Risk factors analysis and establishment of a clinical predictive model for recurrence after first attack of myasthenia gravis: a single-center retrospective study

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Research Article

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Risk factors analysis and establishment of a clinical predictive model for recurrence after first attack of myasthenia gravis: a single-center retrospective study

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Abstract

Background: Myasthenia gravis (MG) is a rare and recurrent disease. The purpose of this study was to investigate the risk factors for relapse in MG patients after their first attack and establish a clinical predictive model. We conducted a retrospective study of 86 MG patients, followed and reviewed the clinical data of patients from the first onset to the first relapse, including age of onset, site of first symptom, MGFA at onset, thymoma, surgical resection of the thymoma, infection history, irregular drug use, combination of other autoimmune diseases, AChR antibody, and anti-Musk antibody, etc. The R software was used for statistical analysis. Univariate analysis and multivariate analysis were used to analyze risk factors. The clinical predictive model was established by Logistic regression analysis.

Results: Within 2 years after the first attack, 61.2% of MG patients relapsed. MGFA at onset, irregular drug use and infection history were independent risk factors for MG relapse within 2 years after the first attack ($p < 0.05$). The clinical predictive model had good discrimination and calibration.

Conclusion: The relapse of MG is affected by a variety of factors. The clinical predictive model that was established in this study can help clinicians predict the

probability of relapse in MG patients, identify early high-risk relapse patients, and serve for high-quality clinical management.

Keywords: Myasthenia Gravis, relapse, Risk factors, Clinical prediction model

Background

Myasthenia gravis (MG) is a recurrent autoimmune disease, which mainly involves acetylcholine receptors (AChR) in the postsynaptic membranes of neuromuscular junctions. Currently, the total incidence of MG in the world is estimated at 4.1-30 cases /1 million, and the prevalence is 150-200 cases /1 million [1]. In China the incidence of MG is approximatively 6.8 cases/1 million, the median hospitalization cost is ¥6859, and the in-hospital mortality is approximatively 14.69‰ [2]. The main causes of death include respiratory failure and pulmonary infection [2]. MG is affected by many factors and most patients are repeatedly hospitalized for longer periods, which have important impacts on the patient's family and a burden to society. Therefore, reducing the relapse of MG is very important. Previous literature has reported that serum autoantibodies, thymus abnormalities, drug use, and other autoimmune diseases are related to the recurrence of MG [3-5]. However, there is still no effective method to evaluate the relapse of MG patients, and therefore, it is very important to clarify the risk factors of MG relapse and establish an effective clinically predictive model. Through collecting clinical data of MG patients who were hospitalized in Zhejiang Provincial Hospital of Chinese Medicine in the past 10 years, this study established a clinical predictive model that looked at risk factors affecting the relapse of MG patients after the first onset with the aim of providing intervention strategies to reduce the relapse of MG patients.

Results

1. Analysis of basic clinical data of MG patients

A total of 86 MG patients were included in this study, of whom 61.2% relapsed within 2 years after the first MG attack (53 in the relapse group and 33 in the non-relapse group). The interval between symptom relapse and the first MG attack in the relapse group was 3-24 months, and the median time was 1 year. See Table 1 and Table 2 for details.

