbach P, Blanchette V, Kühne T, Ruggeri M, George JN. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. *Blood* 2009;113:2386-2393.
2. Godeau B, Stasi R. Is B-cell depletion still a good strategy for treating immune thrombocytopenia? *Presse Med* 2014;43:e79-85.
3. George JN, Woolf SH, Raskob GE, Wasser JS, Aledort LM, Ballem PJ, Blanchette VS, Bussel JB, Cines DB, Kelton JG, Lichtin AE, McMillan R, Okerbloom JA, Regan DH, Warrior I. Idiopathic thrombocytopenic purpura: a practice guideline developed by explicit methods for the American Society of Hematology. *Blood* 1996;88:3-40.

4. Kojouri K, Vesely SK, Terrell DR, George JN. Splenectomy for adult patients with idiopathic thrombocytopenic purpura: a systematic review to assess long-term platelet count responses, prediction of response, and surgical complications. *Blood* 2004;104:2623-2634.
5. George JN. Management of patients with refractory immune thrombocytopenic purpura. *J Thromb Haemost* 2006;4:1664-1672.
6. Arnold DM, Dentali F, Crowther MA, Meyer RM, Cook RJ, Sigouin C, Fraser GA, Lim W, Kelton JG. Systematic review: efficacy and safety of rituximab for adults with idiopathic thrombocytopenic purpura. *Ann Intern Med* 2007;146:25-33.
7. Cooper N, Stasi R, Cunningham-Rundles S, Feuerstein MA, Leonard JP, Amadori S, Bussel JB. The efficacy and safety of B-cell depletion with anti-CD20 monoclonal antibody in adults with chronic immune thrombocytopenic purpura. *Br J Haematol* 2004;125:232-239.
8. Zaja F, Iacona I, Masolini P, Russo D, Sperotto A, Prosdocimo S, Patriarca F, de Vita S, Regazzi M, Baccarani M, Fanin R. B-cell depletion with rituximab as treatment for immune hemolytic anemia and chronic thrombocytopenia. *Haematologica* 2002;87:189-195.
9. Bennett CM, Rogers ZR, Kinnamon DD, Bussel JB, Mahoney DH, Abshire TC, Sawaf H, Moore TB, Loh ML, Glader BE, McCarthy MC, Mueller BU, Olson TA, Lorenzana AN, Mentzer WC, Buchanan GR, Feldman HA, Neufeld EJ. Prospective phase 1/2 study of rituximab in childhood and adolescent chronic immune thrombocytopenic purpura. *Blood* 2006;107:2639-2642.
10. Provan D, Stasi R, Newland AC, Blanchette VS, Bolton-Maggs P, Bussel JB, Chong BH, Cines DB, Gernsheimer TB, Godeau B, Grainger J, Greer I, Hunt BJ, Imbach PA, Lyons G, McMillan R, Rodeghiero F, Sanz MA, Tarantino M, Watson S, Young J, Kuter DJ. International consensus report on the investigation and management of primary immune thrombocytopenia. *Blood* 2010;115:168-186.
11. Stasi R, Pagano A, Stipa E, Amadori S. Rituximab chimeric anti-CD20 monoclonal antibody treatment for adults with chronic idiopathic thrombocytopenic purpura. *Blood* 2001;98:952-957.
12. Braendstrup P, Bjerrum OW, Nielsen OJ, Jensen BA, Clausen NT, Hansen PB, Andersen I, Schmidt K, Andersen TM, Peterslund NA, Birgens HS, Plesner T, Pedersen BB, Hasselbalch HC. Rituximab chimeric anti-CD20 monoclonal antibody treatment for adult refractory idiopathic thrombocytopenic purpura. *Am J Hematol* 2005;78:275-280.
13. Santoro C, Biondo F, Baldacci E, De Propriis MS, Guarini A, Paoloni F, Foà R, Mazzucconi MG. Rituximab in previously treated primary immune thrombocytopenia patients: evaluation of short- and long-term efficacy and safety. *Acta Haematol* 2014;132:24-29.
14. Schweizer C, Reu FJ, Ho AD, Hensel M. Low rate of long-lasting remissions after successful treatment of immune thrombocytopenic purpura with rituximab. *Ann Hematol* 2007;86:711-717.
15. Al-Habsi K, Al-Khabori M, Al-Muslahi M, Pathare A, Al-Farsi K, Al-Huneini M, Al-Lamki S, Al-Kindi S. Rituximab leads to long remissions in patients with chronic immune thrombocytopenia. *Oman Med J* 2015;30:111-114.
16. Chugh S, Darvish-Kazem S, Lim W, Crowther MA, Ghanima W, Wang G, Heddle NM, Kelton JG, Arnold DM. Rituximab plus standard of care for treatment of primary immune thrombocytopenia: a systematic review and meta-analysis. *Lancet Haematol* 2015;2:e75-81.
17. Khellaf M, Charles-Nelson A, Fain O, Terriou L, Viillard JF, Cheze S, Gravelleau J, Slama B, Audia S, Ebbo M, Le Guenno G, Cliquennois M, Salles G, Bonmati C, Teillet F, Galicier L, Hot A, Lambotte O, Lefrère F, Sacko S, Kengue DK, Bierling P, Roudot-Thoraval F, Michel M, Godeau B. Safety and efficacy of rituximab in adult immune thrombocytopenia: results from a prospective registry including 248 patients. *Blood* 2014;124:3228-3236.
