EFFECTS OF RITUXIMAB ON PROGNOSIS IN MYASTHENIA GRAVIS: A SINGLE-CENTER EXPERIENCE FROM TURKEY

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Background and purpose – Management of treatment-resistant patients with myasthenia gravis (MG) remains an important issue. This study aimed to evaluate the effects of rituximab (RTX) treatment on the prognosis of patients with acetylcholine receptor autoantibody-positive (AChR-Ab+), muscle-specific kinase autoantibody-positive (MuSK-Ab+), or seronegative or double seropositive MG.
Methods – Nineteen patients treated with RTX between 2015 and 2020 were included in this study. Demographic and clinical characteristics, prognosis, and prognostic predictors of MG were evaluated retrospectively. The Myasthenia Gravis Foundation of America Post-Intervention Status (MGFA-PIS) before RTX treatment (pre-RTX) and after RTX treatment (post-RTX) were recorded.
Results – A total of 10 patients (52.6%) were AchR Ab+, 6 patients (31.6%) were MuSK Ab+, 1 patient (5.3%) was seronegative, and 2 patients (10.5%) were double seropositive. Steroid dose was pre-RTX 38.9±5.7 (25-45), it was post-RTX 10.5±10.3 (0-30) ($p<0.001$). Post-RTX steroid treatment was discontinued in 6 of 19 patients ($p=0.041$). Only three patients received intravenous immunoglobulin at the post-RTX follow-up ($p<0.001$). In post-RTX 12th month, the MGFA-PIS score was as minimally manifestation or better in 9 patients (47.3%) and improved or was better in 18 patients (94.7%) (p-value 0.004; <0.001, respectively).
Conclusion – The improvement in MGFA-PIS scores post-RTX was similar in MuSK-Ab+ and AChR-Ab+ patients. The data are insufficient in seronegative and double seropositive patients and RTX must be considered in the treatment of suitable patients with MuSK-Ab+ and AChR-Ab+ refractory MG.

A RITUXIMAB HATÁSA A MYASTHENIA GRAVIS PROGNÓZISÁRA: EGY KÖZPONTÚ, TÖRÖKORSZÁGI TAPASZTALAT

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Háttér és cél – A terápiára nem reagáló myasthenia gravis- (MG-) betegek kezelése fontos problémát jelent. Vizsgálatunk célja az volt, hogy megvizsgáljuk a rituximab- (RTX-) kezelés hatását az acetilkolin-receptor-ellenes autoantitest-pozitív (AChR-Ab+), az izomspecifikus kináz-ellenes autoantitest-pozitív (MuSK-Ab+), a szeronegatív és a dupla szeropozitív MG-ben szenvedő betegek esetében.
Módszerek – A vizsgálatba 19 olyan MG-beteget vontunk be, akiket 2015 és 2020 között RTX-szel kezeltek. Retrospektív módon összegyűjtöttük a betegek demográfiai és klinikai adatait, valamint értékeltük az MG prognosztikus prediktorait. Feljegyeztük az RTX-kezelés előtti (pre-RTX) és utáni (post-RTX) MGFA-PIS-értékeket (MGFA-PIS: Myasthenia Gravis Foundation of America Post-Intervention Status).
Eredmények – 10 beteg (52,6%) volt AChR-Ab+, hat beteg (31,6%) volt MuSK-Ab+, egy beteg (5,3%) volt szeronegatív, és két beteg (10,5%) volt dupla szeropozitív. A pre-RTX szteroiddózis 38,9 ± 5,7 (25–45) volt, a post-RTX szteroiddózis pedig 10,5 ± 10,3 (0–30) ($p < 0,001$). A post-RTX szteroid-kezelés a 19 betegből hat esetében vált szükségtelenné ($p = 0,041$). A post-RTX-utánkövetés során mindössze három beteg kapott intravénás immunglobulint ($p < 0,001$). Az RTX-kezelés utáni 12. hónapban az MGFA-PIS-pontszám kilenc beteg (47,3%) esetében minimális vagy jobb értékeket mutatott, 18 beteg (94,7%) esetében pedig javuló vagy jobb manifesztációt (p-érték: 0,004; <0,001).
Következtetés – Az RTX-kezelés utáni MGFA-PIS-pontszám-javulás hasonló volt a MuSK-Ab+ és az AChR-Ab+ betegek körében. A szeronegatív és a dupla szeropozitív MG-betegek esetében elégtelen mennyiségű adat

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keletkezett. Az RTX-kezelés megfontolandó a megfelelően kiválasztott, terápiarezisztens MuSK-Ab+ és AChR-Ab+ MG-betegek esetében.