Table 1 Basic clinical data of 86 MG patients

Characteristics	Total（N=86）	Non-relapse group（N=33）	Relapse group（N=53）	*P*-value
Gender				0.852
male	48 （55.8%）	18 （54.5%）	30 （56.6%）	
female	38 （44.2%）	15 （45.5%）	23 （43.4%）	
age of onset[a]	44.50（30.00, 56.25）	46.00（36.00, 59.00）	43.00（29.00, 53.00）	0.918
＜50	62 （72.1%）	24 （72.7%）	38 （71.7%）	
≥50	24 （27.9%）	9 （27.3%）	15 （28.3%）	
Myasthenia Gravis Foundation of America (MGFA) at onset				0.069
Type I	56（65.1%）	25（75.85%）	31（58.5%）	
Type IIa	16（18.6%）	7（21.2%）	9（17.0%）	
Type IIb	11（12.8%）	0（0.0%）	11（29.8%）	
Type IIIa	3（3.5%）	1（3.0%）	2（3.8%）	
site of first symptom				0.003
eye muscle	56（67.5%）	25（75.8%）	31（58.5%）	
eye muscle, limbs and trunk	14（16.3%）	5（15.2%）	9（17.0%）	
eye muscle, medulla oblongata	4（4.7%）	0（0.0%）	4（7.5%）	
limbs and trunk	4（4.7%）	3（9,1%）	1（1.9%）	
limbs and trunk, medulla oblongata	3（3.5%）	0（0.0%）	3（5.7%）	
medulla oblongata	5（5.8%）	0（0.0%）	5（9.4%）	
combination of other autoimmune diseases				0.605
yes	11（12.8%）	5（15.2%）	6（11.3%）	
no	75（87.2%）	28（84.8%）	47（88.7%）	

combination of other diseases				0.415
yes	50（58.1%）	21（63.6%）	29（54.7%）	
no	36（41.9%）	12（36.4%）	24（45.3%）	
thymoma				0.243
yes	30（34.9%）	9（27.3%）	21（39.6%）	
no	56（65.1%）	24（72.7%）	32（60.4%）	
surgical resection of thymoma				0.205
yes	25（29.1%）	7（21.2%）	18（34.0%）	
no	61（70.9%）	26（78.8%）	35（66.0%）	
AChR antibody				0.550
positive	24（27.9%）	8（24.2%）	16（30.2%）	
negative	62（72.1%）	25（75.8%）	37（69.8%）	
anti-Musk antibody				0.076
positive	9（10.5%）	1（3.0%）	8（15.1%）	
negative	77（89.5%）	32（97%）	45（84.9%）	
myasthenic crisis history				0.427
yes	1（1.2%）	0（0.0%）	1（1.9%）	
no	85（98.8%）	33（100.0%）	52（98.1%）	
irregular drug use				0.019
yes	45（52.3%）	12（36.4%）	33（62.3%）	
no	41（47.7%）	21（63.6%）	20（37.7%）	
Infection history				0.016
yes	26（30.2%）	5（15.2%）	21（39.6%）	
no	60（69.8%）	28（84.8%）	32（60.4%）	
glucocorticoid use in remission phase				0.428
yes	22（25.6%）	10（30.3%）	12（22.6%）	
no	64（74.4%）	23（69.7%）	41（77.4%）	
cholinesterase inhibitor use in remission phase				0.479
yes	51（59.3%）	18（54.5%）	33（62.3%）	
no	35（40.7%）	15（45.5%）	20（37.7%）	
Other immunosuppressants use in remission phase				0.732
yes	2（2.3%）	1（3.0%）	1（1.9%）	
no	84（97.7%）	32（97.0%）	52（98.1%）	

Note: [a] Referring to previous literature, the age was 50 years as the boundary [7].

Table 2　Clinical data of 53 MG patients in the relapse group

Characteristics	Relapse group（N=53）
Case of Relapse	
None	43（81.1%）
infection	7（13.2%）
fatigue	1（1.9%）
emotion and stress	1（1.9%）
after thymoma surgery	1（1.9%）
MGFA when relapse	
Type I	14（26.4%）
Type IIa	20（37.7%）
Type IIb	12（22.6%）
Type IIIa	5（9.4%）
Type IIIb	2（3.8%）
Time between relapse and first attack (year)	1.00（0.50, 1.67）

2. Monofactorial analysis

The results showed that the proportion of irregular drug use, infection history, initial symptoms involving limbs and medulla oblongata (MGFA) in the relapse group, was significantly higher than that in the non-relapse group ($P < 0.05$). There were no significant differences between the two groups regarding gender, age of onset, combination of other autoimmune diseases, thymoma, AChR antibody, anti-Musk antibody, and glucocorticoid, cholinesterase inhibitor, and other immunosuppressants usages during the remission phase ($P > 0.05$) (Table 3).

