Remission and relapses of myasthenia gravis on long-term tacrolimus: a retrospective cross-sectional study of a Chinese cohort

Zhuajin Bi (ID)**, Yayun Cao, Chenchen Liu, Mengcui Gui, Jing Lin, Qing Zhang, Yue Li, Suqiong Ji and Bitao Bu**

Abstract

Objective: To identify the factors that predict the remission and relapses in myasthenia gravis (MG) patients improved by prednisone and tacrolimus treatment.

Methods: A retrospective, observational cohort analysis of MG patients who achieved remission after receiving prednisone and tacrolimus were performed at Tongji Hospital. The main outcome measures were the time to remission, prednisone discontinuation, tacrolimus reduction–associated relapse, and treatment outcome.

Results: After adding tacrolimus, 256 patients were able to achieve remission with a mean time to remission of 2.1 ± 1.4 months. After a median follow-up of 2.9 years, 167 patients (65.2%) discontinued prednisone, and 20 patients (7.8%) achieved complete stable remission. Moreover, 53 of the 109 patients who were tapering tacrolimus experienced relapses. In multivariable analysis, female sex, low tacrolimus concentrations, and quantitative myasthenia gravis (QMG) scores have a positive correlation with the time to remission; concomitant additional autoimmune disease (AID) and high anti-acetylcholine receptor antibody (AChR-ab) levels were significantly associated with low probabilities of prednisone discontinuation [odds ratio (OR) = 0.312–0.912, respectively]; rapid tacrolimus decrement speed ($\geqslant 0.76$ mg/year) was an independent predictor for the development of relapse during tapering tacrolimus (OR = 5.662).

Conclusion: Sex, tacrolimus concentrations, and QMG scores can be used as potential predictors of the time to remission in MG patients treated with tacrolimus and prednisone. Prednisone should be tapered slowly, especially in patients with additional AID or high serum titers of AChR-ab. To avoid symptoms recurrence, the dose of tacrolimus should reduce slowly, not exceeding 0.76 mg/year.

Keywords: dose reduction, myasthenia gravis, predictors, prednisone, remission, relapse, tacrolimus

Correspondence to:
Bitao Bu
Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jiefang Avenue, Wuhan 430030, Hubei Province, China.
bubitao@tjh.tjmu.edu.cn

Zhuajin Bi
Chenchen Liu
Mengcui Gui
Jing Lin
Qing Zhang
Yue Li
Suqiong Ji
Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Yayun Cao
Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Department of Radiology, Zhongnan Hospital of Wuhan University, Wuhan, China

Introduction

Myasthenia gravis (MG) is an immune-mediated neuromuscular disorder that leads to fluctuating and fatigable muscle weakness, caused by circulating antibodies, including anti-acetylcholine receptor (AChR), anti-muscle-specific kinase (MuSK) antibodies, and low-density lipoprotein receptor–related protein 4 (LRP4) antibodies.[1] The long-term treatment plan should include immunotherapy and glucocorticoids (steroids) are the main therapeutic options for controlling MG symptoms.[2] Although effective, prolonged use of steroids frequently results in considerable side effects, necessitating the administration of

steroid-sparing agents.[3,4] Tacrolimus, a calcineurin inhibitor, is widely used as a potent immunosuppressor to improve the symptoms of MG patients, especially in the Asian population.[5–7] Several studies have demonstrated that tacrolimus significantly reduced prednisone dose and improved outcomes in various forms of MG, including thymomatous and seronegative MG.[7–9] A few studies, however, have focused on the time required to achieve remission after adding tacrolimus, as well as the factors associated with the initial acting time of tacrolimus and possibility of discontinuing prednisone. Furthermore, once the MG symptoms had been effectively controlled, tacrolimus should be tapered to avoid unnecessary costs and adverse events.[4,10] A rapid dose reduction of tacrolimus may lead to a recurrence of myasthenic weakness. As a result, it is critical to understand the frequency of and factors predicting initial exacerbations with tacrolimus dose reductions.

We present a retrospective cohort analysis of outcomes in MG patients from our clinic who were treated with prednisone and tacrolimus. Our goal is to determine the factors for predicting the time required in MG patients to achieve remission, the frequency of prednisone discontinuation, the tacrolimus reduction–associated relapses, and treatment outcome.

## Materials and methods

Patient selection and study design
This retrospective cohort study was performed for patients with MG who were treated with prednisone and tacrolimus at Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology from January 2012 to May 2021. The diagnosis of MG was based on the clinical history of fluctuating and fatigable muscle weakness together with one or more of the positive results of the neostigmine, repetitive nerve stimulation (RNS), or AChR-ab test.[11] Specifically, a positive response to the neostigmine test was defined as a reduction of QMG score $\geq 60\%$ in any of the tested muscles with significant weakness after intramuscular injection of 1–1.5 mg neostigmine methylsulfate and 0.5 mg atropine, positive RNS was defined as a decrement of greater than 10% between first and fifth compound muscle action potential (CMAP) amplitude in any of tested nerves following 2–5 Hz

stimulation, and anti-AChR antibody (AChR-ab) titers >0.50 nmol/l were defined as positive (RIA kit, RSR Limited, Cardiff, UK). All patients had achieved remission, which was defined as no symptoms of functional limitations of MG but some weakness of extraocular muscles on examination.[12] Patients were excluded if they had any of the following conditions: (a) thymectomy or other steroid-sparing agents were used within 3 months of receiving tacrolimus, (b) patients had an inadequate response to tacrolimus after at least 24-week treatment, (c) tacrolimus was withdrawn due to adverse events, (d) follow-up was less than 1 year, or (e) there was insufficient baseline data.