Keywords: *myasthenia gravis, rituximab, clinical prognosis, acetylcholine receptor autoantibody*

Kulcsszavak: *myasthenia gravis, rituximab, klinikai prognózis, acetilkolin-receptor-ellenes autoantitest*

Myasthenia gravis (MG) is a chronic autoimmune disease involving the neuromuscular junction (NMJ), characterized by muscle weakness and fatigue, with a prevalence of 20-50 per 100.000 people[1, 2]. Autoantibodies that are developed against NMJ proteins especially acetylcholine receptor subunits play roles in its pathogenesis[3].

Acetylcholine receptor antibodies (AChR-Ab), muscle-specific kinase antibodies (MuSK-Ab), antibodies against lipoprotein-associated protein 4 or agrin are detected in MG, but in some cases specific antibody profile cannot be defined[4–6]. AChR-Ab is detected in 85% of MG patients. MuSK-Ab is detected in half of the remaining patients, antibodies cannot be detected with standard methods in the other half, and are considered seronegative MG[7].

Although many immunosuppressive and immunomodulatory treatment modalities are available, 10-20% of MG patients are resistant to conventional treatment. Patients who are resistant to this treatment require intravenous immunoglobulin (IVIg) and plasma exchange (PLEX)[8, 9]. Refractory MG reduces the quality of life of patients and increases hospitalization[10].

Alternative effective treatments e.g. rituximab (RTX) have recently emerged to improve the prognosis[11]. RTX is a chimeric monoclonal antibody that is specific for the B-cell surface antigen CD20[7]. Favorable treatment response with RTX in refractory MG was reported in a meta-analysis that was based on observational studies with small case series[12]. It was given that RTX is safe, effective, and fast-acting in MuSK-Ab+ patients[13]. In contrast, a randomized clinical trial that evaluated the reduction in corticosteroid use as the primary outcome in AChR-Ab+ refractory MG failed to demonstrate a benefit of RTX over the placebo group[14].

The American Academy of Neurology's (AAN) latest updated 2020 guide on MG management is similar to the 2016 Guide in terms of RTX treatment recommendations. Current guidelines recommend RTX treatment as an early treatment option in MuSK-Ab+ patients who do not respond to initial immunotherapy at sufficient levels. However, the benefit of RTX remains unclear in AChR-Ab+ patients. No recommendations were made in this Guide for RTX treatment for seronegative and double seropositive MG (DSPMG) patients[15].

In the present study, the effects of RTX on the prognosis were evaluated in our cohort that included AChR-Ab+, MuSK-Ab+, seronegative, and DSPMG patients.

Patients and methods

Among 350 MG patients who were followed between 2015 and 2020 in the Neurology Department of Karadeniz Technical University Faculty of Medicine, which is the reference center in the northern part of Turkey, 19 RTX- treated patients were included in the study. The approval for the study was obtained from the Karadeniz Technical University Faculty of Medicine Ethics Council (Ethics Committee no: 2020/776, dated 21.12.2020).

The inclusion criteria were: age >18 years, MG with AChR-Ab+ or MuSk-Ab+ or seronegative but confirmed with electroneuromyography, Myasthenia Gravis Foundation of America Clinical Classification (MGFA-CC) III, IV or V, and receiving RTX treatment.

The demographic and clinical characteristics, prognosis, and prognostic predictors obtained from the medical records of the patients were evaluated retrospectively. The baseline MGFA-CC and Myasthenia Gravis Foundation of America Post-Intervention Status (MGFA-PIS), and the RTX treatment (post-RTX) in the 3-6-12 months were recorded[16]. MGFA-CC IIIa and IIIb were grouped as moderate weakness, and MGFA-CC IVa, IVb, and V were grouped as severe weakness. Adequate response to RTX treatment was defined as improved or better (improved, minimal manifestation, pharmacologic remission, complete stable remis-

sion), and minimal manifestation or better (minimal manifestation, pharmacologic remission, complete stable remission) in MGFA-PIS. Secondary outcomes were at least 50% reduction in steroid dose, and no requirement for IVIg or PLEX within 12 months post-RTX.