Table 3　Monofactorial analysis of risk factors for MG relapse within 2 years after the first attack

Characteristics	Relapse group（N=53）	Non-relapse group（N=33）	*OR*	95%*CI*	*P*-value
Gender					
male	30	18			
female	23	15	0.92	0.38-2.22	0.85
age of onset					

＜50	38	24			
≥50	15	9	1.05	0.4-2.86	0.92
MGFA at onset					
Type I/IIa	40	32			
Type IIb/IIIa	13	1	10.4	1.91-193.92	0.03
combination of other autoimmune diseases					
yes	6	28	0.71	0.2-2.68	0.61
no	47	5			
Thymoma					
yes	21	9			
no	32	24	1.75	0.69-4.65	0.24
AChR antibody					
positive	17	8			
negative	37	25	1.35	0.51-3.77	0.55
anti-Musk antibody					
positive	8	1			
negative	45	32	5.69	0.97-108.21	0.11
irregular drug use					
yes	33	12			
no	20	21	2.89	1.19-7.28	0.02
Infection history					
yes	21	5			
no	32	28	3.67	1.3-12.17	0.02
glucocorticoid use in remission phase					
yes	12	10	0.67	0.25-1.82	0.43
no	41	23			
cholinesterase inhibitor use in remission phase					
yes	33	18	1.37	0.57-3.34	0.48
no	20	13			
Other immunosuppressants use in remission phase					
yes	1	1	0.62	0.02-15.93	0.73
no	52	32			

3. Multivariate analysis

The results showed that MGFA at onset (*P*=0.048, *OR*=8.76, 95%*CI*=1.48-168.61), irregular drug use (*P*=0.026, *OR*=3.02, 95%*CI*=1.16-8.26), infection history (*P*=0.036, *OR*=3.53, 95%*CI*=1.15-12.59) were independent risk factors for relapse within 2 years after the first MG attack ($P < 0.05$) (Table 4).

Table 4 Multivariate analysis of risk factors for MG relapse within 2 years after the first attack

Characteristics	regression coefficient	*OR*	95%*CI*	*P*-value
MGFA at onset	2.17	8.76	1.48-168.61	0.048
Irregular drug use	1.11	3.02	1.16-8.26	0.026
Infection history	1.26	3.53	1.15-12.59	0.036

4. Establishment of a clinical predictive model

MGFA onset, irregular drug use, and the infection history were considered as final predicators. An equation that matches the model was established:

$$P=e^X/（1+e^X）$$

X=-0.62+2.17 × MGFA type IIb or above at the onset +1.11 × Irregular drug use +1.26×Infection history (e is Natural logarithm)

Meanwhile, the model is presented as a nomogram for further visualization (Figure 1).