18. Medeot M, Zaja F, Vianelli N, Battista M, Baccarani M, Patriarca F, Soldano F, Isola M, De Luca S, Fanin R. Rituximab therapy in adult patients with relapsed or refractory immune thrombocytopenic purpura: long-term follow-up results. *Eur J Haematol* 2008;81:165-169.
19. Godeau B, Porcher R, Fain O, Lefrère F, Fenaux P, Cheze S, Vekhoff A, Chauveheid MP, Stirnemann J, Galicier L, Bourgeois E, Haiat S, Varet B, Leporrier M, Papo T, Khellaf M, Michel M, Bierling P. Rituximab efficacy and safety in adult splenectomy candidates with chronic immune thrombocytopenic purpura: results of a prospective multicenter phase 2 study. *Blood* 2008;112:999-1004.
20. Patel VL, Mahévas M, Lee SY, Stasi R, Cunningham-Rundles S, Godeau B, Kanter J, Neufeld E, Taube T, Ramenghi U, Shenoy S, Ward MJ, Mihatov N, Patel VL, Bierling P, Lesser M, Cooper N, Bussel JB. Outcomes 5 years after response to rituximab therapy in children and adults with immune thrombocytopenia. *Blood* 2012;119:5989-5995.
21. Zaja F, Volpetti S, Chiozzotto M, Puglisi S, Isola M, Buttignol S, Fanin R. Long-term follow-up analysis after rituximab salvage therapy in adult patients with immune thrombocytopenia. *Am J Hematol* 2012;87:886-889.
22. Gudbrandsdottir S, Birgens HS, Frederiksen H, Jensen BA, Jensen MK, Kjeldsen L, Klausen TW, Larsen H, Mourits-Andersen HT, Nielsen CH, Nielsen OJ, Plesner T, Pulczynski S, Rasmussen IH, Rønnev-Jessen D, Hasselbalch HC. Rituximab and dexamethasone vs dexamethasone monotherapy in newly diagnosed patients with primary immune thrombocytopenia. *Blood* 2013;121:1976-1981.
23. Ghanima W, Godeau B, Cines DB, Bussel JB. How I treat immune thrombocytopenia: the choice between splenectomy or a medical therapy as a second-line treatment. *Blood* 2012;120:960-969.
24. Peñalver FJ, Jiménez-Yuste V, Almagro M, Alvarez-Larrán A, Rodríguez L, Casado M, Gallur L, Giraldo P, Hernández R, Menor D, Rodríguez MJ, Caballero D, González R, Mayans J, Millán I, Cabrera JR; Multi-institutional Retrospective Spanish Study Group on the Use of Rituximab in Refractory ITP. Rituximab in the management of chronic immune thrombocytopenic purpura: an effective and safe therapeutic alternative in refractory patients. *Ann Hematol* 2006;85:400-406.
25. Zaja F, Vianelli N, Sperotto A, De Vita S, Iacona I, Zaccaria A, Masolini P, Tomadini V, Tani M, Molinari AL, Baccarani M, Fanin R. B-cell compartment as the selective target for the treatment of immune thrombocytopenias. *Haematologica* 2003;88:538-546.
26. Aleem A, Alaskar AS, Algahtani F, Rather M, Almahayni MH, Al-Momen A. Rituximab in immune thrombocytopenia: transient responses, low rate of sustained remissions and poor response to further therapy in refractory patients. *Int J Hematol* 2010;92:283-288.
27. Zaja F, Vianelli N, Battista M, Sperotto A, Patriarca F, Tomadini V, Fili C, Tani M, Baccarani M, Fanin R. Earlier administration of rituximab allows higher rate of long-lasting response in adult patients with autoimmune thrombocytopenia. *Exp Hematol* 2006;34:571-572.
28. Pasa S, Altintas A, Cil T, Danis R, Ayyildiz O. The efficacy of rituximab in patients with splenectomized refractory chronic idiopathic thrombocytopenic purpura. *J Thromb Thrombolysis* 2009;27:329-333.
29. Mueller BU, Bennett CM, Feldman HA, Bussel JB, Abshire TC, Moore TB, Sawaf H, Loh ML, Rogers ZR, Glader BE, McCarthy MC, Mahoney DH, Olson TA, Feig SA, Lorenzana AN, Mentzer WC, Buchanan GR, Neufeld EJ; Pediatric Rituximab/ITP Study Group; Glaser Pediatric Research Network. One year follow-up of children and adolescents with chronic immune thrombocytopenic purpura (ITP) treated with rituximab. *Pediatr Blood Cancer* 2009;52:259-262.
30. Garcia-Chavez J, Majluf-Cruz A, Montiel-Cervantes L, Esparza MG, Vela-Ojeda J; Mexican Hematology Study Group. Rituximab therapy for chronic and refractory immune thrombocytopenic purpura: a long-term follow-up analysis. *Ann Hematol* 2007;86:871-877.
31. Arnold DM, Heddle NM, Carruthers J, Cook DJ, Crowther MA, Meyer RM, Liu Y, Cook RJ, McLeod A, MacEachern JA, Mangel J, Anderson D, Vickers L, Tinmouth A, Schuh AC, Kelton JG. A pilot randomized trial of adjuvant rituximab or placebo for nonsplenectomized patients with immune thrombocytopenia. *Blood* 2012;119:1356-1362.
32. Neunert C, Lim W, Crowther M, Cohen A, Solberg L Jr, Crowther MA; American Society of Hematology. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. *Blood* 2011;117:4190-4207.