Rituximab was administered to the cases if the immunotherapy dose could not be reduced, the desired clinical improvement could not be achieved, and/or there were serious side effects because of immunosuppressive treatment, and if the patient received IVIg or PLEX in the last 1 year. Since there was no established treatment protocol for RTX in MG, "rheumatological disease-like treatment protocol" was used as the treatment protocol in the study. In other words, 1000 mg RTX IV infusion was administered on the 1st and 15th days, and 1000 mg RTX infusion every 6 months according to the clinical status[9]. All patients were premedicated with Methylprednisolone and Acetaminophen before the infusion. Adverse events that were associated with RTX treatment and overall mortality were recorded.

STATISTICAL ANALYSIS

Statistical analyzes were made in the IBM SPSS for Windows Version 22.0 package program. Numerical variables were summarized as mean±standard and [Min-Max] values, and categorical variables were summarized as numbers and percentages. The difference between the two groups in terms of numerical variables was investigated with the Mann-Whitney U test. The Chi-Square Test was used to determine whether there were relations between the categorical variables. The McNemar test was used to compare the categorical variables in dependent groups. The significance level was taken as $p<0.05$.

Results

Among the 19 generalized MG patients included in our study, 9 were female (47.4%), and 10 were male (52.6%). The mean age was 48.6±12.3 (28-74 years). When classified according to antibody, 10 patients (52.6%) were AchR-Ab+, 6 patients (31.6%) were MuSK-Ab+, 1 patient (5.3%) was seronegative, and 2 patients (10.5%) were DSPMG. Before the RTX treatment (pre-RTX), 12 patients (63.2%) had a moderate weakness, and 7 patients (36.8%) had a severe weakness. The demographic and clinical characteristics of the patients are given in **Table 1**.

The cumulative dose of RTX was 3.4±1.6 (1-6) g in AChR-Ab+ patients, and 3.3±1.5 (1-5) g in MuSK-Ab+ patients (p=1.000). Seronegative patients received a total of 5 g RTX, and DSPMG patients received a total of 2.5±2.1 (1-4) g RTX. When the time until RTX initiation in MG patients was compared, it was 10.4±7.5 (4-26) years in AChR-Ab+ patients, and 4.8±4.6 (1-12) years in MuSK-Ab+ patients (p=0.056). Seronegative patients were followed for 13 years, and DSPMG patients were followed for 3.0±2.8 (1-5) years with MG diagnosis **(Table 1)**.

Before RTX, although the steroid dose was 38.9±5.7 (25-45) in all MG patients, 42.0±3.5 (35-45) in AChR-Ab+ patients, 38.3±4.1 in MuSK-Ab+ patients (30-40), post-RTX steroid dose was 10.5±10.3 (0-30) in all MG patients, 14.0±9.4 (0-30) in AChR-Ab+ patients, 8.3±12.1 (0-30) in MuSK-Ab+ patients (p-value <0.001; 0.005; 0.027, respectively). Post-RTX steroid treatment was discontinued in 6 of the 19 patients who received pre-RTX steroids (p=0.041). A patient with seronegative MG who received pre-RTX 25 mg steroid was discontinued from post-RTX steroid. Post-RTX steroid was discontinued in one of the 2 patients with DSPMG, and a ≥50% reduction was observed in steroid requirement in the other patient. Overall, the steroid dose was reduced by more than 50 percent in 84.2% of patients with MG, in 80% of AChR-Ab+ patients, and 83.3% of MuSK-Ab+ patients. Although 19 patients needed IVIg treatment in pre-RTX one year, only 3 patients had to receive IVIg treatment in post-RTX one year ($p < 0.001$). No patient required PLEX within one-year post-RTX. The treatments of pre-RTX and post-RTX patients are given in **Table 2**.

When the MGFA-PIS scores before and at 3-6-12th months after RTX treatment were compared, the MGFA-PIS score at 3 months post-RTX had minimal manifestation or was better in 3 patients (15.7%), and was improved or better in 18 patients (94.7%), (p-value 0.250; <0.001, respectively); at 6 months post-RTX, the MGFA-PIS score was minimally manifested or better in 6 patients (31.6%), and improved or better in 18 patients (94.7%) (p-value 0.031; <0.001, respectively). At 12 months post-RTX, the MGFA-PIS score was minimally manifested or better in 9 patients (47.3%) and improved or better in 18 patients (94.7%) (p-value 0.004; <0.001, respectively). The pre-RTX and post-RTX MGFA-PIS scores of MG patients are given in **Table 3**.