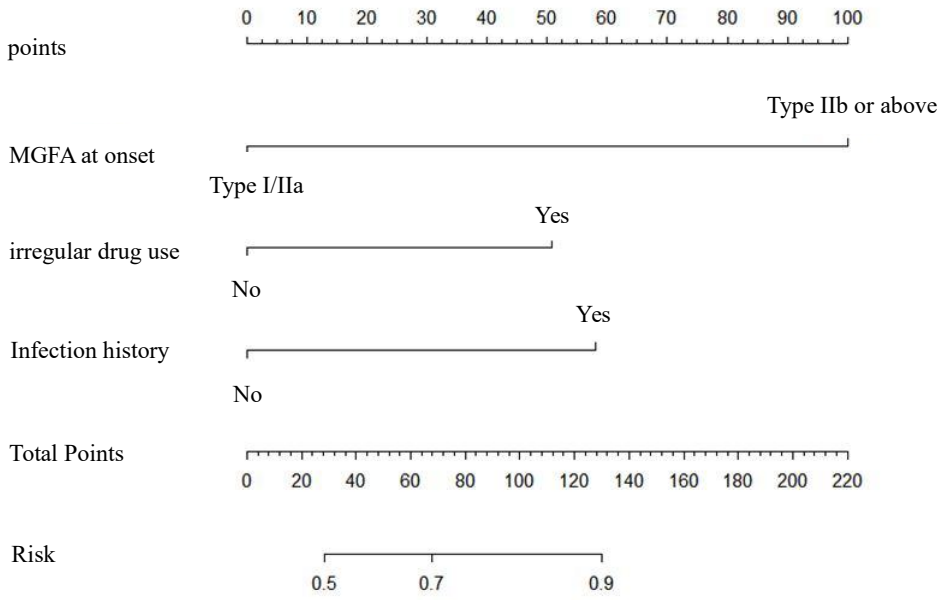

Figure 1 The clinical predictive model for MG relapse within 2 years after the first attack

5. Evaluation of the predictive model

5.1. Discrimination evaluation

In this study, ROC curve was drawn based on the above prediction model and as shown in Figure 2 (Area under the curve (AUC) =0.750，95% *CI*：0.649-0.851). The prediction model had a significant degree of discrimination and could distinguish the relapse and non-relapse of MG patients.

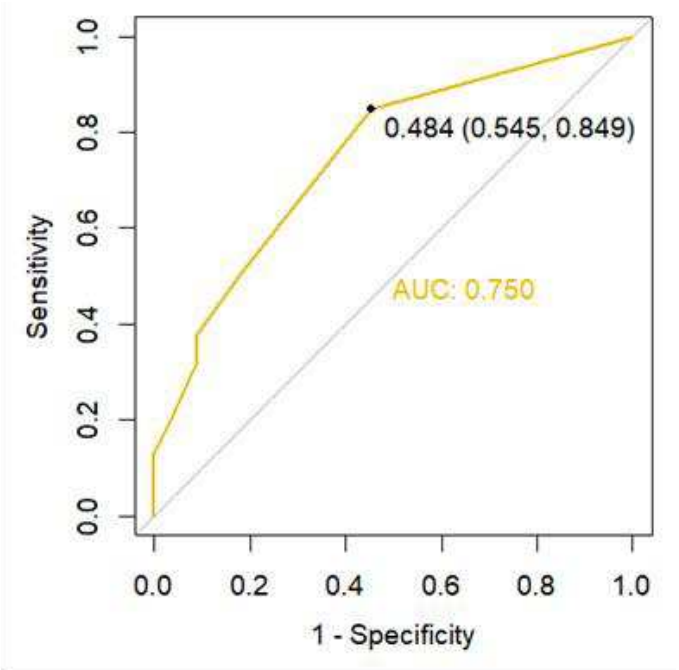

Figure 2 ROC curve of the prediction model

5.2 calibration evaluation

The Hosmer-Lemeshow Test was used to test the validity of fit of the prediction model. The *p*-value of the Hosmer-Lemeshow Test for the study clinical predictive model was 0.93 ($P > 0.05$), indicating that the fitting effect of prediction model was relatively good.

The calibration curve indicates that the closer the predicted value is to the observed value, the better the calibration degree of the model is. The calibration curve of this predictive model reflects a significant consistency between the actual observed value and the predicted probability (Figure 3).