A subsequent follow-up was performed to identify those who had undergone a taper of tacrolimus. The patients with tacrolimus reduction were divided into two groups based on whether or not they experienced relapse: relapsed group and nonrelapsed group. The relapse of MG was defined as the reappearance or worsening of one or more MG symptoms or signs of muscle weakness with QMG scores ≥ 3 points that lasted more than 24 h. Furthermore, the duration between the MG relapse and the last remission should be more than 3 months.[6,13] Patients were excluded if their symptoms relapsed with other definite factors (such as infection, inappropriate drug, fatigue, or pregnancy) or if their follow-up was less than 6 months after tapering tacrolimus. The selection procedure is depicted in Figure 1.

Clinical parameters and therapy administration
The following patient characteristics were collected from medical records: onset age, sex, symptoms at onset, disease duration from onset to starting tacrolimus, concomitant autoimmune disease (AID), the serum levels of AChR-ab and anti- muscle-specific kinase antibody (MusK-ab) titers estimated by radioimmunoassay, initial tacrolimus trough concentration measured by liquid chromatography-mass spectrometry, thymus type, thymectomy, drug dose reduction and withdrawal, the Myasthenia Gravis Foundation of America (MGFA) clinical classification at maximum severity,[12] quantitative myasthenia gravis (QMG) scores evaluated at tacrolimus administration,[14] and MGFA postintervention status at the last visit.[12] Patients with negative AChR-ab (<0.5 nmol/l) were referred to as seronegative myasthenia gravis (SNMG). According to the MGFA postintervention status, complete stable
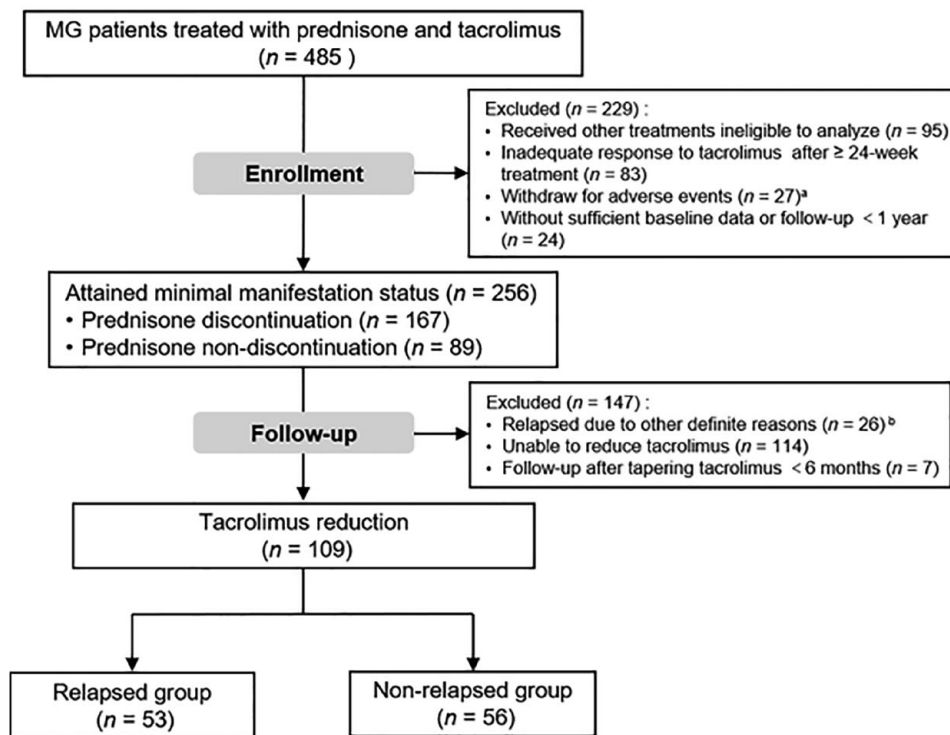
Figure 1. Flowchart of participants' recruitment.
[a]In 27 patients, tacrolimus was withdrawn due to adverse events after a median of 3.0 months (ranged from 1.0 to 20.0 months): hepatic dysfunction in seven, renal insufficiency in six, hyperglycemia in four, infection in four, stomachache in two, tremor in two, and allergic to tacrolimus in two.
[b]In 26 patients, symptoms are aggravated by infection (n = 16), fatigue (n = 3), inappropriate drugs (n = 3), and others (n = 4).

remission (CSR) was defined as no symptoms or signs of MG for at least 1 year without the use of any pharmacologic medications.[12]

All patients were treated with pyridostigmine bromide and prednisone at the same as or before tacrolimus. Tacrolimus (Hangzhou Zhongmei Huadong Pharmaceutical Co., Ltd., Hangzhou, China; H20094027) at a dose of 1–3 mg/day was prescribed to all patients, with a target trough concentration of 5–10 ng/ml. Once significant improvements in MG symptoms were observed, prednisone reduction was started at a rate no faster than 5 mg every 2 weeks for doses higher than 20 mg/day, 5 mg every month for lower doses, and finally adjusted as necessary to maintain remission. If patients continued to do well after at least 3 months of minimum dose or discontinuation of prednisone, a gradual reduction in tacrolimus dose would be initiated. The reduction speed is calculated as follows: the beginning dose diminished by the final dose divided by the duration of taper. Duration of taper was defined as the periods from the starting of tacrolimus use

to relapse, stable tacrolimus dose, or tacrolimus withdrawal. If the clinical symptoms recurred, the dose of prednisone or tacrolimus was increased immediately until the symptoms improved and stabilized.