The effects of time elapsed before RTX treatment, gender, antibody titer before and after RTX treatment, AChR-Ab or MuSK-Ab positivity, pre-

Table 1. *Clinical and demographic characteristics of patients with myasthenia gravis*

		AChR-Ab+ (n=10)	MuSK-Ab+ (n=6)	Seronegative (n=1)	Double seropositive (n=2)
Gender (M/F)		4/6	5/1	–/1	1/1
Age		49.3±9.7 (28 – 63)	52.5±16.2 (30 – 74)	47	34.5±9.2 (28 – 41)
Duration of diagnosed MG before RTX (years)		10.4±7.5 (4 – 26)	4.8±4.6 (1 – 12)	13	3.0±2.8 (1 – 5)
MGFA-CC	IIIa	4 (40%)	–	–	–
	IIIb	3 (30%)	3 (50%)	1 (100%)	1 (50%)
	IVa	2 (20%)	2 (33.3%)	–	–
	IVb	1 (10%)	–	–	1 (50%)
	V	–	1 (16.7%)	–	–
Thymus pathology	No	2 (20%)	6 (100%)	1 (100%)	1 (50%)
	Thymoma	7 (70%)	–	–	1 (50%)
	Thymus hyperplasia	1 (10%)	–	–	–
Thymectomy		7 (70%)	–	–	1 (50%)
Coexisting extrathymic malignancy		–	1 (16.7%)	–	–
Coexisting autoimmune disease		4 (40%)	–	–	2 (100%)
Medication before	Pyridostigmine	10 (100%)	6 (100%)	1 (100%)	2 (100%)
RTX treatment	Steroid	10 (100%)	6 (100%)	1 (100%)	2 (100%)
	Azathioprine	7 (70%)	4 (66.7%)	1 (100%)	2 (100%)
	Mycophenolate mofetil	2 (20%)	1 (16.7%)	–	-
Use of IVIg before RTX (previous 1 year)		10 (100%)	6 (100%)	1 (100%)	2 (100%)
Use of PLEX before RTX (previous 1 year)		2 (20%)	2 (33.3%)	–	–
Cumulative dose of RTX (gr)		3.4±1.6 (1–6)	3.3±1.5 (1–5)	5	2.5±2.1 (1–4)
Advers effect of RTX		–	–	–	1 (50%)
Advers effect of steroid		2 (20%)	1 (16.7%)	–	–
Death		1 (10%)	–	–	–

MG: myasthenia gravis, MGFA-CC: Myasthenia Gravis Foundation of America Clinical Classification, RTX: rituximab, IVIg: intravenous immune globulin, PLEX: plasma exchange

RTX MG severity, ≥50% reduction in steroid dose, and thymus pathology were evaluated on the MGFA-PIS score after RTX treatment at 12 months. No statistically significant relations were detected between the MGFA-PIS score and these parameters **(Table 4)**.

One patient had a bladder tumor as an extrathymic malignancy that accompanied Myasthenia Gravis. As an autoimmune disease, MG was accompanied by autoimmune Thyroid Disease (Graves' Disease or Hashimoto's Thyroiditis) in 5 patients and Pemphigus Vulgaris in 1 patient. As adverse events, urticaria and chest tightness because of RTX infusion developed in 1 patient with accompanying autoimmune disease. The infusion was stopped and was then continued at a slower rate with premedication after the complaints were gone. One patient with thymoma and pemphigus vulgaris, who last received RTX treatment two years ago, but continued to take other immunosuppressive treatments, died because of esophageal candidiasis.

Discussion

Rituximab studies are generally retrospective in MG. In the present study, the demographic data, clinical findings, and the effects of RTX treatment on the prognosis of 19 patients who were diagnosed with generalized MG, treated with RTX in a reference center in the northern part of Turkey were presented. It was found according to the data obtained in our study that the use of IVIg and/or PLEX, the number of patients using steroids, and the steroid dose decreased in the first year after RTX treatment. It was also observed that the improvement in MGFA-PIS scores starting from the 3rd month was more pronounced in the 12th month, and the improvement in the MGFA-PIS scores was similar in MuSK-Ab+ and AChR-Ab+ patients. The present study is important in that it includes seronegative and DSPMG patients.