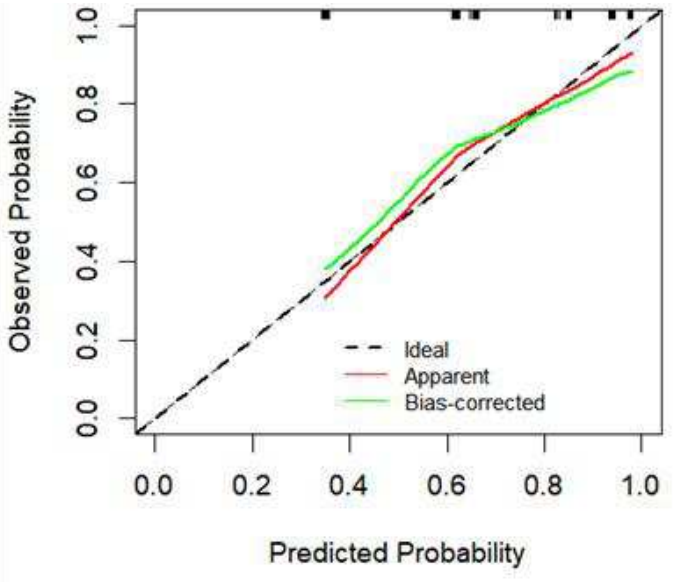

Figure 3 Calibration curve of the predictive model

Discussion

MG is an autoimmune neuromuscular disease which clinical course can include remission, relapse, deterioration, and death. Current clinical studies have different definitions of the MG relapse [4,5,8,9]. Referring to previous literature, this study defines "relapse" as the reappearance of any symptoms and signs of muscle weakness such as extraocular muscle, medulla oblongata, and limb muscles within 2 years after the first onset of remission symptoms, and for a duration of more than 30 days between relapse and final remission [4].

The pathogenesis of MG is extremely complex and its occurrence is the result of the interaction between the genetic susceptibility of the host, the influence of environmental infectious factors, and the body's own immune response. AChR antibody [10-11], other major pathogenic autoantibodies, and THymus CD4+ T lymphocytes [12] play an important role in MG pathogenesis. Although the mechanism of MG relapse may be related to the β 2 adrenergic receptor, this mechanism is still unclear and rarely studied [13]. Therefore, further research on the mechanism of MG relapse will improve our understanding of MG pathogenesis,

relieve patients' pain, provide patients with daily living ability, and reduce the burden of the disease on society and patients.

Clinical predictive models have been widely used in diagnosis and prognosis and have certain significance for the treatment of diseases. In this study, logistic regression analysis was used to preliminarily establish a clinical predictive model for relapse within 2 years after the first attack of MG and in single-center patients. Among the patients, 61.2% relapsed within 2 years after the first MG attack. In the relapse group, the interval between relapse and first attack ranged from 3 to 24 months, and the median time was 1 year. Meanwhile, this study showed that MGFA, irregular drug use and infection were independent risk factors for MG relapse. The model has significant discrimination and calibration, but there is no external validation. Therefore, the model needs validation in a wider range of MG patients.

A previous study reported that 50%-80% of ocular MG often develop into a systemic MG within 1-2 years [14]. In this study, the MGFA of 11 cases of MG patients in the relapse group was changed from type I to type II or type III, which was consistent with the results of the previous study. Meanwhile, the MGFA of most MG patients in the non-recurrence group was type I, and the affected site was limited to the eye muscle. Most of the literature reported that patients with a limited site and mild degree of involvement generally have a good prognosis. Meanwhile, studies have shown that patients with ocular MG have a lower risk of relapse after receiving glucocorticoid treatment compared with that of patients with systemic MG [15]. Yu et al. found that MGFA type I was an independent predictor of complete clinical remission in MG patients [16]. Patients with MGFA type IIB or above may have weakness of the muscle innervated by the medulla oblongata, which is mainly manifested as dysphagia, dysarthria, cough when eating, tongue muscle weakness, and other symptoms [17]. The clearing ability of the upper respiratory tract of these patients is weakened, and therefore, the accumulated secretions in the oropharynx cannot be timely removed. Thus, an upper airway obstruction is extremely likely to occur, which significantly increases the frequency of aspiration, aspiration pneumonia, atelectasis, and other conditions, causing infection and affecting the relapse of MG

[18].