Statistical analysis
Numerical data are presented as mean value ± standard deviation (SD) or median [interquartile range (IQR)], and categorical data are presented as frequencies with absolute numbers and percentages. Correlations between clinical factors were evaluated using Spearman's rank correlations. Kaplan–Meier curve was performed to visualize the rate of prednisone discontinuation during the tacrolimus treatment. Multiple linear regression was applied to measure the association between all clinical variables and the time to attain remission. Logistic regression was used to identify possible factors correlated with the prednisone discontinuation and tacrolimus reduction–associated relapse; univariate associations with a p value < 0.20 were used to build a multivariate

model. The relapse risk was further evaluated with receiver-operating characteristic (ROC) curves, and the optimal cut-off values were defined as the point at which Youden's index was maximal. Using parameters estimated from the sample,[15] a post hoc power calculation demonstrated that this study had 100% power to investigate predictors of the time required to achieve remission and prednisone discontinuation, and had 86% power to identify the predictors of the tacrolimus reduction–associated relapses. All data analyses were performed with IBM SPSS statistics version 22.0 (Armonk, NY, USA) and G∗ Power 3.1, and a two-tailed $p < 0.05$ was deemed to indicate statistical significance.

Results

Baseline characteristics of participants

Among 485 MG patients treated with prednisone and tacrolimus in our center, 83 patients were resistant to tacrolimus and 27 patients discontinued tacrolimus due to severe adverse events after a median of 3.0 months (range = 1.0–20.0 months) (Figure 1). These patients were excluded from the study, and a total of 256 patients [median (IQR) age at onset: 26.9 (5.9–46.6) years; 65.1% female] were enrolled in the study, with a follow-up for a median of 2.9 years (IQR = 2.2–4.1). According to the diagnostic tests of MG, there are 196 (76.6%) MG patients with positive AChR-ab, 8 (3.1%) SNMG patients with positive MuSK-ab, 19 (7.4%) SNMG patients with positive RNS, and 33 (12.9%) SNMG patients without any electrophysiological or serological evidence of MG (seen in Supplementary Table S1). At the initial stage, ocular MG was present in 162 patients, 72 of whom converted to generalized myasthenia gravis(GMG) after a median of 0.96 years (IQR = 0.17–6.69). Before tacrolimus treatment, the maximum disease severity was classified as ocular MG (MGFA class I) in 90 patients (35.2%), mild MG (MGFA class II) in 80 patients (31.2%), and moderate or severe MG (MGFA class III–V) in 86 patients (33.6%). The basic clinical data of the 256 study patients are summarized in Table 1.

Remission and prednisone discontinuation

The patients achieved remission after a mean time of 2.1 ± 1.4 months, with a mean reduction of 8.0 ± 4.3 in QMG scores. In multivariate linear

Table 1. Baseline characteristics of 256 patients with remission after adding tacrolimus.

Characteristics	Patients
Sex	
Male	89 (34.9)
Female	166 (65.1)
Age at onset (years)	26.9 (5.9–46.6)
≤18	98 (38.3)
18–50	107 (41.8)
>50	51 (19.9)
Concomitant AID	50 (19.5)
Duration before Tac administration, years	2.8 (0.6–7.5)
Symptoms at onset	
Ocular MG	162 (63.3)
Generalized MG	94 (36.7)
Generalized disease development, years[a]	0.96 (0.17–6.69)
Within 2 years	44 (61.1)
After 2 years	28 (38.9)
MGFA classification (most severe)	
I	90 (35.2)
II	80 (31.2)
III–V	86 (33.6)
QMG scores at Tac administration	8.39 ± 4.26
Reduction in QMG scores	8.04 ± 4.27
Neostigmine test (+)	249 (97.3)
RNS (+)	69/98
Autoantibody status[b]	
AChR-ab (+)	196 (76.6)
MuSK-ab (+)	8/168
Highest measured AChR-ab, nmol/l	4.19 (0.69–9.23)
Thymus type[c]	
Normal	119 (46.5)

(Continued)

Table 1. (Continued)

Characteristics	Patients
Hyperplasia	47 (18.3)
Thymoma	90 (35.2)
Thymectomy	98 (38.3)
Time from onset to thymectomy, years	0.8 (0.2–2.1)
Duration of Pre before Tac, years	0.3 (0.0–2.7)
Pre reduction speed, mg/month	2.85 ± 3.13
Age at Tac administration, years	31.5 (16.5–50.1)
Tac concentrations before tapering, ng/ml	5.58 ± 1.59
Time to attain remission, months	2.09 ± 1.43
Postintervention status	
CSR	20 (7.8)
Other status	236 (92.2)
Follow-up time, years	2.9 (2.2–4.1)

AChR-ab, anti-acetylcholine receptor antibody; AID, autoimmune disease; CSR, complete stable remission; MG, myasthenia gravis; MGFA, Myasthenia Gravis Foundation of America; MuSK-ab, anti-muscle-specific kinase antibody; Pre, prednisone; QMG, quantitative myasthenia gravis; RNS, repetitive nerve stimulation; Tac, tacrolimus.

Data are given as *n* (%), median (interquartile range), or mean value ± standard deviation.

[a]Because only patients with ocular forms at onset can develop a generalized disease, the denominators are the number of patients with ocular forms at onset.

[b]The AChR-ab titers >0.50 nmol/l and MuSK-ab titers >0.05 nmol/l were defined as positive (RIA kit, RSR Limited, Cardiff, UK).