In a multicenter prospective study, 14 (58%) of the 24 patients with MuSK-Ab+ MG, who were

Table 2. *Comparison of treatments before and after rituximab treatment*

	All (n=19)			AChR-Ab (n=10)			MuSK-Ab (n=6)		
	Pre-rituximab	Post-rituximab	p-Value	Pre-rituximab	Post-rituximab	p-Value	Pre-rituximab	Post-rituximab	p-Value
Steroid dose Mean±SD	38.9±5.7	10.5±10.3		42.0±3.5	14.0±9.4		38.3±4.1	8.3±12.1	
(min-max)	(25–45)	(0–30)	<0.001	(35–45)	(0–30)	0.005	(30–40)	(0–30)	0.027
Steroid	19	13	0.041	10	9	1.000	6	3	0.248
Pyridostigmine	19	17	0.479	10	10	1.000	6	4	0.479
Mycophenolate mofetil	3	1	0.500	2	1	1.000	1	0	NA
Azathioprine	14	12	0.687	7	5	0.500	4	5	1.000
Steroid dose reduced by at least 50%	16 (84.2%)			8 (80%)			5 (83.3%		
Use of IVIg	19	3	<0.001	10	1	0.008	6	2	0.134
Use of PLEX	4	0	0.134	2	0	0.479	2	0	0.479

IVIg: intravenous immune globülin, PLEX: plasma exchange

treated with RTX, and 5 (16%) of 31 patients without RTX treatment achieved minimal manifestation and received only low-dose immunosuppressive treatment at follow-ups. The MuSK-Ab+ MG patient showed improvement in symptoms after RTX when compared with patients not receiving RTX treatment[17]. In a retrospective, multicenter study of MuSK-Ab + MG patients, RTX that was administered at a dose of 375 mg/m^2 once a week for 4 weeks and monthly for the next 2 months had a lower recurrence rate than with an infusion of 1 g RTX administered 2 weeks apart[18]. In our study, RTX infusion was applied as a rheumatic disease-like treatment protocol, and a statistically significant improvement in MGFA-PIS scores and a ≥50% decrease in daily steroid requirements were detected in MuSK-Ab+ patients. Also, two patients using PLEX before RTX treatment did not need PLEX after RTX treatment, and only two of the six patients using IVIg continued to require IVIg. However, no statistically significant differences were detected. This may be because of the low number of MuSK-Ab+ patients.

In a single-center retrospective study involving 21 AChR-Ab+, 3 MuSK-Ab+, and 4 patients with seronegative MG, muscle strength was found to have increased at significant levels from baseline at 6 months, then stabilized by 36 months, and MGFA-PIS improved in 43% of patients at 6 months[9]. In a prospective study that included 22 refractory MGs, MG manual muscle test (MMT) scores showed significant improvements compared to the baseline values at a mean follow-up of 29±19 months in the AChR-Ab+ and MuSK-Ab+ groups[8]. It was shown in another prospective study involving 14 refractory MG patients with MuSK-Ab+ and seronegative MG that there was an improvement in MMT scores at a mean follow-up of 22 months[19]. In our study, improvement rates were observed in MGFA-PIS, which increased over time from the 3rd to the 12th month, in line with the literature data.

In Austria, in a retrospective study that included 56 patients with AChR-Ab+ and MuSK-Ab + MG, it was reported that 26% of patients were in remission 3 months after the treatment with varying RTX dose protocols, and the remission rate increased over time[13]. In another retrospective multicenter study that was conducted in France, MGFA-PIS was improved or better in 86.2% of the patients[20]. In our study, 42.1% of the patients achieved minimal manifestation at 12 months, and 5.3% of them entered remission. MGFA-PIS was improved or better in 94.7% of patients. In the present study, although the number of patients in remission was

Table 3. *MGFA-PIS score of patients receiving rituximab therapy*

Variables	MGFA PIS before Rituximab (n=19)	MMGFA PIS 3 months after Rituximab (n=19)	MMGFA PIS 6 months after Rituximab (n=19)	MMGFA PIS 12 months after Rituximab (n=19)
Unchanged	8 (42.1%)	1 (5.3%)	1 (5.3%)	1 (5.3%)
Worse	6 (31.6%)	–	–	–
Exacerbation	5 (26.3%)	–	–	–
Improved	–	15 (78.9%)	12 (63.2%)	9 (47.4%)
Minimal manifestation MMP–1	–	–	1 (5.3%)	1 (5.3%)
Minimal manifestation MMP–3	–	3 (15.8%)	5 (26.3%)	7 (36.8%)
Pharmacologic remission	–	–	–	1 (5.3%)

MGFA-PIS: Myasthenia Gravis Foundation of America post-intervention status

less than that reported in the literature, there were more patients who improved or who were better. This can be explained by the fact that the time elapsed before the RTX treatment was longer, and our study included refractory patients.