At present, irregular drug use for the treatment of MG mainly refers to insufficient dosage of cholinesterase inhibitors, random increase or decrease of glucocorticoid dosage, drug omission, unreasonable use of antibiotics. Wakata et al. found that early and standardized use of prednisolone could reduce the relapse rate in MG patients [5]. The study results of Yang et al. showed that irregular drug use is an independent influencing factor for relapse or exacerbation of MG symptoms in non-hospitalized patients, which is largely due to patients' insufficient understanding of the disease and failure to take drugs according to the normal usage methods [9]. In this study, there were 45 patients with irregular drug use (33 in the relapse group and 12 in the non-relapse group), and the number of patients in the relapse group was significantly higher than that in the non-relapse group. We speculated that irregular drug use might lead to an immune system disorder in patients and a rise of serum autoantibody titer; thereby, causing the relapse of MG.

Infection plays a crucial role during MG. In Australia, one-third of patients reported that infections would lead to the worsening of MG symptoms, and as many as half of patients showed seasonal changes in MG severity [19]. In the United States, a large cohort study confirmed the seasonal change of MG, with the symptoms being more severe in winter than in summer [20]. This change was speculated to be closely related to respiratory tract infection. In China, a recent study of children with MG found that infection was the second major relapse factor after the withdrawal of immunotherapy [21]. Yang et al. found that infection was an independent influencing factor for relapse or deterioration in both hospitalized patients and non-hospitalized patients with MG [9]. For non-hospitalized patients, infections had higher risk impacts. The results of this study showed that infection was an independent risk factor for relapse, which was consistent with the results of previous studies. We found that 30.2% of MG patients had a history of infection, and that the probability of relapse within 2 years increased by 3.53 times. The mechanism may be related to the disorder of autoimmune system caused by infections as they can cause local inflammatory responses, which increase the expression of molecules involved in antigen recognition

[22, 23]. Infections can also induce polyclonal activation of immunocompetent cells, including autoreactive B lymphocytes and T lymphocytes. This is a potential mechanism for inducing autoimmune diseases, but it has not been proven in MG, and therefore, further basic research is needed. Thus, it is proposed that MG patients should avoid infections. Once an infection occurs, a timely, effective, and standardized treatment should be carried out.

This study evaluated the discrimination and calibration of the prediction model, indicating its good differentiation and calibration, and its effect on predicting relapse of MG patients within 2 years after the first attack. this study is a retrospective study, and there are some limitations. The sample size is small, and there may be selection bias, confounding bias, and other errors. Meanwhile, there may be a certain degree of recall bias, especially recall distortion for relapse time point and a relapse situation may affect the reliability of the results. Therefore, more cases are required for future studies.

Conclusions

In summary, we established a clinical predictive model according to the prediction factors related to MG patients, which can help clinicians predict the probability of relapse within 2 years after the first attack in MG patients. For patients with the first MG attack, we should pay attention to their MGFA, guide patients to use standardized drugs, prevent and treat the occurrence of infection, and provide a reliable tool for predicting the short-term prognosis of MG patients to improve their prognosis and better carry out high-quality clinical management.

Methods

Research subjects

Our investigation involved 86 patients who were diagnosed with MG at Zhejiang Provincial Hospital of Chinese Medicine, from January 2010 to September 2018. The inclusion criteria were as follows: (1) Consistent with the diagnosis of MG (Based on clinical, electrophysiological and laboratory tests [6]): a) The symptoms of muscle

weakness in the affected skeletal muscles are fluctuating and fatigable. They are lighter in the morning and more severe in the evening. They worsen after exercise and can be relieved after rest; b) The neostigmine test is positive; c) When a repeated electrical stimulation is performed, the amplitude of low frequency stimulation decreases by more than 10%; d) AChR antibody, anti-musk antibody or anti Lrp4 antibody can be detected in serum. Based on the typical clinical symptoms of MG, the diagnosis can be made if any point in b-d is satisfied; (2) Included relapse group: patients have their first relapse within 2 years after remission of their first symptoms; (3) Included in the non-relapse group: the patients are followed up for 2 years without relapse after the first onset of symptoms remission; and (4) Complete clinical data. The exclusion criteria were as follows: (1) Lambert-Eaton syndrome, motor neuron disease, congenital myasthenia syndrome, chronic progressive extraocular palsy, Graves' disease, ocular muscular dystrophy and other diseases, should be excluded; (2) During the course of the disease, the symptoms are progressive without remission; and (3) Incomplete clinical data.