[c]Thymus status was evaluated by chest computed tomography (CT) scan in nonthymectomized patients and thymus histology in thymectomized patients.

regression analysis, female sex, QMG scores, and tacrolimus concentrations were found to be independent predictors of the time to remission (Table 2). Tacrolimus concentration was correlated with the reduction in QMG scores by Spearman's test ($p = 0.029$, $r = -0.137$).

Before tacrolimus administration, all patients had been on prednisone for a median (IQR) duration of 0.3 (0.0–2.7) years. The prednisone dose decreased significantly after the addition of tacrolimus, at a speed of 0.97 mg/month, from a mean of 22.87 ± 12.14 mg/day at baseline to 3.07 ± 5.08 mg/day at the final visit ($p < 0.001$). Prednisone was eventually withdrawn from 167 patients (65.2%) after a mean time of 1.24 ± 1.10 years (Figure 2). The 256 patients were further divided into two groups: prednisone discontinuation group ($n = 167$) and nondiscontinuation group ($n = 88$). The clinical data of the two groups were available in Supplementary Table S2. Age at onset, initial symptoms, concomitant AID, AChR-ab titers, MGFA classification, age at tacrolimus administration, and the periods of follow-up were found to be associated with the prednisone discontinuation using univariate logistic regression analysis. Finally, multivariate logistic regression analysis indicated that concomitant AID [odds ratio (OR) = 0.312, 95% confidence interval (CI) 0.163–0.599; $p < 0.001$] and AChR-ab levels (OR = 0.912, 95% CI = 0.864–0.962; $p = 0.001$) were independent predictors of prednisone discontinuation (Table 3).

Tacrolimus reduction–associated relapse

All 256 patients received a daily dose of 2–3 mg of tacrolimus with a mean trough concentration of 5.58 ± 1.59 ng/ml. One hundred nine of 256 (42.5%) patients who were well controlled with tacrolimus underwent a taper, with a follow-up for a median of 1.6 years (range = 0.5–6.7) (Table 4). These patients were divided into two groups: the relapsed MG group ($n = 53$) and the nonrelapsed MG group ($n = 56$), and the clinical data of the two groups were available in Supplementary Table S3. All patients had no symptoms or signs of MG before tacrolimus reduction. The QMG scores for 53 MG relapsed patients significantly increase at the time of relapse compared with that in baseline, with a mean QMG score of 9.6 ± 5.6 points (range = 3–24 points). The mean lag time from tacrolimus reduction to relapse in relapsed patients was 2.1 ± 1.6 months (range = 0.4–6.0 months). Univariate results showed that age at onset, sex, concomitant AID, AChR-ab titers, combination with prednisone, age at tacrolimus administration, and tacrolimus reduction speed were all associated with reduction-associated relapse. In the multivariate model, only tacrolimus reduction speed (OR = 5.662, 95% CI = 2.613–12.267; $p < 0.001$) remained significant (Table 5). The best cut-off value determined in an ROC curve analysis for the tacrolimus reduction speed was 0.76 mg/year (sensitivity = 90.6%; specificity = 76.8%) (shown in Figure 3).

Table 2. Multiple linear regression model for the influencing factors of the time to remission.

Variable	Unstandardized coefficients		Standardized coefficients	*t*	*p* value
	B	SE	β		
Constant	0.376	0.575		0.655	0.513
Age at onset, years	−0.006	0.005	−0.091	−1.344	0.180
Female sex	0.462	0.152	0.154	3.042	**0.003***
Duration before Tac therapy, years	−0.004	0.012	−0.022	−0.365	0.715
Symptoms at onset (GMG)	0.076	0.186	0.026	0.411	0.681
Concomitant AID	0.030	0.178	0.008	0.170	0.865
Neostigmine test (+)	0.605	0.437	0.069	1.385	0.167
QMG scores at Tac administration	0.222	0.023	0.660	9.761	**<0.001***
AChR-ab titers, nmol/l	−0.028	0.017	−0.097	−1.646	0.101
Thymus type	−0.030	0.117	−0.019	−0.259	0.796
Thymectomy	0.216	0.219	0.073	0.988	0.324
MGFA classification	−0.213	0.133	−0.123	−1.604	0.110
Duration of Pre usage before Tac, years[a]	0.009	0.027	0.020	0.322	0.748
Pre dose, mg/kg/day	0.838	0.490	0.099	1.708	0.089
Tac concentrations, ng/ml	−0.116	0.046	−0.129	−2.503	**0.013***

$R = 0.666$; $R^2 = 0.444$; adjusted $R^2 = 0.411$; $p = 0.000$

AChR-ab, anti-acetylcholine receptor antibody; AID, autoimmune disease; GMG, generalized myasthenia gravis; MGFA, Myasthenia Gravis Foundation of America; Pre, prednisone; QMG, quantitative myasthenia gravis; Tac, tacrolimus.
[a]There was a strong correlation between the duration of prednisone therapy before tacrolimus initiation and the QMG scores at tacrolimus administration (Spearman's correlation coefficient = −0.366, $p < 0.001$).
*$p < 0.05$.

Treatment outcome and prognosis
For 53 patients with tacrolimus reduction–associated relapse, symptoms were reversed in 45 (84.9%) patients within 2 months of increasing the tacrolimus dose to the previous maintenance level. Six (11.3%) patients, however, required hospitalization for treatment with intravenous immunoglobulin (IVIG), and two patients developed acquired resistance to tacrolimus. Fortunately, no patients experienced a respiratory crisis. In terms of the treatment outcome of patients treated with prednisone and tacrolimus, only 20 out of 256 (7.8%) patients enter CSR after a variable period, ranging from 1.46 to 6.07 years. No factors, however, were found significantly associated with CSR by multivariate logistic regression.