It was reported in a systematic review of previously published case reports of 169 patients between January 2000 and August 2015 that 72% of MuSK-Ab+ MG patients treated with RTX and 30% of AChR-Ab+ MG patients achieved minimal manifestation or better[21]. The number of RTX cycles varied but had no effect on the response. A systematic review of recently published previous studies that involved 165 RTX-treated patients with AChR-Ab+ MG reported that 113 patients (68%) had significant clinical improvement and 36% achieved remission despite heterogeneous outcome measures[11]. In our study, 60% of the patients with AChR-Ab+ and 33.3% of MuSK-Ab+ patients achieved minimal manifestation or better. However, all patients with MuSK-Ab+ MG achieved improvement or better. The fact that the clinical status of MuSK-Ab+ patients was more severe than AChR-Ab+ patients in our patient cohort may explain this situation.

A Phase II randomized controlled trial (Beat-MG) evaluated the efficacy and safety of RTX in 52 patients with non-thymomatous AChR-Ab+ generalized MG[14]. Two RTX cycles 6 months apart were compared with placebo, and it was found that there was a ≥75% reduction in daily steroid requirements. It was demonstrated that RTX is unlikely to have a clinically significant steroid protective effect for 12 months in mild-moderately symptomatic generalized AChR-Ab+ MG[14, 15]. In our study, 84% of AChR-Ab+ patients had a ≥50% reduction in daily steroid requirements, and pre-RTX steroid doses were higher than in the literature. Also, more than half of the patients in the Beat-MG study were MGFA-CC II. The patients in our study consisted of MGFA-CC III and more severe patients.

The side effect rates of the patients with MG who received RTX treatment and those who received RTX treatment for other autoimmune diseases were similar. It was shown that the most common side effects are infusion reactions that are easily preventable by antihistamines and steroids, and progressive multifocal leukoencephalopathy was detected in only one patient[3]. Similarly, an infusion reaction developed in one of our patients. RTX treatment was continued by premedicating the patient and decreasing the infusion rate.

A case of double seropositive myasthenia crisis that responded to RTX was reported in the literature. It is still a matter of debate whether DSPMG must be evaluated as MuSK-Ab+ or as a separate subtype of MG[22]. In a review that included 28 DSPMG patients, it is reported that it is more common in women, bulbar involvement is prominent, and the risk of myasthenia crisis is higher. It is also reported that these patients require more immunosuppressants and have a low pharmacological remission rate[23]. Cases of DSP-MG were reported at the time of diagnosis or years after their diagnoses[22, 24]. In our study, the 2 DSPMG patients were also double seropositive at the time of diagnosis, and they are the first cases reported in the Turkish population treated with RTX. The MGFA-PIS score of one patient with DSPMG was improved at 12 months in our study, the other patient reached minimal manifestation. Post-RTX steroid was discontinued in one of the 2 patients with DSPMG, and a ≥50% reduction was observed in steroid requirement in the other patient.

Studies have shown that the demand on steroid is reduced after RTX in seronegative patients. However, while statistically significant improvements were observed in the clinics of patients in some studies, no significant improvement was