Research methods

Inpatient electronic medical record system was used to query and collect clinical data of the 86 patients from the first onset to the first relapse, including age of onset, site of first symptom, MGFA at onset, thymoma, surgical resection of the thymoma, infection history, myasthenic crisis history, irregular drug use, combination of other autoimmune diseases, combination of other diseases, AChR antibody, and anti-Musk antibody.

Follow-up and outcome indicators: by December 2020, the information of MG patients was obtained through reaching out to the outpatient, telephone or consulting the patient's electronic medical record to determine whether there was relapse of MG symptoms within 2 years after the first attack.

Definition of relapse: the reappearance of any symptoms and signs of muscle weakness such as extraocular muscle, medulla oblongata, and limb muscles within 2 years after the first onset of symptoms remission with a duration of more than 30days between relapse and final remission.

Statistical analysis

In this study, the R software (version 4.0.3) was used for statistical analysis. The continuous variables subject to normal distribution are expressed as mean ± standard deviation and analyzed by *t*-test. The continuous variables of skew distribution were expressed by median (interquartile range) and analyzed by rank sum test. The Chi-square test was used for categorical variables and the trend Chi-square test was used for ordered categorical variables. Univariate analysis and multivariate analysis were used to analyze risk factors. The clinical predictive model was established by Logistic regression analysis and presented in the form of a nomogram. Receiver operating characteristic (ROC) curve, calibration curve and goodness of fit were used to evaluate the predictive value of the prediction model. P <0.05 was considered statistically significant.

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Authors' contributions

Z-X Z and J-Y L designed the study. All authors contributed to the generation, collection, assembly, analysis and/or interpretation of data. J-Y L wrote the manuscript, Z-X Z revised the manuscript. All authors read the manuscript and approved the final manuscript.

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Availability of data and materials

The data and materials can be obtained from the first author.

Ethics' approval and consent to participate

The study is in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Zhejiang Provincial Hospital of Chinese Medicine (Hangzhou, China) (Ethical approval No. 2020-KL-165-02).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Dresser L, Wlodarski R, Rezania K, Soliven B. Myasthenia Gravis: Epidemiology,

Pathophysiology and Clinical Manifestations. J Clin Med. 2021; 10(11):2235.

2. Chen J, Tian DC, Zhang C, Li Z, Zhai Y, Xiu Y, et al. Incidence, mortality, and economic burden of myasthenia gravis in China: A nationwide population-based study. Lancet Reg Health West Pac. 2020; 5: 100063. DOI: 10.1016/j.lanwpc.2020.100063.

3. Suzuki S, Nishimoto T, Kohno M, Utsugisawa K, Nagane Y, Kuwana M, et al. Clinical and immunological predictors of prognosis for Japanese patients with thymoma-associated myasthenia gravis. J Neuroimmunol. 2013; 258(1-2): 61-6.

4. Wang L, Zhang Y, He M. Clinical predictors for the prognosis of myasthenia gravis. BMC Neurol. 2017; 17(1): 77.

5. Wakata N, Iguchi H, Sugimoto H, Nomoto N, Kurihara T. Relapse of ocular symptoms after remission of myasthenia gravis—a comparison of relapsed and complete remission cases. Clin Neurol Neurosurg. 2003; 105(2): 75-7.

6. Neuroimmunology branch of Chinese Society for Immunology. Chinese guidelines for the diagnosis and treatment of myasthenia gravis (2020 edition). Chin J Neuroimmunol Neurol. 2021; 28(01): 1-12.