Discussion
This was a retrospective study of the clinical outcomes in a large and heterogeneous group of MG patients who achieved remission after receiving prednisone and tacrolimus over a long-term follow-up. Unlike other traditional immunosuppressants, such as azathioprine, mycophenolate, and methotrexate, which had a delayed therapeutic response between 3 and 12 months,[1,2,16,17] our findings demonstrated that the improvement began soon after starting tacrolimus and peaked
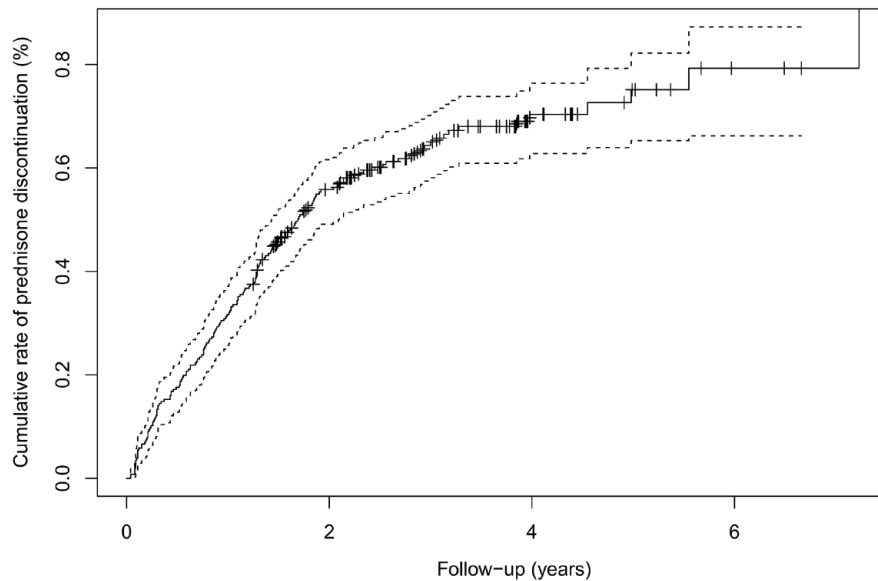
Figure 2. The Kaplan–Meier curve for time to discontinue prednisone in MG patients. Regression line (solid) and 95% confidence limits (dotted) are shown.

after a mean of nearly 2 months. Ponseti *et al.*[18] reported that in addition to changes in muscle strength, the rapid immunomodulatory effect of tacrolimus on MG patients could also be reflected by a progressive reduction of serum AChR-ab levels. In addition, considering that tacrolimus has a biological role to increase glucocorticoid receptor transactivity from cytoplasm to nucleus,[9,18] the favorable early results in this study may be partially attributable to the facilitatory effect of this drug on steroids pharmacology. Importantly, our results suggested that abruptly discontinuing tacrolimus may increase the risk of MG relapse, lending credence to the hypothesis that tacrolimus plays a role in the maintenance of MG remission.[1,10]

Sex, QMG scores, and tacrolimus concentrations were statistically identified as clinical predictors of the time to remission in this study. Our findings revealed that females took longer than men to achieve remission after taking tacrolimus. This sex bias could be explained by the fact that sex hormones like estrogens or progesterone can aggravate the MG disease by promoting the production of AChR-ab and expansion of AChR-specific Th1 cells.[19] Furthermore, the QMG scores were primarily used to assess the therapeutic efficacy of MG.[17] This study demonstrated a negative correlation between tacrolimus concentration and absolute value reduction of QMG

scores, indicating that the tacrolimus efficacy has been considered to be concentration-dependent in MG treatment, with an adequate tacrolimus concentration being more effective than low tacrolimus concentration.[8]

In the case of MG patients who responded well to combined immunotherapy, there is often a tendency to taper steroids dose first due to concerns about side effects associated with long-term use.[5] Our data showed that 65% of patients were able to discontinue prednisone after responding well to tacrolimus. This result may suggest that tacrolimus has stronger immunosuppressive and prednisone-sparing effects than azathioprine[16] and cyclosporine.[17] However, because there were insufficient data to make specific recommendations regarding the comparison of steroid-sparing effects between different immunosuppressants, it was concluded that a larger, prospective, randomized controlled trial would be required.[20] Moreover, we found that complete discontinuation of prednisone was more likely to fail in patients with other AID or high AChR-ab levels. This observation may indicate that MG patients with associated AID or high AChR-ab levels have a more generalized autoimmune disturbance that necessitates more effective combined immunotherapy.[21–24]

Similarly, in studies of azathioprine[25] or mycophenolate mofetil dose tapering,[26] fast tapering was

Table 3. Univariate and multivariate analyses for the influencing factors of prednisone discontinuation.