Table 4. *Relationship between clinical features of patients and MGFA-PIS*

		MGFA-PIS minimal manifestation or better*			MGFA-PIS improved or better**		
		No (n=10)	Yes (n=9)	p	No (n=1)	Yes (n=18)	p
Time from diagnosis of MG to initiation of RTX (years)		6.0±4.2 (1–13)	10.2±8.5 (1–26)	0.356	4	8.2±7.1 (1–26)	NA
Gender	Male	5 (50%)	5 (50%)	1.000	–	10 (100%)	0.474
	Female	5 (55.6%)	4 (44.4%)		1 (11.1%)	8 (88.9%)	
Decrease of antibody titer after RTX therapy[a]	Yes	3 (33.3%)	6 (66.7%)	1.000	1 (11.1%)	8 (88.9%)	0.523
	No	2 (66.7%)	1 (33.3%)		–	3 (100%)	
	Antibody was not studied again	5 (71.4%)	2 (28.6%)	–	7 (100%)		
Antibody[b]	AChR–Ab+	4 (40%)	6 (60%)	0.608	1 (10%)	9 (90%)	1.000
	MuSK–Ab+	4 (66.7%)	2 (33.3%)		–	6 (100%)	
	Seronegative	1 (100%)	–		–	1 (100%)	
	Double seropositive	1 (50%)	1 (50%)		–	2 (100%)	
The severity of the disease before RTX treatment	Moderate disease	7 (58.3%)	5 (41.7%)	0.650	1 (8.3%)	11 (91.7%)	1.000
	Severe disease	3 (42.9%)	4 (57.1%)		–	7 (100%)	
Steroid dose reduced by at least 50%	No	2 (66.7%)	1 (33.3%)	1.000	–	3 (100%)	1.000
	Yes	8 (50%)	8 (50%)		1 (6.3%)	15 (93.8%)	
Thymectomy	No	7 (70%)	3 (30%)	0.179	–	10 (100%)	0.474
	Thymoma	3 (37.5%)	5 (62.5%)		1 (12.5%)	7 (87.5%)	
	Thymus hyperplasia	–	1 (100%)		–	1 (100%)	

MGFA-PIS: Myasthenia Gravis Foundation of America post-intervention status, RTX: rituximab, AChR-Ab: acetylcholine receptor antibody, MuSK-Ab: muscle-specific tyrosine kinase antibody, Moderate disease: MGFA-CC IIIa and IIIb, severe disease: MGFA-CC IVa, IVb, and V, MGFA-CC: Myasthenia Gravis Foundation of America Clinical Classification

*MGFA-PIS minimal manifestation or better: minimal manifestation, pharmacologic remission, complete stable remission

**MGFA-PIS improved or better: improved, minimal manifestation, pharmacologic remission, complete stable remission

a: The titers of the patients with autoantibodies against the acetylcholine receptor were measured and compared statistically.

b: The p value was calculated for AChR-Ab+ and MuSK-Ab+.

observed in some studies[1, 8, 9, 25]. The most important limitation of all these studies is that they included a small number of seronegative patients. In our study, the patient with seronegative MG achieved improved MGFA-PIS or better and post-RTX steroid was discontinued.

It is shown in the literature that RTX is similarly effective in reducing the steroid dose in patients with AChR-Ab+ MG or MuSK-Ab+ MG[1]. In the current MG Treatment Guide, it is emphasized that RTX is an early treatment option in MuSK-Ab+ patients who do not respond adequately to initial immunotherapy, but the uncertainty in the response to RTX treatment continues in AChR-Ab+ patients. This Guide does not recommend RTX treatment for seronegative and DSPMG patients[15]. In our study, the use of IVIg and/or PLEX, the number of patients using steroids, and the steroid dose decreased with RTX treatment. The improvement in MGFA-PIS scores was similar in both MuSK-Ab+ and AChR-Ab+ patients.

In a study that included refractory AChR-Ab+ MG patients where RTX treatment was effective, no statistically significant differences were detected between pre-RTX and post-RTX antibody AChR-Ab titers[26]. It was found in some studies reported in the literature that antibody titers decreased after RTX treatment, which may be related to the response to treatment[21, 27, 28]. However, it was also found that titers did not rise at the time of recurrence[27]. Further studies are needed to determine the frequency of RTX repeat doses and to establish a generalizable approach[29]. In this study, in which the RTX repeat doses were evaluated according to the patient's clinical course, no differences were detected between the antibody levels in patients with AChR-Ab+ MG with and without MGFA-PIS minimal manifestation or better. These findings

suggest that additional markers are needed in patients to determine treatment response and RTX repeat dose intervals.

The limitations of the study were its retrospective design and the low number of seronegative and DSPMG patients. Also, the lack of serological confirmation of other antibodies (e.g. anti-LRP4, anti-Agrin) in a seronegative MG patient can be considered one of the limitations of the study.

In conclusion, RTX treatment can be applied safely in selected MG patients and should be considered in the treatment of suitable patients with MuSK-Ab+ and refractory AChR-Ab+. In seronegative and DSPMG patients, clinical improvement and reduction in steroid dose were observed in small patient groups. However, multicenter studies including patients with seronegative and DSPMG are needed because of the small patient population in the present study.

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CONFLICTS OF INTEREST

The authors report no conflicts of interest related to the manuscript. The authors alone are responsible for the content and writing of the paper. This article does not contain any studies with animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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