7. Aarli JA, Romi F, Skeie GO, Gilhus NE. Myasthenia gravis in individuals over 40. Ann N Y Acad Sci. 2003; 998: 424-31.

8. Bogdan A, Barnett C, Ali A, AlQwaifly M, Abraham A, Mannan S, et al. Prospective study of stress, depression and personality in myasthenia gravis relapses. BMC Neurol. 2020; 20(1): 261.

9. Yang Y, Liu F, Hou Z, Zhu A, Rao X, Gu C. Analysis of factors influencing relapse or exacerbation of myasthenia gravis. J Nurs (China). 2012; 19(04): 60-5.

10. Aguirre F, Manin A, Fernandez VC, Justo ME, Leoni J, Paz ML, et al. C3, C5a and anti-acetylcholine receptor antibody as severity biomarkers in myasthenia gravis. Ther Adv Neurol Disord. 2020; 13: 1756286420935697. DOI: 10.1177 /1756286420935697.

11. Rodolico C, Bonanno C, Toscano A, Vita G. MuSK-associated myasthenia gravis: clinical features and management. Front Neurol. 2020; 11: 660.

12. Çebi M, Durmus H, Aysal F, Özkan B, Gül GE, Çakar A，et al. CD4 + T cells of myasthenia gravis patients are characterized by increased IL-21, IL-4, and IL-17A

productions and higher presence of PD-1 and ICOS． Front Immunol. 2020; 11: 809.

13. Guereschi MG, Araujo LP, Maricato JT, Takenaka MC, Nascimento VM, Vivanco BC, et al. Beta2-adrenergic receptor signaling in CD4+ Foxp3+ regulatory T cells enhances their suppressive function in a PKA-dependent manner. Eur J Immunol. 2013;43:1001–1012.

14. Hong YH, Kwon SB, Kim BJ, Kim BJ, Kim SH, Kim JK, et al. Prognosis of ocular myasthenia in Korea: a retrospective multicenter analysis of 202 patients. J Neurol Sci. 2008; 273(1-2): 10-4.

15. Okada H, Kuhn C, Feillet H, Bach JF. The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. Clin Exp Immunol. 2010; 160(1): 1-9.

16. Yu S, Li F, Chen B, Lin J, Yang M, Fu X, et al. Eight-year follow-up of patients with myasthenia gravis after thymectomy. Acta Neurol Scand. 2015; 131(2): 94-101.

17. Rabinstein AA, Wijdicks EF. Warning signs of imminent respiratory failure in neurological patients. Semin Neurol. 2003; 23(1): 97-104.

18. Jani-Acsadi A, Lisak RP. Myasthenic crisis: guidelines for prevention and treatment. J Neurol Sci. 2007; 261(1-2): 127-33.

19. Blum S, Lee D, Gillis D, McEniery DF, Reddel S, McCombe P. Clinical features and impact of myasthenia gravis disease in Australian patients. J Clin Neurosci. 2015; 22(7): 1164-9.

20. Melamed R D, Khiabanian H, Rabadan R. Data-driven discovery of seasonally linked diseases from an Electronic Health Records system. BMC Bioinformatics. 2014; 15(Suppl 6): S3.

21. Gui M, Luo X, Lin J, Li Y, Zhang M, Zhang X, et al. Long-term outcome of 424 childhood-onset myasthenia gravis patients. J Neurol. 2015; 262(4): 823-30.

22. Barzago C, Lum J, Cavalcante P, Srinivasan KG, Faggiani E, Camera G, et al. A novel infection- and inflammation-associated molecular signature in peripheral blood of myasthenia gravis patients. Immunobiology. 2016; 221(11): 1227-36.

23. Bach J F. The hygiene hypothesis in autoimmunity: the role of pathogens and commensals. Nat Rev Immunol. 2018; 18(2): 105-20.