Variable	Univariable		Multivariable	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Age at onset, years	0.989 (0.977–1.002)	0.088*		
Female sex	1.189 (0.694–2.037)	0.528		
Duration before Tac, years	0.987 (0.954–1.022)	0.463		
Symptoms at onset (GMG)	0.677 (0.399–1.149)	0.148*		
Concomitant AID	0.331 (0.175–0.623)	0.001**	0.312 (0.163–0.599)	0.000**
Neostigmine test (+)	1.422 (0.311–6.497)	0.650		
QMG scores at Tac administration	1.189 (0.694–2.037)	0.528		
Time to attain remission, months	0.948 (0.794–1.132)	0.558		
AChR-ab titers, nmol/l	0.917 (0.870–0.966)	0.001**	0.912 (0.864–0.962)	0.001**
Thymus type		0.335		
Normal	1 (Reference)			
Hyperplasia	1.324 (0.629–2.786)	0.459		
Thymoma	0.759 (0.430–1.340)	0.342		
Thymectomy	1.164 (0.683–1.983)	0.576		
MGFA classification		0.005**		
I	1 (Reference)			
II	0.672 (0.344–1.314)	0.245		
III–V	0.355 (0.187–0.674)	0.002		
Duration of Pre usage before Tac, years	1.032 (0.949–1.124)	0.460		
Pre reduction speed, mg/month	0.972 (0.897–1.053)	0.490		
Age at Tac administration, years	0.984 (0.971–0.998)	0.027**		
Tac concentrations (ng/ml)	0.983 (0.836–1.155)	0.835		
Follow-up (years)	1.134 (0.937–1.372)	0.198*		

AChR-ab, anti-acetylcholine receptor antibody; AID, autoimmune disease; CI, confidence interval; GMG, generalized myasthenia gravis; MGFA, Myasthenia Gravis Foundation of America; OR, odds ratio; Pre, prednisone; QMG, quantitative myasthenia gravis; Tac, tacrolimus.
*$p < 0.2$; **$p < 0.05$.

associated with an increased risk of exacerbations in well-controlled MG patients. Our data showed that the speed of oral tacrolimus reduction was the most important factor possibly related to the relapse of MG symptoms in the late phase. To avoid disease rebound, the tacrolimus dose should be tapered down slowly, not exceeding 0.76 mg/year. In this study, relapses occurred in nearly half of the patients with well-controlled MG after tapering tacrolimus, which is considerably higher than the 4% relapses in the series of Nishida *et al.*[6] This could be because the latter limited definition

Table 4. Baseline characteristics of 109 patients with tacrolimus reduction.

Characteristics	Patients
Sex	
Female	63 (57.8)
Male	46 (42.2)
Age at onset, years	31.2 (14.2–47.9)
≤18	30 (27.5)
18–50	56 (51.4)
>50	23 (21.1)
Concomitant AID	17 (15.6)
Neostigmine test (+)	104 (95.4)
Duration before Tac therapy, years	2.0 (0.4–5.6)
Symptoms at onset	
Ocular MG	67 (61.5)
Generalized MG	42 (38.5)
MGFA classification	
I	33 (30.3)
II	40 (36.7)
III–V	36 (33.0)
QMG scores at Tac administration	8.88 ± 4.27
AChR-ab titers, nmol/l	5.70 (0.67–9.72)
Thymus type[a]	
Normal	45 (41.3)
Hyperplasia	19 (17.4)
Thymoma	23 (41.3)
Thymectomy	48 (44.0)
Combination with Pre	33 (30.3)
Age at Tac administration, years	35.0 (23.2–51.2)
Tac concentrations before tapering, ng/ml	5.78 ± 1.41

(Continued)

Table 4. (Continued)

Characteristics	Patients
Time to attain remission, months	1.99 ± 1.43
Tac reduction speed, mg/years	1.42 ± 1.47
Follow-up time, years	1.6 (1.1–2.5)

AChR-ab, anti-acetylcholine receptor antibody; AID, autoimmune disease; MG, Myasthenia gravis; MGFA, Myasthenia Gravis Foundation of America; NRMG, nonrelapsed myasthenia gravis; Pre, prednisone; QMG, quantitative myasthenia gravis; RMG, relapsed myasthenia gravis; Tac, tacrolimus.
Data are given as *n* (%), median (interquartile range) or mean value ± standard deviation.
Analysis of continuous data was done with the Mann–Whitney *U* test or Student's *t* test. Analysis of dichotomous data was done with the Chi-square test or Fisher's exact test as appropriate.
[a]Thymus status was evaluated by chest computed tomography (CT) scan in nonthymectomized patients and thymus histology in thymectomized patients.
*$p < 0.05$.

of exacerbations to 3 months after tacrolimus reduction. In addition, relapses typically occurred within 6 months of dose reduction (mean 2.1 ± 1.6 months), indicating that the improvement induced by tacrolimus does not usually last after tapering off. Thus, physicians should be cautious when rapidly tapering immunotherapy and conduct regular follow-ups to monitor for signs of recurrence in MG patients.[5,26,27]

Consistent with previous studies that medications reduction–associated exacerbations were often mild and rarely required hospitalization,[6,25,26] the majority of relapsed patients (84.9%) in our cohort achieved remission again within a few months after increasing tacrolimus dose. Notably, 2 of 53 relapsed patients developed resistance to tacrolimus and required more aggressive therapies, but the underlying mechanism for this observation remains unclear and needs to be further explored.[4] Furthermore, only 20 of 256 patients in this study achieved CSR at the last follow-up, which was similar to that of previous research works.[27] Mantegazza *et al.*[28] reported that factors influencing CSR included female sex, early onset age, and thymectomy therapy. No clinical factors, however, were found to be significantly associated with CSR in tacrolimus-treated MG patients.

Table 5. Univariate and multivariate analyses for the influencing factors of MG relapse during tapering of tacrolimus dose.

Variable	Univariable		Multivariable	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Age at onset (years)	1.023 (1.002–1.044)	0.031**		
Female sex	0.423 (0.194–0.921)	0.030**		
Duration before Tac, years	0.980 (0.918–1.046)	0.542		
Symptoms at onset (GMG)	0.937 (0.433–2.027)	0.868		
Concomitant AID	2.183 (0.744–6.401)	0.155*		
Neostigmine test (+)	0.617 (0.099–3.848)	0.605		
QMG scores at Tac administration	1.042 (0.954–1.139)	0.361		
Time to attain remission, months	0.887 (0.673–1.168)	0.392		
AChR-ab titers, nmol/l	1.085 (1.009–1.167)	0.029**		
Thymus type		0.533		
Normal	1 (Reference)			
Hyperplasia	0.558 (0.186–1.677)	0.299		
Thymoma	1.000 (0.438–2.286)	1.000		
Thymectomy	1.103 (0.518–2.353)	0.799		
MGFA classification		0.102*		
I	1 (Reference)			
II	2.211 (0.852–5.735)	0.103		
III–V	2.800 (1.050–7.470)	0.040		
Combination with Pre	0.542 (0.237–1.239)	0.146*		
Age at Tac administration, years	1.026 (1.003–1.051)	0.027**		
Tac concentrations (ng/ml)	1.011 (0.774–1.319)	0.938		
Tac reduction speed, mg/year	5.662 (2.613–12.267)	<0.001**	5.662 (2.613–12.267)	<0.001**
Follow-up (years)	1.171 (0.839–1.635)	0.354		

AChR-ab, anti-acetylcholine receptor antibody; AID, autoimmune disease; CI, confidence interval; GMG, generalized myasthenia gravis; MGFA, Myasthenia Gravis Foundation of America; OR, odds ratio; Pre, prednisone; QMG, quantitative myasthenia gravis; Tac, tacrolimus.
*$p < 0.2$; **$p < 0.05$.

Several limitations still exist in this study, for instance, (1) the treatment of tacrolimus among participants with intractable symptoms may contribute to selective bias; (2) despite the overall large size of our cohort, recruitment of subjects from a single center may not be highly representative; (3) the low AChR-ab titers in our cohort may be caused by population biases; (4) because of the lack of a control group in this study, the efficacy of tacrolimus in reducing QMG scores
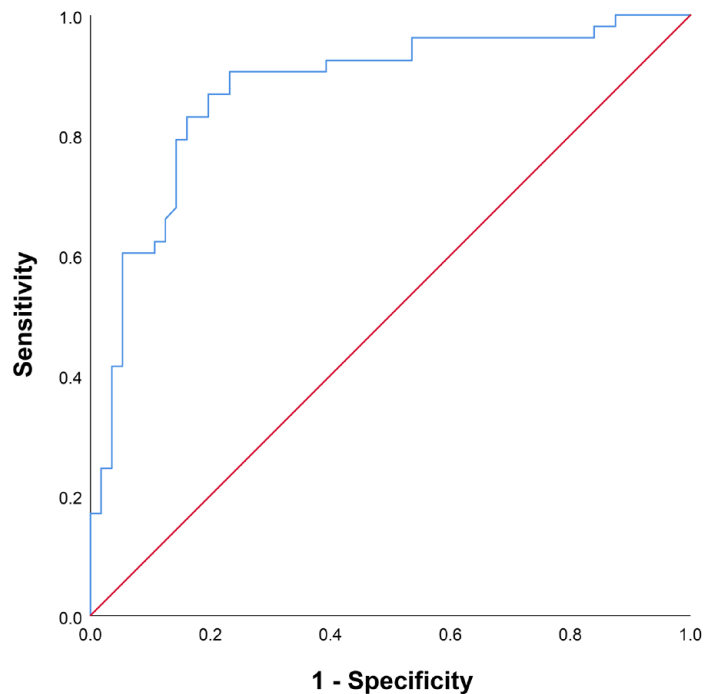
Figure 3. Receiver operating characteristic curves analysis for MG relapse after tapering tacrolimus. With regard to the speed of tacrolimus reduction, the AUC is 0.875 and 95% CI is 0.806–0.943.
AUC, area under the ROC curve; CI, confidence interval.

and prednisone dose needs to be further validated in long-term prospective randomized controlled trials.

Conclusion

In conclusion, our findings demonstrated that sex, tacrolimus concentrations, and QMG scores are clinically independent factors influencing time to remission in MG patients treated with tacrolimus. Although prednisone can be gradually tapered off in the majority of MG patients, it should be done with caution, particularly in patients with other AID or high serum titers of AChR-ab. Tacrolimus, as a maintenance drug to prevent disease relapses, should be tapered down slowly, not exceeding 0.76 mg/year.

Declarations

Ethics approval and consent to participate

This study has been approved by the Committee of Clinical Investigation at Tongji Hospital, Tongji Medical College, Huazhong University of

Science and Technology, Wuhan, China (no. TJ-IRB20190414). Written informed consent was obtained from each participant or their parents when underage according to the principles of the Declaration of Helsinki.

Consent for publication

Consent for publication was not required.

Author contributions

Zhuajin Bi: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing – original draft.

Yayun Cao: Data curation; Formal analysis; Investigation; Software.

Chenchen Liu: Data curation; Formal analysis; Methodology.

Mengcui Gui: Conceptualization; Formal analysis; Investigation.

Jing Lin: Data curation; Formal analysis.

Qing Zhang: Data curation; Formal analysis.

Yue Li: Data curation; Formal analysis.

Suqiong Ji: Data curation; Formal analysis.

Bitao Bu: Conceptualization; Supervision; Writing – review & editing.

Competing interests
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ORCID iD
Zhuajin Bi https://orcid.org/0000-0002-2002-6633

Supplemental material
Supplemental material for this article is available online.

References
1. Dalakas MC. Immunotherapy in myasthenia gravis in the era of biologics. *Nat Rev Neurol* 2019; 15: 113–124.

2. Gilhus NE and Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. *Lancet Neurol* 2015; 14: 1023–1036.

3. Lascano AM and Lalive PH. Update in immunosuppressive therapy of myasthenia gravis. *Autoimmun Rev* 2021; 20: 102712.

4. Bi Z, Cao Y, Lin J, *et al*. Long-term improvement in a Chinese cohort of glucocorticoid-resistant childhood-onset myasthenia gravis patients treated with tacrolimus. *Front Neurol* 2022; 13: 820205.

5. Morren J and Li Y. Maintenance immunosuppression in myasthenia gravis, an update. *J Neurol Sci* 2020; 410: 116648.

6. Nishida Y, Takahashi YK, Kanai T, *et al*. Safety of tapering tacrolimus dose in patients with well-controlled anti-acetylcholine receptor antibody-positive myasthenia gravis. *Eur J Neurol* 2020; 27: 100–104.

7. Cruz JL, Wolff ML, Vanderman AJ, *et al*. The emerging role of tacrolimus in myasthenia gravis. *Ther Adv Neurol Disord* 2015; 8: 92–103.

8. Kanai T, Uzawa A, Kawaguchi N, *et al*. Adequate tacrolimus concentration for myasthenia gravis treatment. *Eur J Neurol* 2017; 24: 270–275.

9. Shimojima Y, Matsuda M, Gono T, *et al*. Tacrolimus in refractory patients with myasthenia gravis: coadministration and tapering of oral prednisolone. *J Clin Neurosci* 2006; 13: 39–44.

10. Zhang C, Bu B, Yang H, *et al*. Immunotherapy choice and maintenance for generalized myasthenia gravis in China. *CNS Neurosci Ther* 2020; 26: 1241–1254.

11. Meriggioli MN and Sanders DB. Myasthenia gravis: diagnosis. *Semin Neurol* 2004; 24: 31–39.

12. Sanders DB, Wolfe GI, Benatar M, *et al*. International consensus guidance for management of myasthenia gravis: executive summary. *Neurology* 2016; 87: 419–425.

13. Wang L, Zhang Y and He M. Clinical predictors for the prognosis of myasthenia gravis. *BMC Neurol* 2017; 17: 77.

14. Jaretzki A 3rd, Barohn RJ, Ernstoff RM, *et al*. Myasthenia gravis: recommendations for clinical research standards. Task Force of the Medical Scientific Advisory Board of the Myasthenia Gravis Foundation of America. *Ann Thorac Surg* 2000; 70: 327–334.

15. Faul F, Erdfelder E, Buchner A, *et al*. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav Res Methods* 2009; 41: 1149–1160.

16. Palace J, Newsom-Davis J and Lecky B. A randomized double-blind trial of prednisolone alone or with azathioprine in myasthenia gravis. Myasthenia Gravis Study Group. *Neurology* 1998; 50: 1778–1783.

17. Ciafaloni E, Nikhar NK, Massey JM, *et al*. Retrospective analysis of the use of cyclosporine in myasthenia gravis. *Neurology* 2000; 55: 448–450.

18. Ponseti JM, Gamez J, Azem J, *et al*. Tacrolimus for myasthenia gravis: a clinical study of 212 patients. *Ann N Y Acad Sci* 2008; 1132: 254–263.

19. Delpy L, Douin-Echinard V, Garidou L, *et al.* Estrogen enhances susceptibility to experimental autoimmune myasthenia gravis by promoting type 1-polarized immune responses. *J Immunol* 2005; 175: 5050–5057.

20. Sharshar T, Porcher R, Demeret S, *et al.* Comparison of corticosteroid tapering regimens in myasthenia gravis: a randomized clinical trial. *JAMA Neurol* 2021; 78: 426–433.

21. Kanazawa M, Shimohata T, Tanaka K, *et al.* Clinical features of patients with myasthenia gravis associated with autoimmune diseases. *Eur J Neurol* 2007; 14: 1403–1404.

22. Christensen PB, Jensen TS, Tsiropoulos I, *et al.* Associated autoimmune diseases in myasthenia gravis. A population-based study. *Acta Neurol Scand* 1995; 91: 192–195.

23. Ullah U, Iftikhar S and Javed MA. Relationship between low and high anti-acetylcholine receptor antibody titers and clinical severity in myasthenia gravis. *J Coll Physicians Surg Pak* 2021; 31: 965–968.

24. Heldal AT, Eide GE, Romi F, *et al.* Repeated acetylcholine receptor antibody-concentrations and association to clinical myasthenia gravis development. *PLoS ONE* 2014; 9: e114060.

25. Dube M, Sodani A and Chouksey D. Outcome of Myasthenia gravis treated with high-dose prednisolone and azathioprine: a single centre ambispective study from India. *Acta Neurol Taiwan* 2017; 26: 106–119.

26. Oskarsson B, Rocke DM, Dengel K, *et al.* Myasthenia gravis exacerbation after discontinuing mycophenolate: a single-center cohort study. *Neurology* 2016; 86: 1159–1163.

27. Sanders DB and Evoli A. Immunosuppressive therapies in myasthenia gravis. *Autoimmunity* 2010; 43: 428–435.

28. Mantegazza R, Baggi F, Antozzi C, *et al.* Myasthenia gravis (MG): epidemiological data and prognostic factors. *Ann N Y Acad Sci* 2003; 998: 413–423.